THE ACIDITIES OF EXOCYCLIC AMINO GROUPS IN HETEROAROMATIC SYSTEMS

BY

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ABSTRACT

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The acidities of 37 amino-substituted heterocyclic compounds have been determined using measurements of degree of ionization in DMSO/water/0.011 M hydroxide ion and recently developed extrapolative procedures which are based on the Bunnett-Olsen (B.O.) and the Marziano-Cimino-Passerini (M.C.P.) methods. The compounds studied include 19 aminopyridines and pyrimidines and 18 derivatives of the biologically important nucleotide bases, cytosine, adenine and guanine.

The UV/vis spectra of the neutral and anionic forms of the aminopyridines and pyrimidines are well separated so that in evaluating the ionization ratios, changes of the spectra with medium composition were accounted for by measuring at the wavelength of maximum anionic absorption in each solution and by assuming that e_{A^-} and e_{HA} vary linearly with H_-^N. For the majority of the nucleotide derivatives, the neutral and anionic spectra were closely overlapping although different in shape. The ionization ratios were best evaluated by using the area under the spectral curve; spectral changes with solvent composition were accounted for by an adaptation of the method described above. Ionization ratios evaluated by the area method agreed well with those calculated by other methods as long as the anionic area was at least one and one-half times as great as the neutral area (or vice versa).

The aminopyrimidines and purines define a new acidity function H_-^P. This function covers the range from water to 87.6 mole % DMSO and was established using 15 aminopyrimidines and purines. When H_-^P is plotted against mole % DMSO, it rises less steeply than H_-^N. This has

been attributed to less extensive delocalization of charge in the aminopyrimidine anion relative to that of an aniline (usually a nitroaniline) ionizing at the same solvent composition.

The B.O. pK_{HA} values of unsubstituted 2- and 4-aminopyridine, 2- and 4-aminopyrimidine and 2-amino-s-triazine, ranging from 23.50 to 14.91 are well-correlated by a Hammett plot using σ values recently established for aza substituents and assuming the additivity of aza substituent effects, ρ. 4.99. The B.O. pK_{HA} values of five substituted anilines, ranging from 25.50 to 23.13, fall close to this plot. All ten compounds are accommodated by a straight line, ρ value 5.10, which suggests that the aminoheterocycles may be considered to be aza-substituted anilines. It appears that the sensitivity of aniline and diphenylamine acidity to substituent effects is greater than previously believed.

The findings in this work suggest that transmission of substituent effects through a heterocyclic and a benzene nucleus are equal and that an aza group may be regarded as a normal ring substituent. From the acidities of substituted aminopyridines and pyrimidines it appears that, to a first approximation, aza groups do not perturb the effects of other substituents on the same nucleus, although there may be a small resonance interaction between a +R group, such as chloro, and an aza group lying para to it. The same conclusion was reached from examination of the basicities of these compounds.

There is no evidence of increased through-resonance between the amino group and a 4-aza substituent, as is observed between an amino and a 4-nitro group in the anilines. The effects of 2- and 4-aza groups are correlated by Hammett σ values rather than by σ^- constants. Unlike the effect of nitro groups on aniline acidity, the effect of aza groups is additive. In the aminoheterocycles, a nitro group para to the amino group exerts a large acid-strengthening effect, as in the anilines, but this effect is less than in the latter and decreases with the number of aza groups already present in the ring.

The carbonyl group in cytosines and isocytosines exerts an expected acid-strengthening effect. If the aza group vicinal to the carbon bearing the amino group is methylated, the acidity is greatly enhanced. Thus 3-methylcytosine, pK_{HA} 13.37, is a stronger acid than 1-methylcytosine, pK_{HA} 16.69. It is suggested that the neutral form of these compounds is stabilized when a hydrogen-bonding interaction between the amino group protons and the aza group is possible. A similar explanation is advanced for the observation that 7-methyladenine derivatives are stronger acids than the corresponding 9-methyladenines.

The substituent effects of C_2- and C_8-substituted 7- and 9-methyladenines were well-correlated using Hammett's σ_m constants for C_2-substituents and σ_7^+ constants (derived for naphthalene) for C_8-substituents. In aminopurines, the imidazole ring exerts both an acid-strengthening effect, (increased delocalization of charge in the purine anion) and an acid-weakening effect, (increased electron-donation into the pyrimidine ring) relative to an aminopyrimidine. In unsubstituted

7- and 9-methyladenine, the acid-stengthening effect predominates since they are stronger acids than 4-aminopyrimidine. Electronegative groups at C_2, however, increase electron-donation into the pyrimidine ring. In fact, 1,7- and 1,9-dimethylguanine are slightly weaker acids than 3-methylisocytosine.

In the cytidine examined the ribosyl moiety appears to exert a -I effect on amino group acidity.

Little correlation is observed between the pK_{HA} and pK_{BH^+} values of the aminoheterocycles. This is not surprising since the sites of protonation and deprotonation are different.

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DEDICATION

This thesis is dedicated to my three colleagues Dr. Robin A. Cox, Dr. Trevor W. (Pud) Toone and Dr. R. Srinivasan whose good friendship and guidance made completion of this work possible.

INTRODUCTION

A. Definition of the Acid Ionization Constant

The simplest reaction for studying the effect of molecular structure on reactivity is the ionization of Brönsted acids, or proton transfer between solute and solvent.[1]

The Arrhenius definition of an acid is a substance which produces protons in aqueous solution and of a base, a substance which produces hydroxide ions in aqueous solution. The Brönsted-Lowry concept defines an acid as a proton donor and a base as a proton acceptor. The Lewis sytem defines a base as an electron pair donor and an acid as an electron pair acceptor.[2]

In this work the Brönsted definitions of acid and base will be used. This definition includes acid ionizations in aqueous media (Arrhenius acids) as well as ionizations in nonaqueous solvents such as anhydrous dimethyl sulfoxide. Thus for a given neutral acid HA, the acid ionization in water may be written,

$$HA \rightleftharpoons H^+ + A^- \qquad (1)$$

where A^- is the conjugate base of HA. All species in equation (1) are considered to be solvated. Then it follows that

$$K_{eq} = \frac{[H^+][A^-]}{[H_2O][HA]} \qquad (2)$$

where [ ] expresses the concentration of X in moles per liter. In practice $[H_2O]$ is incorporated into the equilibrium constant such that

$$K^c_{HA} = \frac{[H^+][A^-]}{[HA]} \qquad (3)$$

K^c_{HA} is the concentration ionization constant[3].* However, the behavior of a solute X in solution is better expressed by its activity a, where f_x is

$$a_x = f_x[X] \qquad (4)$$

the activity coefficient of the given solute X.

The thermodynamic ionization constant, which is independent of concentration, is expressed by the equation,

$$K_{HA} = \frac{a_{H^+} \cdot a_{A^-}}{a_{HA}} \qquad (5)$$

The activity and concentration of X deviate more and more as the concentration of X or other solutes increases. At infinite dilution, however, activity and concentration become equivalent and f_x is equal to unity.[2] When K_{HA} is determined spectrophotometrically the concentration of HA is usually very

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* Throughout this work K_a will represent the general acid ionization constant, K_{HA} the acid ionization constant of a neutral acid HA and K_{BH^+} the acid ionization constant of a cationic acid BH^+.

small, of the order 10^{-3} to 10^{-5} moles per litre, so that the activities of the species in (1) may be considered equal to their respective concentrations. The thermodynamic constant K_{HA} is thus defined as the ionization constant of HA at infinite dilution in water at 25°. For convenience, K_{HA} is usually expressed as its negative logarithm pK_{HA}.

$$-\log K_{HA} = pK_{HA} \qquad (6)$$

B. Definition of Acidity Function. The Hammett Principle of Overlapping Indicators.

Equation (5) may be rewritten such that,

$$pK_{HA} = \log\left(\frac{a_{HA}}{a_{A^-}}\right) - \log a_{H^+} = \log \frac{[HA]}{[A^-]} - \log\left(\frac{a_{H^+} \cdot f_{A^-}}{f_{HA}}\right) \qquad (7)$$

At very low concentrations in water, the activity coefficients equal unity and (7) reduces to

$$pK_{HA} = \log \frac{[HA]}{[A^-]} - \log a_{H^+} \qquad (8)$$

The last term in equation (8) is approximately equal to the pH of the solution, determined potentiometrically. At zero or low ionic strength equation (8) may be used to calculate the pK_{HA} of acids ionizing in the pH region 1-13.

Very weak acids, though not detectably ionized in water, may be measurably

ionized in strongly basic media such as ternary mixtures of dimethyl sulfoxide (DMSO)/water/0.011 M tetramethylammonium hydroxide (TMAH). The deprotonating power of this medium towards a given indicator molecule cannot be expressed by the pH of the solution but, within a set of structurally similar indicators, it may be expressed by an acidity function H_-. H_- is defined,[4,5]

$$H_- = - \log \left(\frac{a_{H^+} \cdot f_{A^-}}{f_{HA}} \right) = pK_{HA} - \log \frac{[HA]}{[A^-]} \qquad (9)$$

For two indicators HA_1 and HA_2 both measurably ionized at the same solvent composition,

$$pK_{HA_1} - pK_{HA_2} = \log \frac{[HA_1]}{[A_1^-]} - \log \frac{[HA_2]}{[A_2^-]} + \log \left(\frac{f_{HA_1} f_{A_2^-}}{f_{A_1^-} f_{HA_2}} \right) \qquad (10)$$

If the difference in the ionization ratios of the two indicators is constant over a specified range of solvent composition, the activity coefficient term of (10) may be considered to be independent of the medium in the region considered. For two acids which ionize in a near aqueous medium, it is assumed that this constant difference in the ionization ratios extends to pure water.[4] At infinite dilution in water, the activity coefficients are unity and the last term in equation (10) is zero.

The Hammett activity coefficient postulate or overlap assumption requires this term to be zero over the whole solvent composition range in which the indicators are both ionized, that is, the activity coefficients of HA_1 and HA_2 must cancel. This is true only within a narrow set of

structurally similar indicators. If Hammett's postulate holds then (10) reduces to,

$$pK_{HA_1} - pK_{HA_2} = \log \frac{[HA_1]}{[A_1^-]} - \log \frac{[HA_2]}{[A_2^-]} \quad (11)$$

Hammett's postulate is the basis of determining acidity constants, pK_{HA}'s, by the method of overlapping indicators. An anchor compound HA_1, whose thermodynamic pK_{HA} is known and whose ionization ratios can be measured both in water and in dilute solutions of the basic medium, is selected. Another compound, HA_2, ionizes measurably over a range of solvent composition in the dilute region, but not in water. However, HA_1 and HA_2 both ionize measurably in some of this range, that is, they overlap. If, in this region of overlap, the Hammett postulate holds, the thermodynamic pK_{HA} of HA_2 may be calculated using equation (11). A test of the postulate is that the difference in their ionization ratios be constant in the region of overlap.

For a weaker acid HA_3, overlapping with HA_2, if the difference between its ionization ratios and those of HA_2 remains constant, its thermodynamic pK_{HA} may also be calculated from (11) using the pK_{HA} of HA_2 previously calculated. The procedure may be extended to include yet weaker acids, and as long as Hammett's postulate is obeyed their pK_{HA}'s so determined will be the thermodynamic constants referred to standard state water.

By a suitable choice of indicators, no more than 1 pK unit apart[6], the whole solvent composition range may be spanned. Using the pK_{HA}'s calculated and the measured ionization ratios, H_- may be calculated from equation (9). If a compound "obeys" H_- thus constructed, that is belongs

to the set of the overlapping indicators, then a plot of its ionization ratios against H_- will be a straight line of slope 1.00 and its pK_{HA} will be the value of H_- at half-ionization.

C. The H_- Function in Ternary Mixtures of DMSO/water/0.011 M TMAH

The compounds whose acid ionizations were studied in this work are acids too weak to be measurably ionized in water. A strongly basic or deprotonating medium is required to generate a measurable amount of the indicator anion. The pK_{HA}'s were determined by the acidity function approach of Hammett.[4,5]

The subject of strongly basic systems has been reviewed by Rochester,[7] Bowden[8] and Jones[6]. Strongly basic systems may be created by using (a) an aqueous solution of an alkali metal hydroxide, e.g. concentrated sodium hydroxide solution (b) an alcoholic solution of alkoxide, e.g. methanolic sodium methoxide (c) an aqueous solution of a basic organic compound, e.g. hydrazine in water (d) an aqueous solution of a polar aprotic solvent made basic by the addition of small fixed amounts of base, e.g. 0.011 M TMAH in aqueous DMSO. Several of these systems were investigated by Edward[38] and Stewart and O'Donnell[9,10] but aqueous DMSO solutions containing small fixed amounts of TMAH offered the most promise for the construction of a wide-ranging H_- function. DMSO was felt to be a good choice by virtue of its weak acidity, pK_a 32-33[11]; its high autoprotolysis constant[12], its ability to dissolve a large variety of compounds[162] and its high dielectric constant (48.9)[13]. The high dielectric constant prevents ion pair association, which interferes adversely with accurate pK_a determinations.[14]

The system is usually made more basic by increasing the proportion of DMSO rather than the concentration of base. Dolman and Stewart[15] established an H_- scale from pure water (H_- = 12) to 99.6 mole % DMSO (H_- = 26) using primary anilines and diphenylamines as indicators. Other H_- functions that have been established in this system include: H_- functions for carbon acids[16,17] and phenols[18]; the H_{2-} scale for diphenylamine carboxylic acids[19]; the $H_{-(q)}$ scale for quaternary amine methyl iodides using base concentration 0.047 M TMAH[20]; and the H_{GC} function using both 0.011 M and 0.047 M TMAH, determined electrochemically by measuring the potential between a glass electrode and a dropping mercury electrode at which cobalticinium ion was reduced.[21]

The strongly basic nature of the DMSO/water/0.011 M TMAH system may be explained by consideration of the equilibrium below,

$$HA + OH^-(H_2O)_n \rightleftharpoons A^- + (n+1)H_2O$$

where n is the solvation number of the hydroxide ion, often taken to be equal to 3,[6] or the difference in solvation numbers between the left and right hand side of the equilibrium.[22]

As the proportion of DMSO is increased, the concentration of water is decreased and the equilibrium is shifted to the right by the mass action effect. The equilibrium is also shifted to the right by a decrease in the activity of water, brought about by association between DMSO and free water, that is, water not involved in solvating the species above. A 2:1 hydrate of water:DMSO, postulated by Yagil[22] and others,[23] has been observed by studying the ESR spectrum of the di-t-butyl nitroxide radical, as a function

of medium composition, in aqueous DMSO.[24]

DMSO competes effectively with the hydroxide ion for water. The hydroxide ion is solvated by at least three water molecules held to it by hydrogen bonds, but DMSO cannot solvate the ion by hydrogen bonding. At very high concentrations of DMSO, it would be less solvated and its activity would increase, driving the equilibrium to the right. From studies of equivalent conductivities of hydroxide ion at 90 mole % DMSO, it appears that the hydroxide ion is still heavily solvated. Thus its increased activity must be due to the decrease in the activity of the water molecules solvating it, rather than to actual desolvation.[25]

To further explain the increased activity of the hydroxide ion, Dolman[15] postulated that DMSO broke down the ice-like structure of free water in these media, whereas hydroxide ion was believed to promote this structure. Such a competition would greatly increase the activity coefficient of the hydroxide ion. However, recent evidence from the spin-lattice relaxation times of both DMSO and water protons in the NMR spectra of DMSO/water mixtures suggests that DMSO actually promotes this ordering effect, contrary to Dolman's explanation.[26]

In the above discussion, the effects of DMSO on altering the activity coefficients of the indicator were neglected, but these may have considerable effect on the apparent basicity.[27] It is now well-recognized that in acid systems the original H_0 function established by Hammett and Deyrup[5] cannot describe the protonating power of aqueous sulfuric acid towards all bases. Rather a series of H_0 functions, each defined by a set of structurally similar indicators, is required. This fact has been termed the "acidity function failure" by Arnett.[28a] Even structurally very similar indicators

such as primary and tertiary amines (and possibly secondary amines[15]) require different acidity functions H_o' [29] and H_o'" [28b] respectively. The difference was attributed to differences in the activity coefficients of their conjugate acids, due to their differing degrees of solvation. The conjugate acid of a primary amine may hydrogen-bond to three water molecules since it possesses three protons, whereas that of a tertiary amine may bind only to one.

Cox and Stewart[12] have proposed a method for the calculation of pK_{HA}'a of weak acids in basic DMSO/water for which the appropriate acidity function H_- has not been determined. Bunnett and Olsen[30] derived an extrapolative procedure, requiring the knowledge of H_o,[29] for calculating pK_{BH+} of bases protonating in sulfuric acid, but not necessarily obeying H_o. The method of Cox and Stewart, adapted to basic DMSO/water media, requires that the activity coefficients of the two overlapping indicators in (10) be linear functions of one another, rather than cancelling each other out as required by the Hammett postulate.[4] Using the H_- function of Dolman and Stewart,[15] good agreement was found between pK_{HA}'s calculated by this method and those calculated by the method of overlapping indicators, for both nitrogen and carbon acids.

D. Criticisms of H_- Functions Established in DMSO/water/0.011 M TMAH

Kreevoy and Baughman have recently criticized application of the Hammett approach to ternary mixtures of DMSO/water/0.011 M TMAH.[31] They state that it follows from the Hammett activity coefficient postulate that the pK_a's of a given set of acids referred to a solution S as standard state, namely pK_a^s, must be a linear function of the corresponding pK_a referred to standard state water, namely pK_a^o, with a slope of unity. They found that

pK_a^s of either carboxylic acids or phenols determined in aqueous or pure DMSO was indeed a linear function of pK_a^o but with a slope greater than unity.[32]

The Hammett assumption, in fact, need apply only to those solutions wherein the anchor compound is less than 90% ionized. Valid pK_a^o's may be obtained even if deviations from unit slope appear in solutions where the compounds are highly ionized.[12,18,33] However, the right hand side of equation (11), must be constant for each pair of overlapping indicators at all solvent compositions S ranging from water to that DMSO/water solution in which the more acidic indicator is 90% ionized for the pK_a's thus calculated to be equal to pK_a^o.

Hallé and coworkers have also criticized the use of the acidity function approach in these media.[34,35] They determined the pK_{HA}'s of nitrodiphenylamines, anilines and aminopyridines as a function of the DMSO content of the medium (weight percent) by a combination of potentiometric and spectrophotometric techniques. A series of buffers was chosen such that pK_a^s could be determined over a wide range of solvent composition. They were standardized, that is, the hydrogen ion activity determined, by using a hydrogen electrode. The ionization ratios, I, of a given indicator at composition S were determined spectrophotometrically. The curves of pK_a^s against medium composition were not always parallel, as required by the Hammett postulate as expressed by (11), and often deviated sharply from one another at high DMSO content. However, as described above, it is only necessary to establish that such parallelism exists between each pair of overlapping indicators up to compositions where the stronger acid is 90% ionized.

Hallé[35] found that extrapolation of the plots of pK_a^s of diphenylamine carboxylic acids to pure water to give pK_a^o, did not give values in good agreement with those determined by the acidity function method.[19] Poor agreement was also observed between the values of pK_a^s in pure DMSO, obtained by extrapolation of the plots to 100% DMSO,[34] and those determined potentiometrically by Ritchie and Uschold[36] in pure DMSO by means of the glass electrode. Bordwell[37] has suggested that pK_a's determined at high DMSO content by the Hammett procedure are closer in value to those obtained in pure DMSO rather than to those referred to pure water (pK_a^o) and that those determined in dilute solutions of DMSO lie closer to pK_a^o.

The absolute pK_a for a given compound may be higher or lower in water than in DMSO. DMSO solvates anions with delocalized charge effectively and would thereby help to increase the acidity of compounds that give rise to such ions. Generally, its greater basicity would increase the acidity over that observed in water, but its lower dielectric constant and poorer hydrogen bonding ability would tend to decrease the acidity.[36c]

In any spectrophotometric determination of the ionization ratio, I, small changes in the spectra due to changes in medium composition must be separated from those larger changes due to the ionization of the neutral indicator. Failure to account for such medium changes may result in considerable error in log I.[34] Since the basicity of DMSO/water systems is increased by increasing the proportion of DMSO, the medium changes considerably with consequent changes in the UV/vis absorption spectra of the neutral and ionized form of the indicator. Several empirical methods have been devised to correct such solvent effects and are critically compared in this work.[39-41]

If the spectral changes upon ionization are slight or of comparable magnitude to the medium effect, the ionization ratio cannot be determined by means of spectrophotometry. Jones, Cockerill et al.[42] have proposed a kinetic method for determining pK_{HA}'s in DMSO/water/0.011 M TMAH based on the rate of detritiation of a standard carbon acid at a given mole % DMSO. A given indicator whose pK_{HA} is to be determined is placed in a DMSO solution, wherein it is appreciably ionized, together with a standard carbon acid. The change in rate of detritiation depends on the decreased concentration of hydroxide ion, used to deprotonate the indicator. Thus the concentration of the anion may be determined from this change in rate and hence the pK_{HA} of the acid indicator.

Calculations require the knowledge of the acidity function obeyed by the given indicator. Good agreement was found between the pK_{HA}'s determined and those established by Hammett's method for anilines and diphenylamines.[15] However, in the calculations it was assumed that H_- depended linearly on the changing hydroxide ion concentration,[8] an assumption that has been seriously questioned.[12,21,43]

E. Use of Pure DMSO as the Standard State

Recently, pure DMSO has been suggested as reference solvent for the pK_{HA}'s of organic compounds rather than pure water, since a wide range of acidities may be measured in this medium, from 0 to 30,[37a] since the pK_{HA} of DMSO itself is about 33.[17]

Ritchie and Uschold have used the glass electrode to measure the acidities of several weak carbon[36a] and nitrogen[36b] acids, standard state DMSO. The acids were potentiometrically titrated with cesium dimsyl as base

and although the response time of the electrode was slow, it appeared to respond reversibly over the range studied. The pK_{HA}'s of nitroanilines were found to be the same whether referred to water or DMSO,[36a] this result being attributed to cancellation of acid strengthening and weakening effects on going from DMSO to water.[36c] Hallé and Schaal[34] have questioned the reversibility of the glass electrode in DMSO. Kreevoy and Baughman[32] found that the conventional glass electrode and calomel electrode pH meter responded slowly and sometimes irreproducibly in aqueous DMSO. However, Janata[21] concluded, from his polarographic measurements of hydrogen ion activities in mixed aqueous and non-aqueous basic media, that a properly hydrated glass electrode gave results identical to a hydrogen electrode.

Spectrophotometric measurements of the relative equilibrium acidities of carbon acids in pure DMSO have been made by Bordwell and coworkers.[37] The solution of a given indicator whose anion is spectrophotometrically visible is titrated with the solution of another acid whose anion is colorless. Condensation reactions were avoided by first titrating the potassium dimsyl solution with the colored indicator such that its anion deprotonated the desired compound. The pK_{HA}'s obtained agreed poorly with those obtained potentiometrically,[36] but correlated well with the heats of deprotonation observed in DMSO.[1]

There are several experimental difficulties involved in using pure DMSO. It is very hygroscopic and trace amounts of water can alter the basicity considerably. The curve of H_- [15] plotted against mole % DMSO approaches the y-axis beyond 95 mole % almost asymptotically.[12] Furthermore, the conjugative base of DMSO is very sensitive to oxygen.[1] Thus operations must be carried out on the vacuum line or in the dry box. For biologically

important compounds, such as are studied in this work, pK_{HA}'s are only meaningful if referred to standard state water.

F. Other Methods of Determining pK_a

The subject of pK_a determinations in general have been recently reviewed by Cookson[14] and by Russian workers.[17]

The most promising new method for determining pK_a's is by calorimetry and is useful for compounds whose absorption spectra show little change upon ionization. Arnett observed a linear correlation between the pK_{HA}'s of several nitroanilines and diphenylamines, determined by the Hammett method,[15] and their heats of deprotonation in DMSO at 25° with potassium dimsyl acting as base. The slope of this correlation was close to that observed between the pK_{BH}^+'s of these compounds and their heats of protonation in super acid media.[44] Thus by measuring the heat of deprotonation of a given nitroaniline or diphenylamine in DMSO, an estimate of its pK_{HA}, standard state water, may be made using the linear relation and the assumption that ΔH and ΔG are identical in these systems.

G. The Hammett Equation

The Hammett equation is an empirical relation which attempts a quantitative correlation between the reactivity of a side chain R of a benzene derivative and a substituent X on that nucleus. Hammett observed[4] that the magnitude of the reaction rate constants in a given homologous series of aromatic compounds responded in the same way to the influence of a particular meta or para substituent as did the equilibrium constants of another homologous aromatic series. Hence a well-known linear free energy

equation, is defined,

$$\log k/k_o = \sigma\rho \qquad (12)$$

where k = the rate or equilibrium constant of a meta or para substituted benzene derivative, k_o = the rate or equilibrium constant of the unsubstituted benzene, σ = a substituent constant depending on the nature and location of X, ρ = a reaction constant which depends on the nature of R and on the experimental conditions.

Equation (12) is a linear free energy relationship. Since

$$\Delta G^\circ = -2.3\ RT \log K_{eq} \quad [45]$$

equation (12) expresses the change in free energy on going from reactants to products or, in the case of rate correlations, from reactants to transition state.

The standard reaction defining the substituent constants σ, i.e. for which ρ is set equal to one, is the ionization of substituted benzoic acids in water at 25°. Thus,

$$\sigma = \log K - \log K_o \qquad (13)$$

where K is the ionization constant of a substituted benzoic acid and K_o is the ionization constant of benzoic acid itself.

Sigma values measure the change in electron density at the reaction site produced by substituent X.[46] They are independent of solvent[47a]

except for groups such as NH_2 which may hydrogen-bond to certain solvents. They may be considered a sum of inductive and resonance effects. The inductive effect I of X is due to its electronegativity transmitted through space, the solvent or the sigma bond framework of the molecule. For example, since oxygen is more electronegative than carbon the methoxy group $-OCH_3$ is expected to be electron-withdrawing, i.e., a -I substituent. The resonance effect R is due to the interaction of the pi-orbitals of the benzene ring with the orbitals of X lying in the same plane, for instance the p-orbital of N containing the non-bonding electrons. Thus the amino group $-NH_2$ is a +R substituent. This effect is transmitted only to positions ortho or para to X.

The inductive effect of X, σ_I, is considered to be nearly equal at the meta and para positions. Since the resonance effect is not felt, to a first approximation, at the meta position,[45]

$$\sigma_m = \sigma_I \qquad (14)$$

$$\sigma_p = \sigma_I + \sigma_R \qquad (15)$$

Thus the resonance contribution σ_R of substituent X is given by[45]

$$\sigma_R = \sigma_p - \sigma_m \qquad (16)$$

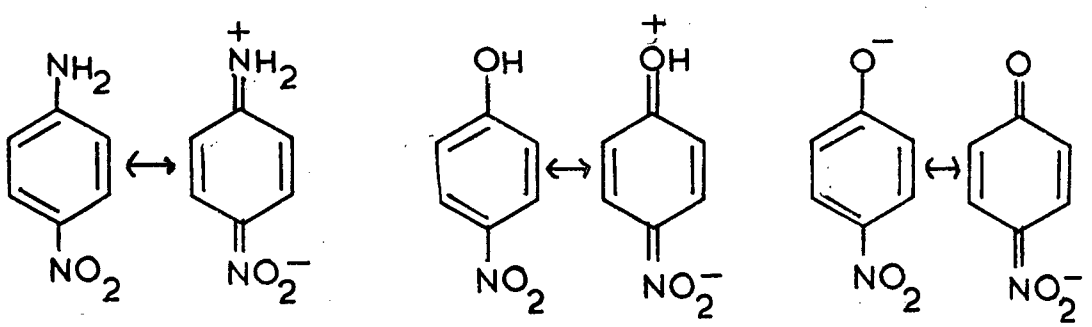
(σ_m may have a small resonance contribution because of inductive charge transmission from ortho and/or para resonance forms.) σ_I has been more accurately evaluated from the acidities of alicyclic acids such as

4-substituted bicyclo[2.2.2]octane carboxylic acids,[48] where resonance effects are precluded by the absence of pi-bonds.

The reaction constant ρ was defined as unity for the ionization of benzoic acids in water at 25°. Its magnitude is a measure of the sensitivity of the equilibrium or reaction to substituent effects. Its sign determines whether the reaction is favored by low electron-density at the reaction site.[46] If ρ is positive then electron-withdrawing substituents favor the reaction and electron-donating substituents retard it.

1. The σ^- constant

The σ constants thus defined do not correlate all the experimental data available, particularly where there is a large change in resonance interaction between the side chain and a para substituent from one side of an equilibrium to another. The σ values of substituents whose effects are primarily inductive do not vary within experimental error,[49,50] i.e. meta substituents and para substituents which do not conjugate with the side chain, such as trifluoromethyl $-CF_3$. Rather than redefine σ_x as the value for X best describing the presently-known body of experimental data as once proposed by Jaffe,[46] two new sets of σ_p constants have been defined for -R and +R substituents, σ^- and σ^+ respectively.



In the anilinium ion resonance between the amino nitrogen and the nitro group is not possible as it is in the neutral molecule. Thus the neutral molecule is stabilized and the basicity is reduced by the para nitro group more than would be predicted from the ionization constant of 4-nitrobenzoic acid. The marked acid-strengthening effect of the para nitro group in phenols is attributed to greater through-conjugation in the anion. The apparent σ_p values required to fit the pK_a's of these compounds bearing a -R para substituent to the line (established using only meta and para substituents without a -R effect) were defined as σ_p^- constants.[2,4,45]

+R substituents in the para position also require modified σ values when through-conjugation of a +R substituent with an electron-withdrawing reaction site is enhanced on one side of the equilibrium relative to the other.[47]

2. The σ^o constant:

For +R substituents such as the methoxy group, through-conjugation is incorporated into the original σ_p values since such para substituents will resonate in the neutral benzoic acid molecule and in the anion with the carboxylic function. To obtain values free of such through-conjugation the carboxylic group was isolated from the benzene ring by a methylene group and the σ values redetermined.[52] For the most part, such σ^o constants are equal to the original σ values except for strong +R substituents in the para position.

Wepster[50] obtained much better correlation of experimental data with substituent constants if para +R and -R substituents capable of resonance with the side chain were omitted. He demonstrated that the σ_p values of such substituents actually constitute a continuous range of values. However, the

two modified sets σ^- and σ^+ correlate much data to a first approximation.[47,51]

H. Ionization Constants of Nitrogen Heterocyclic Compounds

1. Basicities of Nitrogen Heterocyclic Ring Systems

The first major survey of the basic ionization constants of nitrogen heterocycles was made by Albert in 1948.[53] Many of these constants lie in the pH region, pH 1-13, and are easily determined by potentiometric titration. Since 1948 the subject has been extensively reviewed.[54-58]

Piperidine 1 has a basicity comparable to secondary aliphatic amines, pK_{BH^+} = 11.3,[49] but pyridine 2, pK_{BH^+} = 5.2,* is a much weaker base since the lone electron pair of the nitrogen atom now occupies an sp^2 orbital and is less available for conjugation with a proton. Introduction of a second nitrogen into the pyridine ring, meta to the first gives pyrimidine 3, an even weaker base, pK_{BH^+} = 1.31, owing to the -I,-R properties of endocyclic nitrogen. The electron-withdrawing power of an aza group is often equated to that of a nitro group,[49,54,57] and the basicity of pyrimidine is, indeed, close to that of 3-nitropyridine 4, pK_{BH^+} = 0.8.

The purine ring is formed by fusion of an imidazole ring to a pyrimidine ring at positions C_4 and C_5. Purine 6 has a somewhat greater basicity, pK_{BH^+} = 2.39, than pyrimidine which has been explained by the net transfer of negative charge from the electron-rich imidazole ring, 5, to the electron deficient pyrimidine ring.[55a,57,59] Unlike pyrimidine or pyridine, however, purine possesses an ionizable proton at N_7 (or N_9).[54,56] It is a stronger acid, pK_{HA} = 8.93 than imidazole, pK_{HA} = 13, by virtue of the electron-

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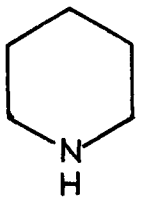
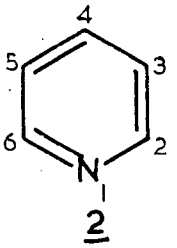
* All pK_a's are taken from reference 54 unless otherwise stated.



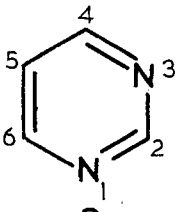
1

pK_{BH^+} 11.3



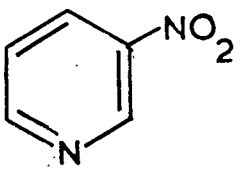
2

pK_{BH^+} 5.2[54]



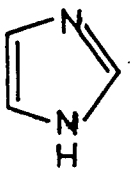
3

pK_{BH^+} 1.31[54]



4

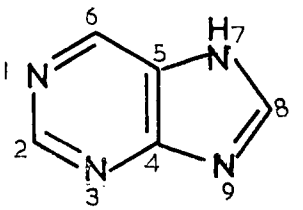
pK_{BH^+} 0.8[54]



5

pK_{BH^+} 7.0[54]

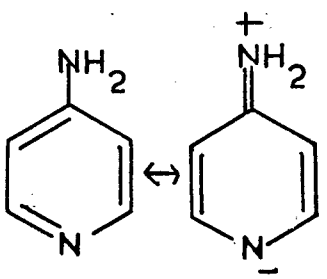
pK_{HA} 13[54]



6

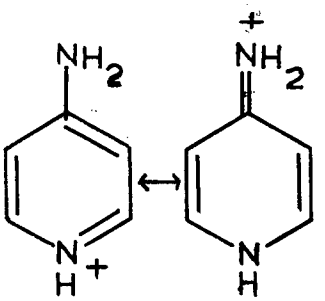
pK_{BH^+} 2.39[54]

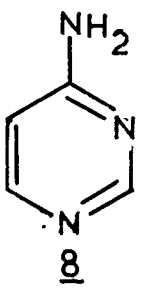
pK_{HA} 8.93[54]



7

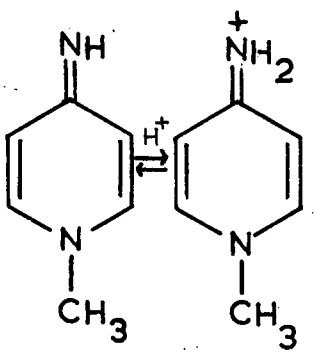
pK_{BH^+} 9.17[60]





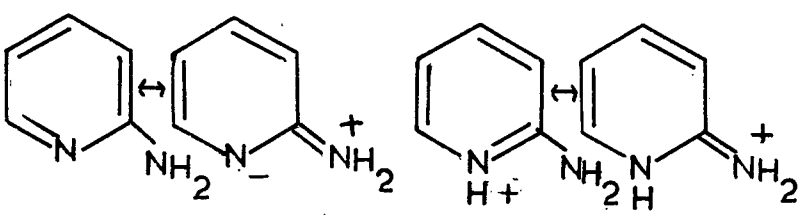
8

pK_{BH^+} 5.71[54]



9

pK_{BH^+} 17.87[20]



10

pK_{BH^+} 6.71[62]

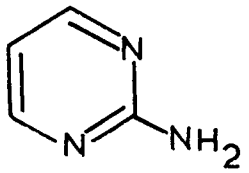
attracting pyrimidine ring. In this work ionization of this proton was precluded by substitution of the labile proton with a methyl group at N_7 or N_9.

2. Effect of Amino and Oxo Substituents on Basicities

Substitution of a primary amino group, a powerful +R substituent,[45] at C_2 or C_4 of the pyridine or pyrimidine ring greatly enhances the compound's basicity. The high basicity of 4-aminopyridine 7, $pK_{BH^+} = 9.17$,[60] and of 4-aminopyrimidine 8, $pK_{BH^+} = 5.71$, is explained by the presence of through-resonance, illustrated for 7, which is much preferred in the cation since a similar resonance in the neutral molecule leads to charge separation.[54] Dipole moment studies suggest a sizeable contribution of this charge separated form in 4-aminopyridine.[61] The greater stabilization of the cation relative to the neutral molecule is the cause of this increased basicity. In the 1-methylimine derivative 9 of 7 such through-resonance is possible in the cation only, which accounts for its very high basicity, $pK_{BH^+} = 17.87$.[20]

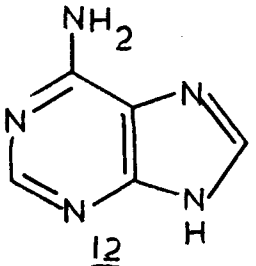
Similarly, the enhanced basicities of 2-aminopyridine 10, $pK_{BH^+} = 6.71$,[62] and of 2-aminopyrimidine 11, $pK_{BH^+} = 3.54$, relative to pyridine and pyrimidine is doubtless due to resonance in the cations.[54] The increase in basicity is less in the 2-amino compounds because the charge separation in the neutral resonance form is less than for the 4-amino compounds, as illustrated for 2-aminopyridine 10.

Introduction of an amino group at C_6 in purine, resulting in adenine 12, $pK_{BH^+} = 4.25$, does not increase the basicity as dramatically as in the monocyclic compounds.



11

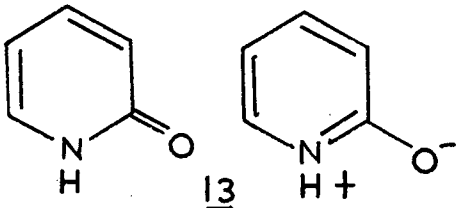
pK_{BH^+} 3.54[54]



12

pK_{BH^+} 4.25[54]

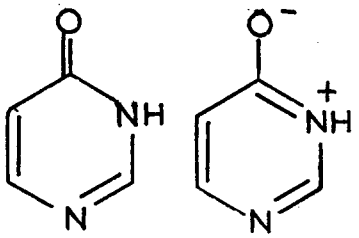
pK_{HA} 9.83[54]



13

pK_{BH^+} 0.75[54]

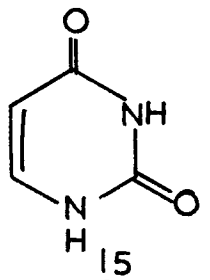
pK_{HA} 11.6[54]



14

pK_{BH^+} 1.85[54]

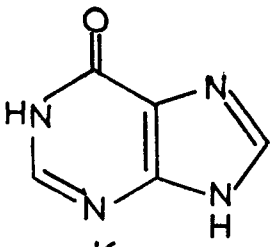
pK_{HA} 8.59[54]



15

pK_{BH^+} −3.4[54]

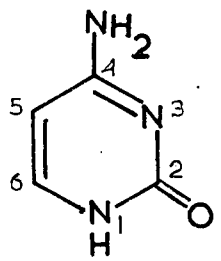
pK_{HA} 9.38[54]



16

pK_{BH^+} 1.98[54]

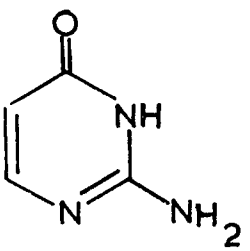
pK_{HA} 8.94[54]



17

pK_{BH^+} 4.58[54]

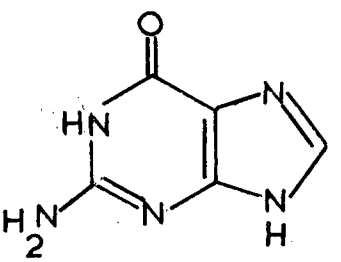
pK_{HA} 12.15[54]



18

pK_{BH^+} 4.00[54]

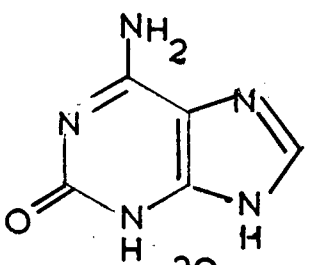
pK_{HA} 9.59[54]



19

pK_{BH^+} 3.0[54]

pK_{HA} 9.32[54]



20

pK_{BH^+} 4.5[54]

pK_{HA} 9.0[54]

Introduction of a hydroxy group at C_2 or C_4 of the pyrimidine ring is also expected to increase the basicity because of this group's +R properties. But the basicities of 2- and 4-hydroxypyrimidine are lower than expected, pK_{BH^+} = 2.24 and 1.85 respectively. The reason is that hydroxypyridines and pyrimidines are no longer aromatic but adopt a cyclic amide form in solution as evidenced by UV,[55a] IR,[55a] and NMR[55b] spectra. The form is stabilized by a zwitterionic contribution as shown for 2-hydroxypyridine 13 and 4-hydroxypyrimidine 14.[54,63] The ortho site of the proton is favored over the para form in 14 [54b] since this involves a smaller charge separation in the zwitterionic form. Protonation occurs at the nitrogen not participating in the cyclic amide structure. In uracil 15 where both aza groups are involved, the basicity is very low, pK_{BH^+} = -(3.4).

Hydroxypurines also adopt a cyclic amide structure, as shown for 6-hydroxypurine or hypoxanthine 16 whether the oxo group is at C_2, C_6 or C_8 as evidenced from IR[64,65] and UV[56] spectra. Unlike the pyrimidine case an oxo group at C_2 or C_6 actually lowers the basicity (pK_{BH^+} for hypoxanthine is 1.98), but an oxo group at C_8 has little effect, pK_{BH^+} for 8-oxopurine is 2.58 and 2.39 for purine itself. This has been interpreted as evidence of protonation in the pyrimidine rather than in the imidazole ring.

Aminohydroxypyrimidines and purines also adopt the cyclic amide form.[54,56] Substitution of an amino group into a hydroxypyrimidine has the expected base-strengthening effect since resonance is possible with the nitrogen not participating in the cyclic amide structure. Thus pK_{BH^+} for 2-pyrimidone equals 2.24 and for cytosine 17 4.58, for 4-pyrimidone 14 1.85, and for isocytosine 18 4.00. An increase in basicity over the corresponding hydroxypurine is also observed for guanine 19, pK_{BH^+} = 3.0, and for isoguanine 20,

pK_{BH^+} 4.5.

3. Acidities of Nitrogen Heterocyclic Systems

The aromatic heterocycles pyridine and pyrimidine possess no detectably ionizable protons but measurably acidic compounds result from the introduction into the ring of functions such as OH, SH, COOH or NH_2.

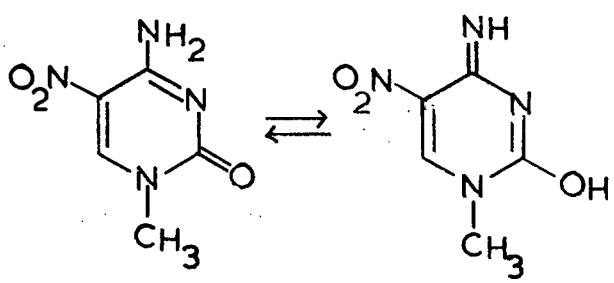
From the equivalent electron-withdrawing powers of the aza and nitro groups, hydroxypyridines and pyrimidines might be expected to have acidities close to those of the corresponding nitrophenols. In fact, their acidities are much lower, since, as explained in the previous section, the true structure of these "hydroxy" heterocycles is that of a cyclic amide. Proton loss occurs from the nitrogen rather than the oxygen atom, accounting for the lower acidity.

Cytosine 17 and isocytosine 18, being amino-pyrimidones, are expected to be slightly weaker acids than the corresponding pyrimidone because of the +R amino group. Isocytosine 18, pK_{HA} 9.59, is slightly weaker than 4-pyrimidone 14, pK_{HA} 8.59, but cytosine, pK_{HA} 12.15, is nearly three pK units weaker than 2-pyrimidone, pK_{HA} 9.17. The reason for the weaker acidity of cytosine relative to isocytosine is not clear.

There is difficulty[54b,56] in assigning the site of proton loss in hydroxypurines. The acid ionization constant of hypoxanthine 16, pK_{HA} 8.94, is close to that of 4-pyrimidone which suggests that first proton loss occurs at N_1 of the pyrimidine ring. The second pK_{HA} of 12.10 would then be loss of a proton from the imidazole ring. The pK_{HA} of guanine 19, 9.32, is close

to that of isocytosine 18 suggesting that proton loss also occurs first from N_1. However, the pK_{HA} of isoguanine is 9.0, much closer to isocytosine 18 than to cytosine 17 whose pK_{HA} one would expect it to approach. It was noted previously that adenine did not show the greatly enhanced basicity over purine that did 4-aminopyrimidine over pyrimidine itself. These two observations suggest that the through-resonance postulated for the aminopyrimidines and pyridines exists to a much lesser extent in the corresponding aminopurines, if indeed it exists at all.

In highly basic media, the primary amino group is also capable of losing a proton, but acid ionization of exocyclic amino groups has rarely been reported. Brown observed a pK_{HA} of 10.57 for 1-methyl-5-nitrocytosine,[66] shown below, but considered proton loss from the amino group so unlikely that he originally suggested the participation of a tautomeric equilibrium in which the hydroxy form is the predominant neutral species such that proton loss occurred from the hydroxy group.[55a]



Since that time Fox and coworkers have reported amino group deprotonations for 1-methyl-5-nitrocytidine, pK_{HA} 9.12,[67] 3-methylcytosine, pK_{HA} 13-14,[68] and some secondary amino-oxopyrimidines.[68] Hirata[69] reported the acid ionizations for 1- and 3-methylisocytosine and for an isocytosine nucleoside, although the work in this thesis will show that these values are in error.

Hallé and coworkers[34] have reported the pK_{HA}'s of 2-amino-3-nitro- and 2-amino-5-nitropyridine in aqueous DMSO as a function of the medium composition rather than the pK_{HA} in pure water as standard state.

In the present work interference of the acid ionization of the cyclic amide proton with the ionization of the amino group proton was prevented by replacing the labile proton of the former by a methyl group. For the same reason the imidazole proton of purine was replaced by a methyl group. Thus 1-methylcytosine was used as a surrogate for cytosine, 1,9-dimethylguanine for guanine and 9-methyladenine for adenine. It was assumed that the acid weakening effect of the methyl group was negligible. It is also noted that the three methylated nucleotide bases are also used to represent the corresponding nucleoside since the hydroxyl protons of the ribosyl moiety are half-ionized at about pH 12.[54]

I. Structure of Aminoheterocycles in Solution

1. Predominant Tautomer in Solution

Since pK_{HA} is dependent on the type of tautomerism present in the solution of the indicator,[49] it must be determined if the aminoheterocycles studied exist predominantly in the amino or amino-oxo form in aqueous solution.

From Pauling's bond energy values, amide structure (a) below is calculated to be 10 Kcal/mole more stable than the hydroxy-imine structure (b). This gain in bond energy compensates for the loss of resonance energy when hydroxypyridines and pyrimidines adopt the cyclic amide form (a). A similar gain in energy is not possible between forms (c) and (d) so that in the aminopyridines and pyrimidines the amino form (c) and aromatic

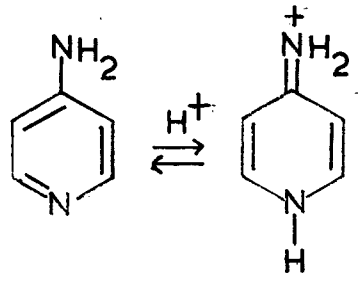
ring structure is retained.[70]

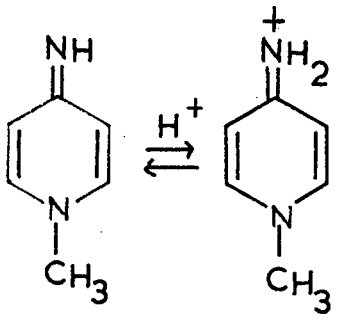
(a) (b) (c) (d)

To determine the predominant tautomer in solution, as well as the site of protonation, the ultraviolet absorption or NMR spectrum of the given aminoheterocycle is compared to those of appropriately N- or O-methylated derivatives, where the methyl group represents the location of the labile proton in a particular neutral or cationic form. The spectra of the methylated derivatives are thought to closely resemble those of the parent compound.[71]

The UV/vis absorption spectra of 2- and 4-aminopyridines closely resemble those of 2- and 4-N,N-dimethylaminopyridine, from which it is concluded that they adopt the amino form in solution, since the dimethylamino compounds must be in the amino form.[57] Similarly the spectra of 2- and 4-aminopyrimidine in water also closely resemble those of their N,N-dimethylamino derivatives,[54b,63,72] and show no resemblance to those of their 1-methylimino derivatives. Hence, these compounds also exist primarily in the amino form in aqueous solution.

The ratio of amino to imino form may be estimated as follows. Both 4-aminopyridine and 1-methyl-4-pyridone imine, shown below, protonate to form the same cation, if a methyl group be considered equivalent to a proton.





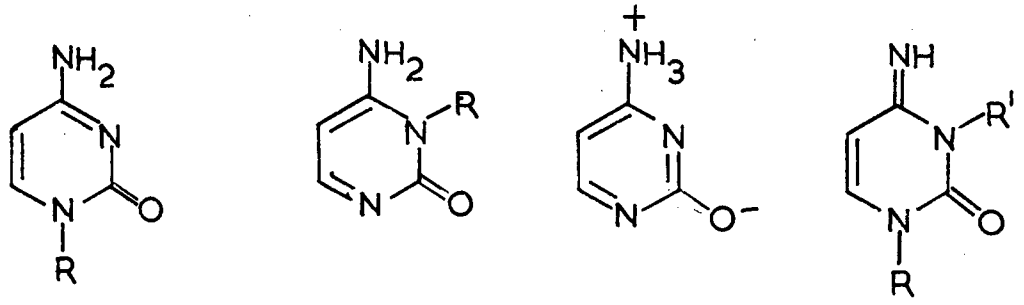
The tautomeric equilibrium constant K_T is therefore given by,

$$K_T = K_a(\text{amino})/K_a(\text{imino}) \quad 70$$

Using pK_{BH^+} of 4-N,N-dimethylaminopyridine (9.70)[73] to approximate the pK_a of the amino form and the pK_{BH^+} of 1-methyl-4-pyridone imine (17.87)[20] for the pK_a of the imino form, K_T is estimated to be 10^8 in favor of the amino form in water.

Using Perrin's lists[73] of pK_{BH^+} values to obtain the pK_{BH^+} of the corresponding N,N-dimethylamino and 1-methyl imine derivatives, K_T was calculated to be about 10^6 and 10^7 in favor of the amino form for 4- and 2-aminopyrimidine respectively.

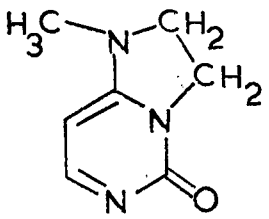
Katritzky[74] and Brown[75] have compared the UV/vis absorption and NMR spectra of cytosine to some of its methylated derivatives both in water and DMSO. These methylated derivatives represented the many possible tautomers of cytosine, of which the most important are given below.



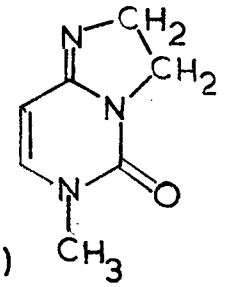
(a) (b) (c) (d)

The imino forms were eliminated since 1,3-dimethylcytosine, (d) R,R' = CH_3, is a much stronger base, pK_{BH^+} 9.3,[74] than cytosine, pK_{BH^+} 4.58. The spectrum of neutral cytosine in water resembled that of 1-methylcytosine, (a) R = CH_3, rather than that of 3-methylcytosine, (b) R = CH_3, or the anion of 2-pyrimidone, which the zwitterionic form (c) would resemble.

1-Methylcytosine is assumed to be frozen in the amino-oxo form (a) because the labile amide proton has been replaced by a methyl group. Ueda and Fox[68] have also established that 3-methylcytosine exists primarily in amino-oxo form (b) in water, rather than in the imino form, because its UV absorption spectrum is closely similar to imidazo-pyrimidine (e), below, rather than that of (f) which is fixed in the imino form.



(e)



(f)

Adenine also exists primarily in the primary amino form, as concluded from IR studies[56] and by comparison of its UV spectrum to its dimethylamino derivative.[54b,65] From dipole moment studies 7- and 9-methyladenine can also be shown to exist as primary amines.[76] From the similarity of the UV absorption spectrum of guanine to those of 1,7- and 1,9-dimethylguanine, assumed to be fixed in the amino-oxo form, it is concluded[77] that guanine adopts the cyclic amide structure in solution with the imidazole proton shared equally between N_7 and N_9.

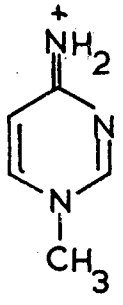
2. Site of Protonation in Aminoheterocycles

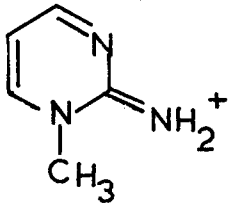
Protonation in aminoheterocycles occurs almost exclusively on the endocyclic nitrogen rather than on the exocyclic amino group.[53] This has been rationalized as being due to the delocalization of the lone electron pair of the amino group nitrogen into the aromatic system, rendering it unavailable for protonation.[71] Attachment of a proton to the exocyclic amino group has been observed in the aminopyridines at very high acidities.[62,78-81] An adenine derivative, 6-dimethylamino-3-methylpurine, has been shown to protonate at the exocyclic amino group.[56] Bŏrresen,[90] from fluorescence studies of the adenine monocation, suggested that a tautomer, protonated at the exocyclic amino group, contributed to the structure of the monocation.

NMR spectra of these compounds, either as hydrochloride salts in DMSO or as protonated species in super acid media[82,83] are useful in deciding the site and order of protonation if more than one heteroatom or basic group is present. German workers[82] have studied the protonation of aminopyrimidines and purines in superacids. In general, protonation occurs first at any available endocyclic nitrogen, namely, one not participating in a cyclic amide structure, then at the oxygens of the oxo groups. Protonation of the exocyclic amino group (or at an endocyclic nitrogen already bearing a proton) was not observed, even at very high acidity.[82]

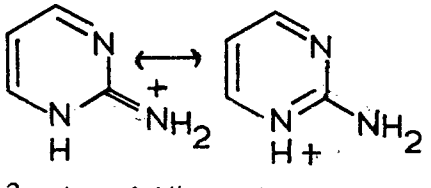
The site of protonation in monoprotonated species is often hard to determine with certainty and the UV/vis absorption spectra of the cations are often compared to the spectra of methylated derivatives to elucidate the site of protonation.

The absorption spectra of the cations of 2- and 4-aminopyrimidine are similar to those of the cations of their 1-methyl imine derivatives below. This suggests that protonation occurs at N_1 in both pyrimidines if the methyl group in the imine derivatives is considered equivalent to a proton.[54]

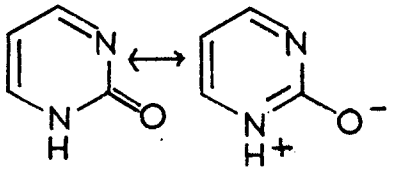




Albert[84] has observed a qualitative similarity between the spectra of neutral 2-pyrimidone and monoprotonated 2-aminopyrimidine. The site of the cyclic amide proton is then equivalent to the site of the proton in 2-aminopyrimidine cation. No similarity was observed between the spectra of 4-pyrimidone 14, whose amide proton is at N_3, and cationic 4-aminopyrimidine. This suggests that the latter protonates at N_1, para to the amino group, rather than at N_3.



2-aminopyrimidine cation

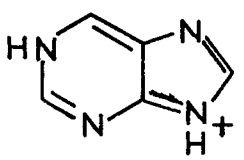


2-pyrimidone

The NMR spectra of 2-amino-4-methylpyridine and 4-aminopyridine have been determined in 100% sulfuric acid by Katritzky,[81] who concluded that initial protonation occurs at N_1 followed by protonation at the amino group at high acidity.

If protonation of cytosine occurred at the exocyclic amino group, resonance of the lone electron pair into the aromatic ring would be prevented and the UV/vis absorption spectrum of its cation would resemble that of neutral 2-pyrimidone, just as the spectrum of the anilinium cation resembles that of benzene.[71] No such similarity has been observed. Several NMR studies of the monoprotonated species indicate that protonation occurs at N_3, the nitrogen not involved in the cyclic amide structure.[55b,74,82]

Determining the site of protonation in purines is even more difficult. Protonation of purine itself is subject to much uncertainty. From NMR studies[82] it appears that protonation occurs at N_1. This at first appears unlikely since the pyrimidine ring is known to be electron-deficient, but can be rationalized by assuming contributions from the resonance form shown below for the cation. Electron-withdrawing groups with a pure -I effect have a much larger effect on basicity when located in the pyrimidine ring than when in the imidazole ring.[54,57]

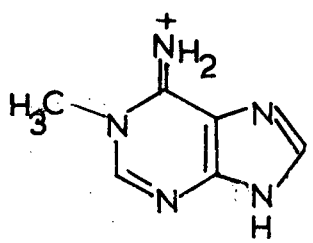


However, from recent detritiation studies of purine, it is suggested that protonation occurs at N_7,[85] in accord with X-ray crystallography studies.[86] However, Albert[87] observed no similarity between the spectra of monoprotonated 7- and 9-methylpurine, which would be expected to be identical if protonation occurred at N_7. From ^{13}C NMR

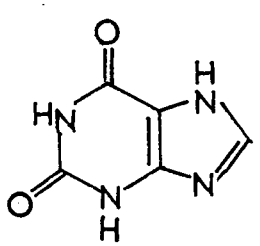
studies it is suggested that protonation occurs at all three endocyclic nitrogens N_1, N_3, N_7 with the N_1 protonated species predominating.[88]

From theoretical calculations[56] and X-ray crystallography[89] adenine is thought to protonate at N_1 although the absorption spectrum of its monocation does not resemble that of the cation of 1-methyladenine.[56] Börresen[90] has suggested the existence of tautomeric species for the monocation.

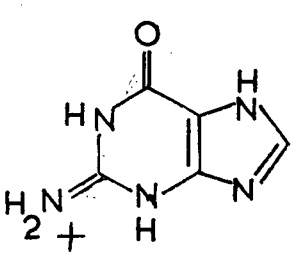
Guanine has been shown to protonate first at N_3 rather than at N_7 as previously thought. This was shown by the similarity of its cationic spectrum to that of monoprotonated 1,7-dimethylguanine.[77] 1,9-Dimethylguanine, however, does protonate at N_7.[77,84] Albert observed a qualitative similarity between the spectra of purine-2,6-dione and monoprotonated guanine, suggesting protonation at N_3.[84] Protonation at the exocyclic amino group has been eliminated by the dissimilarity of its cationic spectrum to that of neutral hypoxanthine 16 or 6-oxopurine.[71]



1-methyladenine cation



purine-2,6-dione



guanine cation

OBJECTS OF THE PRESENT RESEARCH

Establishment of acidity functions in ternary mixtures of dimethyl sulfoxide/water/0.011 M tetramethylammonium hydroxide has allowed the measurement of the acidity constants of many weak nitrogen and carbon acids.[9,13,15,18,20] The most accurately known H_- function for nitrogen acids is that of Dolman and Stewart,[15] established by using primary anilines and diphenylamines. It seemed propitious to extend such measurements to include simple heterocyclic nitrogen acids, whose basicities in water are well-established[53-56] but whose acidities are virtually unknown.

Simple aminopyridines and pyrimidines may be considered primary anilines containing an ortho or para aza substituent. Thus it was hoped that these compounds would obey Dolman's H_- function and consequently that their pK_{HA}'s would be easily determined. Examination of substituted amino heterocycles would aid our understanding of the electronic effects of substituents on the acidities of exocyclic amino groups. Similar studies involving the basicities of these compounds have measured internal effects,[60] since protonation occurs on the endocyclic nitrogen.[53-56] Furthermore, there is some doubt that the aza substituent can be considered an ordinary substituent in that it may perturb the electronic effects of other substituents on the ring. This

would be demonstrated if non-additivity of its effects relative to another aza group or to a given substituent were found.[46,51] Deady and Shanks,[91-94] however, were able to demonstrate additivity of aza substituent effects in the basic hydrolysis of the methyl esters of diazine carboxylates,[93] using σ_N values derived from the basic hydrolysis of methyl pyridyl carboxylates in 85% aqueous methanol at 25°.[92] Correlation of the pK_{HA} values determined in this work with Deady and Shank's σ_N constants could shed light on the problem of the additivity of the aza substituent.

A useful extension to include the biologically important amino-heterocycles adenine, guanine and cytosine was made. The primary amino group of these compounds participates in the hydrogen bonds of the double stranded DNA helix.[167,168] The amino group acidities of these compounds are important to the investigations of von Hippel[168] into hydrogen exchange in DNA.

Appropriately methylated derivatives of adenine, cytosine and guanine were used as models of nucleosides, the methyl group representing the sugar moiety. A substituted cytidine was also examined to test the validity of pK_{HA}'s obtained using such methylated derivatives.

In the course of these investigations sizeable solvent effects were discovered in the UV/visible spectra of the indicators. Several methods of separating such changes from the actual changes occurring in the spectra upon ionization were compared to each other.[39-41] A range of uncertainty in the ionization ratios as a result of these solvent shifts was estimated. Two extrapolative procedures for calculating

pK_{HA}'s in basic media[12] were compared to each other and to the Hammett method[4,5] of overlapping indicators.

EXPERIMENTAL

A. Preparation and Purification of Indicators

Most of the compounds were known and their methods of preparation available in the literature. In a few cases the preparations were altered to give a better yield of the desired compound; all such preparations are given below in detail. The compounds procured from commercial sources were purified by sublimation in vacuo or by recrystallization from an appropriate solvent.

Melting points less than 300° were determined using a Thomas Hoover Unimelt melting point apparatus and were corrected using Thermometric melting point standards. Melting points greater than 300° were determined on a Gallenkamp melting point apparatus and are uncorrected. They should be regarded as approximations only.

NMR spectra were run on a Varian T-60 Spectrometer with TMS (tetramethylsilane) as internal standard in DMSO-d6. Some of the compounds were poorly soluble even in DMSO so that a reliable integration of the proton signals could not be obtained because of the poor signal-to-noise ratio. Chemical shifts could be assigned without difficulty.

If a compound decomposed upon heating, thus showing no well-defined melting point, or if it melted at a temperature differing significantly from literature values, its NMR spectrum was taken and an

elemental analysis performed. Several compounds were prepared using old preparations (1880-1930) and the products obtained were verified by NMR and/or elemental analysis.

1. 2-Aminopyridine: obtained from Aldrich Chemical Co. The yellow color of the commercial product was removed by recrystallization from benzene/ligroin (1:10 v/v) (charcoal). Repeated recrystallization from benzene/ligroin gave pliable, colorless plates, which melted at 56.5-58°. Lit. m.p. 56°[95]; 55-57°[96]; 57°[53]. The compound was protected from light and air to avoid discoloration.

2. 2-Amino-5-chloropyridine: obtained from ICN- K and K Laboratories. Two-fold recrystallization from acetone/water (1:1 v/v) gave white crystal plates melting at 134-135.5°. Lit. m.p. 135-136°.[97]

3. 2-Amino-3,5-dichloropyridine: obtained from Aldrich Chemical Co. Purified by repeated recrystallization from benzene (charcoal). Concentration of the filtrate, followed by prolonged standing at 0° gave a large, brittle, colorless crystal melting at 79.5-81°. Lit. m.p. 84°.[98] The compound had to be protected from air and light to avoid discoloration.

4. 2-Amino-3-nitropyridine: obtained from Aldrich Chemical Co. Recrystallized from water (charcoal) followed by sublimation <u>in vacuo</u> to give lemon-yellow crystals melting at 163.5-165.5°. Lit. m.p. 162-164°.[99]

5. 2-Amino-5-nitropyridine: obtained from Aldrich Chemical Co. Recrystallized from ethanol/water (1:5 v/v) (charcoal) then sublimed in vacuo to give bright yellow crystals, which melted at 185.5-188°. Lit. m.p. 188°[99]; 187°[100].

6. 2-Amino-3-chloro-5-nitropyridine: 2-Amino-5-nitropyridine (1.39 gms) in ethanol (100 mls) was treated with chlorine gas for a few minutes at 0° until the solution turned bright orange. Evaporation of the solvent gave an orange oil. Addition of 20 mls water effected the precipitation of a rust-colored solid, which was collected, washed with water and dried. Two-fold recrystallization from ethanol (charcoal), then from ethanol alone, gave light yellow needles melting at 207-209.5°. Lit. m.p. 211-213°[101]; 205-206°[102].

		C	H	N
Analysis:	calc. for $C_5H_4N_3O_2Cl$	34.60%	2.32%	24.22%
	found	34.54%	2.42%	23.93%

7. 2-Amino-3,5-dinitropyridine: 2-Amino-3-nitropyridine was converted to 2-nitramino-3-nitropyridine by the method of Talik[103] which isomerized to the dinitro compound upon standing in concentrated sulfuric acid for 24 hrs.[104] Recrystallization from ethanol gave orange-yellow needles melting at 189.5-190.5°. Lit. m.p. 190-191°.[104]

8. 4-Aminopyridine: obtained from Aldrich Chemical Co. Repeated recrystallization from benzene or water (charcoal) gave white needles melting at 157.5-159.5°. Lit. m.p. 157-158°[105]; 158°[106]; 159°[107].

9. 4-Amino-3-nitropyridine: 4-Aminopyridine was converted to 4-nitroaminopyridine following the procedure of Koenigs et al., but observations were quite different from those reported.[108]

4-Aminopyridine (10 gms) was added to concentrated sulfuric acid (25 mls) at 0°. The sulfate salt of the pyridine separated out as a white solid. Fuming nitric acid (7 gms) was then added. If addition was rapid, a vigorous reaction occurred: the temperature rose to 50°, there was much fuming and the reaction mixture turned red. If the nitric acid was added slowly, no such reaction was observed. When all the sulfate had reacted after stirring at 25° for 1-2 hrs., the sludgy, reddish-brown reaction mixture, rather than a red solution, was poured onto crushed ice and a small amount of yellow solid isolated. After recrystallization from water it melted at 231° d.

The reaction mixture could be dissolved by warming on the steam bath. If the resulting red solution was poured onto crushed ice no solid precipitated. However, if concentrated aqueous ammonia was added until the solution was weakly acidic a bright yellow compound, also melting at 231°d, was recovered. Later workers report that the nitramine must be precipitated from acidic solution with base and that its melting point is 231°d[109] or 239°d[110].

The following improved preparation is suggested. To concentrated sulfuric acid (20 mls) stirred at 0° was added 4-aminopyridine (5 gms). The resulting suspension was kept at 0-10° and fuming nitric acid (3.5 gms) added over a period of 30 minutes. The reaction vessel was placed in cool water and allowed to warm to room temperature under constant stirring. After stirring at 25° for 1 hour, the red solution

was poured onto 50 gms crushed ice. The resulting yellow solution was kept at 0° overnight. The next day a light yellow solid melting at 183°d was filtered off and discarded. Partial neutralization of the filtrate with concentrated aqueous ammonia precipitated out a bright yellow compound, which after recrystallization from a large volume of water gave yellow needles melting at 235° with violent decomposition.

Conversion of the nitramine to the nitropyridine was essentially according to the method of Koenigs,[111] but again the observations were quite different from those reported.

4-Nitraminopyridine (2 gms) was added carefully to concentrated sulfuric acid (10 mls) to form a dark red solution. No vigorous reaction was observed upon heating the solution to 90° and maintaining it at 90° for over 1 hour. The solution was cooled to room temperature, left overnight, then poured onto crushed ice and made just neutral with concentrated aqueous ammonia. Upon standing at 0°, a granular dark brown solid precipitated. Recrystallization once from 80% ethanol, then twice from water (charcoal) gave a chartreuse-colored powder melting at 203-204.5°. Lit. m.p. 200°d[111]; 200-202°[112]; 204°[113]

		C	H	N
Analysis:	calc. for $C_5H_5N_3O_2$	43.16%	3.63%	30.21%
	found	43.14%	3.81%	30.24%

NMR: d 6.88 δ J6Hz (1H); doublet superimposed on a multiplet at 8.13 δ J7Hz, integration of both (3H); s 8.93 δ (1H).

10. 4-Amino-3,5-dinitropyridine: prepared by the method of Koenigs et al.[111] but using an excess of sulfuric and nitric acids.

4-Aminopyridine (3 gms) was dissolved in concentrated sulfuric acid (30 mls) at 0° and fuming nitric acid (4.5 mls) added while stirring and maintaining the reaction mixture at a temperature less than 10°. It was allowed to warm to room temperature, then heated at 85-90° for 7 minutes and at 170-175° for 5 minutes. It was then poured onto 150 gms crushed ice and a yellow solid recovered. Recrystallization from water gave light yellow plates which decomposed at 333° then melted at 335-337° with gas evolution. This is presumably the hydrolysis product of 4-amino-3,5-dinitropyridine, i.e., 4-hydroxy-3,5-dinitropyridine. The hydroxy-dinitropyridine is usually prepared from 4-aminopyridine using an excess of sulfuric and nitric acid but with prolonged heating above 100°. It formed small yellow plates melting at 325°.[114]

The orange filtrate was treated with concentrated aqueous ammonia. With much foaming a beige precipitate separated, which turned purple upon drying in air. Recrystallization from a large volume of water (charcoal) gave light yellow needles melting at 169-171°. Lit. m.p. 170-171°[111]; 168-169°[115].

NMR: s 9.23 δ (2H); m 8.73 δ (2H).

11. 2-Aminopyrimidine: obtained from Eastman Kodak Co. Purified by sublimation <u>in vacuo</u> at 70° to give white crystals melting at 124-126°. Lit. m.p. 125-126°.[116]

		C	H	N
Analysis:	calc. for $C_4H_5N_3$	50.51%	5.30%	44.19%
	found	50.50%	5.20%	44.16%

12. 2-Amino-4-chloropyrimidine: obtained from ICN- K and K Laboratories. The compound was recrystallized from ethanol, then from water (charcoal) followed by sublimation in vacuo at 50° to give white crystals. The compound turned yellow when heated to 145°, orange at 160.5° and reddish-brown at ca. 250°, but did not melt below 300°. Lit. m.p.: decomposes ca. 168°[117]; turns brown at 160° and sinters at 168° with no clear-cut melting point.[118]

		C	H	N
Analysis:	calc. for $C_4H_4N_3Cl$	37.08%	3.12%	32.44%
	found	37.04%	3.29%	32.55%

NMR: d 6.65 δ J6Hz (1H); d 8.18 δ J6Hz (1H); m 7.08 δ (2H).

13. 2-Amino-4,6-dichloropyrimidine: obtained from Aldrich Chemical Company. Sublimation in vacuo at 80° gave white crystals melting at 223-224.5°. Lit. m.p.: 220-221°[119]; 221°.[120]

		C	H	N
Analysis:	calc. for $C_4H_3N_3Cl_2$	29.29%	1.84%	25.63%
	found	29.39%	1.85%	25.82%

14. 2-Amino-5-nitropyrimidine: obtained from ICN- K and K Laboratories. The compound was recrystallized repeatedly from 50% ethanol (charcoal) to remove the yellow color of the commercial product, then from absolute ethanol. Long standing at 0°, gave the compound as beautiful long colorless needles. The compound decomposed when sublimation in vacuo was attempted. M.p. 234.5-235.5°. Lit. m.p. 236°.[121]

15. 4-Aminopyrimidine: gift from Dr. D.J. Brown. M.p. 149.5-151.5°. Lit. m.p. 149-151°[116]; 150-152°[120]; 151°[122].

		C	H	N
Analysis:	calc. for $C_4H_5N_3$	50.51%	5.30%	44.19%
	found	50.70%	5.28%	44.23%

16. 4-Amino-2-chloropyrimidine: Prepared by heating 2,4-dichloropyrimidine (Aldrich Chemical Co.) with n-butanol and aqueous ammonia in a pressure bottle at 100° for 20 minutes.[123] The solid residue after evaporation of the solvent was recrystallized twice from water (charcoal) to give a solid which decomposed upon heating and partly melted ca. 220°. NMR and UV spectra showed that this solid was a mixture of the 2- and 4-amino isomers. Since 2-amino-4-chloropyrimidine sublimes readily at a temperature much lower than does 4-amino-2-chloropyrimidine separation was easily achieved by sublimation rather than by repeated recrystallization from ethanol as suggested by Ballweg.[123]

The mixture was heated in vacuo at 50° until no more sublimate formed (2-4 hrs.). The sublimate was recrystallized from water; its NMR spectrum was identical to that of 2-amino-4-chloropyrimidine.

The residue of the sublimation was recrystallized twice from water (charcoal) to give colorless needles, whose melting point depended on the rate of heating. Above 200°, if heated rapidly, the compound turned yellow then melted sharply to form a dark red tar. Lit. m.p. 206-207° to a red brown liquid[118]; 219-220°[117]; 209-210°[124].

NMR: d 6.40 δ J6Hz (1H); d 7.95 δ J6Hz (1H); m 7.35 δ (2H).

17. 4-Amino-2,6-dichloropyrimidine: obtained from Biochemical Laboratories, Inc. Direct sublimation of the crude material removed a small amount of solid melting at 221°, presumably 2-amino-4,6-dichloropyrimidine. The crude product was therefore first recrystallized from 80% ethanol (charcoal), sublimed then recrystallized again from ethanol to give white needles. The compound turned yellow when heated above 250°, then melted to a red liquid at 271-273.5°. Lit. m.p. 270-271°[120]; 270-272°[116].

18. 4-Amino-2-chloro-5-nitropyrimidine: All the commercial products were of very poor quality. The compound was therefore synthesized from 2,4-dichloro-5-nitropyrimidine (ICN- K and K Laboratories).

Crude 2,4-dichloro-5-nitropyrimidine (1 gm) was stirred with ether (50 mls), the solid residue filtered off by gravity and the filtrate evaporated to dryness. The resulting yellow oil crystallized upon standing at room temperature. It was redissolved in a minimum of ether then added dropwise to 50 mls of an alcoholic ammonia solution (ammonia bubbled through for 15 min at 25°). After all the ethereal solution had been added, the precipitate was collected, washed with water and dried. The yellow solid was dissolved in 2 M hydrochloric acid (charcoal), filtered, and reprecipitated with aqueous ammonia. For analysis a small portion was sublimed in vacuo at 180° to give yellow crystals.

This product was shown by NMR to be exclusively 2,4-diamino-5-nitropyrimidine, previously synthesized using much more vigorous conditions such as refluxing phenol at 140° under a stream of ammonia.[125]

M.p.: darkens when heated above 200°, 343-344° with decomposition and gas evolution. Lit. m.p. 345-350°[125]; >350°[128].

		C	H	N
Analysis:	calc. for $C_4H_5N_5O_2$	30.97%	3.25%	45.2%
	found	30.94%	3.23%	45.3%

NMR: m 7.43 δ (2H); m 7.92 δ (2H); s 8.83 δ (1H).

Japanese workers observed that amination of 2,4-dichloro-5-nitropyrimidine in methanol/ether gave a mixture of mono- and diamino derivatives. The yield of the monoamino compound was increased to 60% if the reaction was performed at 0°.[126]

2,4-Dichloro-5-nitropyrimidine (0.7 gms), purified as above, was dissolved in ethanol (30 mls), cooled to 0° and 1.5 M aqueous ammonia (2.4 mls) added dropwise. Removal of the reaction vessel from the ice-bath caused immediate formation of a white precipitate, which was collected, washed with water and dried. It was recrystallized twice from water (charcoal) to give granular white crystals, which colored upon heating at 206°, then melted with decompositon and gas evolution at 217.5-218°. Lit. m.p. colors 205°, melts 217°[127]; 220-221°[126].

		C	H	N
Analysis:	calc. for $C_4H_3N_4O_2Cl$	27.52%	1.73%	32.11%
	found	27.37%	1.68%	32.30%

NMR: m 8.52 δ ; s 9.02 δ.

19. 2-Amino-s-triazine: obtained from Peninsular Chemresearch Calgon Corporation (PCR). Purified by recrystallization from water followed by sublimation in vacuo at 80-90° to give colorless crystals, melting at 228-230° with no decomposition. Lit. m.p. 224°d[129]; 228°d

(corr.)[130]; 225-226°[131].

20. 1,7-Dimethylguanine: purchased from Adams Chemical Co. Recrystallization from 95% ethanol gave white needles which were dried at 100° in vacuo for 72 hrs before analysis. M.p.: shrinks and decomposes at 330°, melts at 334-335° with gas evolution. Lit. m.p. 330-331°[77]; 338-340° (343-345° corr.) with slight browning and gas evolution as the liquid is heated[132]; 337-339°[133].

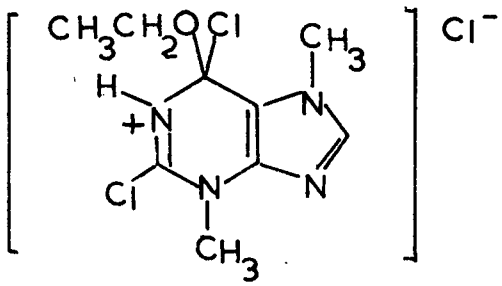
		C	H	N
Analysis:	calc. for $C_7H_9N_5O$	46.92%	5.06%	39.09%
	found	46.82%	5.07%	39.35%

21. 1,9-Dimethylguanine: purchased from Adams Chemical Co. Recrystallized twice from 95% ethanol, then dried at 100° for 72 hrs, before analysis. The white granular crystals melted at 287-289°d. Lit. m.p. 287°.[77]

		C	H	N
Analysis:	calc. for $C_7H_9N_5O$	46.92%	5.06%	39.09%
	found	46.93%	5.18%	39.04%

22. 3,7-Dimethyl-isoguanine: prepared essentially by the method of Nikolaeva and Golovchinskaya,[134] but with some alterations given below.

6-Ethoxy-2,6-dichloro-3,7-dimethyl-3,6-dihydropurine hydrochloride:



Theobromine (5 gms) was refluxed with phosphorus oxychloride ($POCl_3$) (50 mls) overnight. Excess $POCl_3$ was distilled from the resulting brown solution, leaving a brown syrup which was then treated with "super-dry" ethanol (70 mls) at 0°, with careful exclusion of atmospheric moisture. After keeping the resulting solution at 0° for 24 hrs, the fluffy white precipitate was collected, washed with dry ether and dried in vacuo. Upon triturating the solid with 70 mls "super-dry" ethanol at 20°, a yellowish powder remained undissolved. This compound was filtered off and recrystallized from water (charcoal) to give white needles of 2,6-dichloro-7-methylpurine melting at 195° (uncorr.). Lit. m.p. 195-196°.[135]

Addition of 100 mls of dry ether to the filtrate precipitated the compound as a white powder, which decomposed without melting at about 200°. Lit. m.p. 218-220°d.[134]

6-Ethoxytheobromine: The hydrochloride (3 gms) was dissolved in 40 mls of a saturated sodium bicarbonate solution (8 gms bicarbonate in 100 mls water). After a few minutes a vigorous frothing occurred in the solution and a white solid precipitated. The pH was readjusted to 7-8 with more saturated sodium bicarbonate solution and the mixture extracted with chloroform (4 x 50 mls). The white solid dissolved

readily in the chloroform phase. The residue after the evaporation of the chloroform was recrystallized several times from water (charcoal) to give white needles melting at 263-265.5°. Lit. m.p. 261-263°.[134]

3,7-Dimethylisoguanine: 6-Ethoxytheobromine (0.5 gms) was heated with 17% aqueous ammonia (15 mls) in a pressure bottle at 120-130° for 7 hrs.to give the isoguanine. Recrystallization from a small amount of water gave small white needles, which were dried at 100° in vacuo for 12 hrs before analysis. M.p.: yellows 334° then decomposes, sublimes and partially melts ca. 350°. Lit. m.p. 360°[134]; decomposes ca. 340°, then partially melts ca. 380° with rapid heating.[136]

		C	H	N
Analysis:	calc. for $C_7H_9N_5O$	46.92%	5.03%	39.10%
	found	47.09%	4.96%	38.94%

23. 2,8-Dichloro-7-methyladenine: prepared according to the general method of E. Fischer.[137,138]

Theobromine was heated with phosphorus oxychloride and phosphorus pentachloride in a sealed tube at 150-155° for 3 hrs to give 2,6,8-trichloro-7-methylpurine. Since direct amination of this compound gives predominantly the 8-amino isomer[139] it was necessary to convert it to 2,6-dichloro-8-hydroxy-7-methylpurine by boiling for 15 min in 6 N hydrochloric acid. Heating the latter compound with saturated ethanolic ammonia in a sealed tube at 150° for 5 hours gave predominantly 6-amino-2-chloro-8-hydroxy-7-methylpurine, which was converted to the desired product by heating with phosphorus oxychloride in a sealed tube

at 140° for 3 hours. Repeated recrystallization from 80% ethanol (charcoal) gave white needles. M.p.: turns yellow at 269°, orange at 276°, red at 284.5° with no melting point under 300°. Lit. m.p.: colors about 270°, then decomposes at higher temperatures.[137]

		C	H	N
Analysis:	calc. for $C_6H_5N_5Cl_2$	33.03%	2.29%	32.12%
	found	33.09%	2.18%	31.89%

NMR: s 3.93 δ (3H); m 7.55 δ (2H).

24. 2-Chloro-7-methyladenine: Theobromine (25 gms) was converted to 2,6-dichloro-7-methylpurine by refluxing with phosphorus oxychloride ($POCl_3$) (150 mls) and N,N-dimethylaniline (27 gms).[135] The $POCl_3$ was removed by distillation under reduced pressure and the resulting red tar treated with 150 gms crushed ice to give a black solution. The pH was adjusted by the addition of solid sodium carbonate until the solution turned Congo red paper blue and an orange solid precipitated. Recrystallization of this solid from water (charcoal) gave 2,6-dichloro-7-methylpurine as white needles melting at 197-198.5° (uncorr.). Lit. m.p. 195.5-196°.[135]

The dichloropurine was selectively aminated to the desired adenine as follows. Methanol (50 mls) was saturated with ammonia by bubbling the gas through for 15 min at 0°, 0.9 gms of the dichloropurine added and the suspension heated in a sealed tube at 90-100° for 19 hrs. The mixture was evaporated to dryness, triturated with 10 mls of 1 M sodium hydroxide, filtered then allowed to digest in a few mls of glacial acetic acid overnight at 0°. The next day the white solid was recovered, washed thoroughly with water and recrystallized from the

latter with slow cooling to give white needles. The crystals were dried several days at 100°C in vacuo before analysis. M.p.: 284.5° with gas evolution and yellowing. Lit. m.p.: 284° (corr.) with gas evolution.[137,140]

		C	H	N	Cl
Analysis:	calc. for $C_6H_5N_5Cl$	39.23%	3.27%	38.15%	19.35%
	found	39.10%	3.22%	37.89%	19.46%

NMR: s 3.99 δ (3H); m 7.42 δ (2H); s 8.21 δ (1H).

25. 2-Chloro-8-methoxy-7-methyladenine: This previously unknown derivative was prepared by Fischer's method of selective substitution of chloro groups by ethoxy groups in 7-methylpurines.[141]

2,8-Dichloro-7-methyladenine (250 mgms) was dissolved in 150 mls of methanol with the aid of warming. To this was added 5-6 mls of a solution of 500 mgms sodium hydroxide in 50 mls methanol and the whole refluxed for 50 minutes at which time a fine white precipitate had appeared. After heating 30 more minutes, the reaction mixture was allowed to cool to room temperature, 20 mls water added and left to stand overnight. The next day the white, granular solid was collected. The filtrate was no longer alkaline to pH indicator paper and gave a positive chloride ion test with silver nitrate solution. Recrystallization of the product from 80% ethanol gave white, granular crystals which turned yellow at 227° , orange at 239.5°, then melted at 248.5° with gas evolution.

		C	H	N
Analysis:	calc. for $C_7H_8N_5OCl$	39.36%	3.77%	32.78%
	found	39.43%	3.83%	32.58%

NMR: s 3.67 δ (3H); s 4.12 δ (3H); m 7.03 δ (2H).

26. 7-Methyladenine: purchased from Cyclo Chemical Corporation. Recrystallized twice from water to give a granular white powder[136] which melted from 336.5-339° with decomposition and gas evolution. Lit. m.p.: 351°d (corr.)[137]; 349-350°d[142]; 345°[143]; 323°d; 336°d (sealed tube)[144].

		C	H	N
Analysis:	calc. for $C_6H_7N_5$	48.30%	4.73%	46.96%
	found	48.38%	4.51%	47.20%

27. 2,8-Dichloro-9-methyladenine: Uric acid was converted to 2,6,8-trichloropurine by the method of Davoll and Lowy,[145] then methylated in dimethyl sulfoxide with methyl iodide in the presence of potassium carbonate to give exclusively 2,6,8-trichloro-9-methylpurine.[146] Unlike the 7-methyl isomer, 2,6,8-trichloro-9-methylpurine gives predominantly the 6-amino rather than the 8-amino isomer upon treatment with ethanolic ammonia.[139] Recrystallization from 95% ethanol gave white crystalls, which were dried at 100°C in vacuo for 24 hours before analysis. M.p.: 266-268°. Lit. m.p.: 260-261°[139]; 270° (corr.)[137].

		C	H	N
Analysis:	calc. for $C_6H_5N_5Cl_2$	33.03%	2.29%	32.12%
	found	33.08%	2.28%	32.21%

N.R.C.: s 3.65 δ (3H); m 8.21 δ (2H).

28. 2-Chloro-9-methyladenine: 2,6-Dichloropurine (Sigma Chemical Co.) was methylated in dimethyl sulfoxide with methyl iodide in the presence of potassium carbonate to give a mixture of 2,6-dichloro-7- and 9-methyl purines.[147] Separation of the isomers was best effected by chromatography over neutral alumina with ethyl acetate as eluant. Recrystallization of the crude 9-methyl isomer (Rf 0.42) from water gave

lustrous crystal plates which melted at 149.5-151.5° (uncorr.). lit. m.p. 152-153°[147]; 151.5-152.5° [148]. Recrystallization of the 7-methyl isomer from water gave white needles melting at 195.5-196.5° (uncorr.). Lit. m.p.: 195°[147]; 195.5-196°.[135]

The 2,6-dichloro-9-methylpurine thus obtained was aminated by the method of Falconer et al.[148] to give 2-chloro-9-methyladenine. Recrystallization from water (charcoal) gave white crystals which were dried in vacuo at 100° before analysis. M.p. turns yellow ca. 270° but has no melting point under 300°.

		C	H	N
Analysis:	calc. for $C_6H_6N_5Cl$	39.23%	3.27%	38.15%
	found	39.26%	3.14%	38.45%

NMR: s3.70 δ (3H); m 7.68 δ (2H); s 7.97 δ (1H).

29. 2-Chloro-8-methoxy-9-methyladenine: 2,8-Dichloro-9-methyladenine (50 mgms) was dissolved in methanol (30 mls) with the aid of warming, then a solution of 500 mgms of sodium hydroxide in 25 mls methanol added. The solution was refluxed for 5 hours, then cooled to room temperature and allowed to stand overnight. Recrystallization of the granular precipitate from 95% ethanol gave white granular crystals which were dried at 100° in vacúo for 24 hours before analysis. M.p. 270-270.5°d.

		C	H	N
Analysis:	calc. for $C_7H_8N_5OCl$	39.36%	3.77%	32.78%
	found	39.57%	3.90%	32.96%

NMR: s 3.43 δ; s 4.12 δ; m 7.28 δ.

30. 9-Methyladenine: A solution of adenine (0.31 gms) and TMAH·$5H_2O$ (0.43 gms) in water (50 mls) was freeze-dried. Sublimation of the

residue in vacuo at 170-185° for 4-5 hours gave 9-methyladenine.[149] Recrystallization of the sublimate from 95% ethanol gave white crystals which melted at 305-306° with slight browning. Lit. m.p.: 301-302°[149]; 300°[150]; 308-310° no d.[151]; 310°d[152].

31. 2,3-Dihydro-1H-5-oxoimidazo(1,2-c)pyrimidine Hydrochloride: prepared by the method of Ueda and Fox.[68]

Uracil was converted to 4-thiouracil by heating in pyridine with phosphorus pentasulfide for 3 hrs. Recrystallization from water (charcoal) gave orange needles, m.p. 294°d (uncorr.), lit. m.p. 320°d[153]. 4-Thiouracil was converted to 4-methylthio-2-pyrimidone by heating briefly with methyl iodide in methanolic sodium methoxide.[154] N^4-(β-hydroxyethyl)cytosine was synthesized by heating the pyrimidone with ethanolamine in n-butanol, then converted to N^4-(β-chloroethyl)-cytosine by heating with thionyl chloride in chloroform. The latter compound was cyclized to the desired compound by heating in pyridine for 30 min. The compound was purified by trituration with 3 fresh portions of ethanol at room temperature. M.p.: colors slowly as heated above 260°, no melting point less than 300°. Lit. m.p.: greater than 300°.[68]

		C	H	N
Analysis:	calc. for $C_6H_7N_3O.HCl$	41.51%	4.64%	24.20%
	found	41.66%	4.75%	24.09%

32. 1-Methylcytosine: obtained from Cyclo Chemical Corporation. Recrystallization from 95% ethanol gave small, colorless plates which melted at 297-298°d (uncorr.). Lit. m.p. 299-300°[74]; 303°[155].

		C	H	N
Analysis:	calc. for $C_5H_7N_3O$	47.99%	5.64%	33.59%
	found	47.92%	5.56%	33.74%

33. 1-Methyl-5-bromocytosine: A complex between this compound and 9-ethylguanine has been reported but no details of the preparation or physical constants of it were given.[156] It was prepared similarly to 5-bromo-1-ethylcytosine.[157]

1-Methylcytosine (0.63 gms) was dissolved in methanol (50 mls) with the aid of warming and freshly distilled, dry triethylamine added. The white precipitate was redissolved by addition of 10 mls methanol (slight warming), then cooled to 0° with stirring. A further 10 mls of methanol kept the compound in solution at this temperature. Bromine (0.27 mls) was added over a period of 4 minutes and the yellow solution allowed to stir at room temperature overnight. Evaporation of the solution to dryness and two-fold recrystallization of the residue from methanol (charcoal) gave white crystals, m.p. greater than 200°.

Since the compound did not sublime well, bromide ion was removed by chromatography over alumina using aqueous methanol as eluant. The column was washed with water, then methanol. The compound was eluted using methanol/water (80:20 v/v) and recrystallized from 50% methanol (charcoal) to give upon standing beautiful rhombic crystals, which were dried at 60° in vacuo for 6 hrs before analysis. M.p.: yellow 203°, orange 207°, melts to a red liquid at 210-211.5°; the liquid shows much gas evolution and decomposition upon further heating. Rf: (alumina, 75% MeOH) 0.74.

		C	H	N	Br
Analysis:	calc. for $C_5H_6N_3O\,Br\cdot\frac{1}{2}H_2O$	28.18%	3.28%	19.73%	37.50%
	found	28.25%	3.30%	19.51%	37.33%

34. 1-Methylisocytosine: prepared essentially by the method of D.J. Brown and N.W. Jacobsen.[158]

Isocytosine (Sigma Chemical Co.) (1.1 gms) was dissolved in methanolic sodium methoxide (0.23 gms sodium in 12 mls methanol), methyl iodide (1.5 mls) added and the solution refluxed with stirring for 2 rather than 1.5 hours. Cooling to room temperature caused the formation of a white precipitate, but the reaction mixture was evaporated to dryness and the residue dissolved in 25 mls of water with the aid of warming. The aqueous solution was stirred for 15 minutes with silver carbonate (BDH Chemicals Ltd.) (2.7 gms), filtered by gravity then adjusted to pH 7 with concentrated hydrochloric acid. The precipitate of silver chloride was removed by filtration through Celite, the filtrate evaporated to dryness and the white residue extracted with hot ethanol (2 x 100 mls).

The cooled ethanol extract was reduced to 20 mls, but only a small amount of inorganic residue precipitated out on standing at 25°. Further reduction of the filtrate to 5 mls gave a white solid, which was recrystallized from ethanol, then sublimed in vacuo at 150-160°. M.p. 280-280.5°d. Lit. m.p. 283-285°[158]; 275-280°[159].

		C	H	N
Analysis:	calc. for $C_5H_7N_3O$	47.99%	5.65%	33.58%
	found	47.75%	5.70%	33.34%

35. 3-Methylcytosine: obtained from Terra Marine Bioresearch. Purified by three-fold sublimation in vacuo at 130-145° to give colorless crystals, melting at 211-212° with gas evolution. Lit. m.p.: 213°.[74]

36. 3-Methylisocytosine: This compound may be isolated from the reaction mixture of 1-methylisocytosine if refluxing is carried out for exactly 1.5 hours; refluxing for 2 hrs appears to favor the production of the 1-methyl isomer and precludes its isolation from the reaction mixture. Separation is effected by fractional crystallization due to different solubilities of the two isomers in ethanol.[158]

It may also be synthesized unambiguously by amination of 3-methyl-2-methylthiopyrimidin-4(3H)-one with 15 N ammonia.[63,160]

A small sample was donated to us by Dr. D.J. Brown and was used without further purification. M.p.: 257-260°. Lit. m.p.: 262-266°[159]; 257-260°[158].

37. 2',3'-O-Benzylidene-5'-O-tritylcytidine: a gift from Dr. R.C. Lord and used without further purification. M.p.: darkens as heated above 200°; the actual melting point depends on the rate of heating but is about 225°d.

		C	H	N
Analysis:	calc. for $C_{35}H_{31}N_3O_5$	73.28%	5.45%	7.33%
	found	72.80%	5.60%	6.70%

B. Purification of Solvents

Dimethyl sulfoxide (DMSO) was purified essentially by the method of previous workers in this laboratory. DMSO (Mallinckrodt AR Grade) was stirred over powdered calcium hydride (Fisher Scientific Co.) under oil pump vacuum for at least 48 hours. Bubbling of the solvent, either due to degassing or to evolution of hydrogen, generally ceased after a few hours. The solvent was distilled in vacuo at 40-50°. The first and last fractions, each about 10-20% of the total volume, were discarded. The useable fractions were stored over molecular sieve 5A in 500 ml flat-bottomed flasks, sealed with ground glass stoppers and parafilm or electrical tape. DMSO purified in this manner was adequate for the preparation of aqueous solutions containing less than 90 mole % DMSO.

For the more anhydrous solutions, the DMSO was further purified by a simplified zone-freezing method described by Dolman,[15b] then stored in the dry box under helium atmosphere.

Distilled water used in the preparation of DMSO/water solutions was made carbon dioxide-free by boiling it for at least 30 minutes, then cooling it with nitrogen (L-grade) bubbling through it. The cooled water was transferred to a clean, dry glass reagent bottle under a stream of nitrogen and the bottle sealed with a rubber serum stopper.

Tetramethylammonium hydroxide (TMAH), obtained from Eastman Organic Chemicals or from Mallinckrodt Organic Reagents as a 10% or 25% aqueous solution, was used without further purification. The molarity of each solution was exactly determined before use by titration with standardized N/10 hydrochloric acid using phenolphthalein as indicator.

Each solution was retitrated every 4-5 weeks; in no case was the molarity found to be changed. The bottles were kept tightly capped and sealed with electrical tape to avoid carbonate formation. Tetramethylammonium hydroxide pentahydrate (TMAH-$5H_2O$) was obtained in solid form from Eastman Organic Chemicals.

Potassium t-butoxide was obtained as a solid from Aldrich Chemical Co. The bottle was opened and stored in the dry box under helium atmosphere. The formation of a solid crust in the flask indicated contamination with atmospheric moisture and the concomitant formation of potassium hydroxide. The solid was only used if it was a granular, free-flowing solid or a fluffy dry powder.

C. Preparation of Solutions

1. Solutions 0-90 Mole % DMSO

Stock solutions of DMSO/water were prepared in clear glass reagent bottles, which were equipped with plastic screw-caps and teflon liners rather than sealed with rubber serum stoppers. DMSO readily attacked the latter causing contamination of the solutions with a red dye. This impurity has a small absorption at ca. 350 nm, which increased somewhat when the solution was basified, thus interfering with the spectra studied.

The bottle and cap were weighed on a top-loading Mettler balance. Calculated amounts of DMSO and water were added to the bottle via syringe with weighings after each addition. The exact mole % of each solution could thus be calculated from the known weights of DMSO and water added. The bottles were kept tightly capped and stored in a desiccator when

not in use. Exposure of the solution to air was minimized whenever a volume was withdrawn. It was felt that contamination of the solutions by fleeting exposure to atmospheric oxygen and water was less serious than the known danger of the dissolved rubber caps. Albagli[161] observed that DMSO was not sufficiently hygroscopic to warrant the extra precaution of using a dry box in the preparation of more dilute solutions. Thus this method involved fewer manipulations and avoided contamination of the solutions.

2. Solutions 90-100 Mole % DMSO

Glass reagent bottles were weighed, transferred to the dry box, a calculated amount of anhydrous DMSO was syringed in, the bottle was sealed with a rubber serum cap, then removed to the atmosphere and weighed again. Water was added via injection of a 50 mole % DMSO/water solution through the serum cap and the bottle weighed again. The solution was degassed by bubbling nitrogen through it, via a syringe inserted through the rubber cap. The solutions were stored in a desiccator when not in use. Contamination by water and oxygen was considered more significant for solutions rich in DMSO and warranted the use of the rubber caps; care was taken that the solutions did not splash against them, however.

Karl Fischer titration of very anhydrous DMSO tends to underestimate the amount of water present.[162] In fact, titration of consecutive samples of equal volume required progressively smaller volumes of titrant. However, qualitative indications are that the "anhydrous" DMSO contained less than .005% water by weight.

3. Solutions of TMAH in DMSO

Injection of TMAH as a 10% aqueous solution to give a DMSO/water solution 0.011 M in base, diluted 100 mole % DMSO to about 96.3 mole %. However, injection of a solution of TMAH.$5H_2O$ in anhydrous DMSO diluted it to about 99.5 mole %.

TMAH.$5H_2O$ (1.09 gms) was suspended in anhydrous DMSO (50 mls) in a clear glass bottle equipped with a screw-cap and teflon liner. It was left under continuous stirring overnight. Next day any remaining precipitate was allowed to settle and the solution decanted into another glass bottle and sealed with a rubber serum stopper. All the above manipulations were carried out in the dry box. After removal into the atmosphere, the solution turned yellow in 1-2 days if it was not protected from light and oxygen. Flushing the solution with nitrogen as described in Section C(2) for 20 minutes and covering the bottle with foil appeared to be sufficient.

Titration with standardized N/10 hydrochloric acid, using phenolphthalein as indicator, showed that the most concentrated solution possible is about 0.12 M. Karl Fischer titration of the basic solution in methanol-acetic acid gave inconsistent results, greatly underestimating even the minimum possible water content.

4. Solutions of Potassium t-Butoxide in DMSO

Potassium t-butoxide dissolved readily in anhydrous DMSO. Any undissolved material in the solution indicated that the solid used had been contaminated with atmospheric moisture. The solutions, normally a very pale yellow, were sensitive to moisture and oxygen. Upon exposure to air a crystalline precipitate formed immediately and the

solution colored upon a few hours standing to a deep red.

The solutions were made up by weight immediately before use to be as nearly equal to the molarity of the 10% TMAH solution used as possible. They could be kept for short periods of time in Erlenmeyer flasks sealed with a ground glass stopper and protected from light. The solution was never removed from the dry box.

D. Spectral Measurements in the Dimethyl Sulfoxide/Water System

All UV/vis absorption spectra were taken using matched 1 cm silica cells. Complete spectra were taken on a Cary 15 Recording Spectrophotometer and absorbance at individual wavelengths determined using a Cary 16 Spectrophotometer. Both instruments were equipped with constant-temperature cell holders, connected to a water bath, thermostatted at 25.0 ± 0.5°.

Each solution was allowed to thermally equilibrate for several minutes at 25°C before the injection of the indicator since the dissociation constants of nitrogeneous bases (and presumably acids also) are quite temperature sensitive.[3] None of the indicator anions reacted appreciably with oxygen and only a few reacted irreversibly with hydroxide ion.

Consistent results were obtained using the following techniques.

1. Cary 15 UV/vis Spectra

Spectral curves were recorded when the absorption curve of an indicator was complicated and when the ionization ratios were to be estimated by several different methods.

Silicone rubber discs (AR281A-48024 from Armet Industries Ltd., Guelph, Ontario), cut from a silicone rubber sheet with a cork borer, were fitted into the ground glass necks of two matched silica cells. Two syringes were inserted through the rubber disc. Nitrogen (L-grade), passed through Ascarite and silica gel to remove traces of carbon dioxide and water, was passed in through one of the syringes and each cell flushed for two minutes. The nitrogen syringe was withdrawn and about 3 mls of the appropriate DMSO/water solution injected into the cell using a 5-ml glass syringe fitted with a Chaney adaptor. The solution was degassed by flushing with nitrogen for another two minutes, care being taken that no solution was forced into the outlet syringe. Next about 30 µl of a 10% TMAH solution was injected from a 50 µl glass syringe fitted with a Chaney adaptor into each cell such that the solution would be 0.011 M in base after injection of the indicator. The basic solutions were flushed for a further two minutes with nitrogen and the two syringes withdrawn.

If a solution of TMAH in DMSO was used, about 300 µl of such a solution were required to make the final solution 0.011 M in base. Therefore, two injections of about 150 µl each were made into each cell, using a 250 µl glass syringe equipped with a Chaney adaptor.

After equilibration at 25°C, the baseline was obtained by running the two cells against each other in the range of absorption of the indicator usually from 400 nm to 260 nm where DMSO itself begins to absorb. If the baseline was satisfactory, 30 µl of a solution of the indicator in DMSO was injected from a third syringe and the spectrum taken at once. The concentration of the indicator solution was chosen so as to produce an

absorbance of 0.5-0.8 when the indicator was completely ionized. The spectrum was retaken a few minutes later to ensure that no reaction with base was occurring. In most cases no such reaction was ever observed. In fact, the spectrum often remained unchanged even after letting such a solution stand overnight. The final composition of the solution in the cell was calculated from the volumes of the solutions added and their known densities. The densities of the DMSO/water solutions at 25°C were those of Cowie and Toporowski.[23]

The spectrum of the neutral molecule was obtained in a manner identical to the above except for the injection of base. A small concentration correction had to be made because of the slight difference in volume between a neutral and its corresponding basic solution. Adding a volume of water equal to the volume of base injected precluded the need for this correction.

2. Cary 16 Spectra

Measurements were made using the Cary 16 if determination of the ionization ratios was straightforward or if greater accuracy was required, since its reproducibility is about ± 0.005.

The solutions, both basic and neutral, were prepared in exactly the same manner as described in section D(1), except that 3 cells were required: one reference blank and two thermostatted cells, one blank to establish the baseline and the other containing the indicator. Measurements were made at the desired wavelength and any slight absorption in one of the thermostatted cells (A') recorded. The indicator solution was always injected into the cell showing minimal extraneous absorption and the absorbance (A) determined. The value

of the absorbance of the indicator at the wavelength used was thus (A + A'). A' was generally very small, less than 0.010. Three readings were made for each solution and the readings averaged to give final values of A and A'.

If any detectable reaction occurred between the indicator and base, readings were taken at appropriate time intervals, then extrapolated back to the time of injection of the indicator.

3. Measurements using Potassium t-Butoxide in DMSO

A few of the indicators were very weak acids, incompletely ionized even in 99.5 mole % DMSO, 0.011 M in base. To obtain the extinction coefficient of the anion, readings were taken in anhydrous DMSO, 0.011 M in potassium t-butoxide.

The cells were transferred into a dry box containing helium, filled with about 3 mls anhydrous DMSO, then basified with about 30 µl of a 1 M solution of potassium t-butoxide in DMSO. The cells were fitted with silicone rubber discs, then removed from the dry box. Flushing with nitrogen as described in Section D(1) appeared to introduce more oxygen into the solutions than this method.

The baseline, determined by two blank cells before the injection of indicator, was flat in the visible and near UV region, but poor at shorter wavelengths. Fortunately, none of the anions absorbed in this region. Consistent results in the UV region would require careful degassing of anhydrous DMSO on the vacuum line, as described by F.G. Bordwell[37c] in the preparation of potassium dimsyl solutions. The t-butoxide ion is considered to be in equilibrium with the dimsyl anion, i.e., the conjugate base of DMSO.[15,163]

RESULTS AND DISCUSSION

A. Calculation of Ionization Ratios from UV/Vis Absorption Spectra

To evaluate the acidity constants of the indicators two operations must be carried out. Firstly, the ionization ratio, I, is estimated from spectral data, in this work from UV/vis absorption spectra, and secondly, the acidity constant is evaluated from the ionization ratio data by means of extrapolative[12] or overlap[4,5] procedures.

1. UV/Vis Absorption Spectra of the Indicators

Most of the indicators exhibit significant spectral changes upon ionization and these changes were used to estimate each compound's ionization ratio, $I = [A^-]/[HA]$.

The spectra of both the ionized and unionized forms of the aminopyridines and pyrimidines were generally simple Gaussian bands. The changes in the absorption spectra upon ionization were large and the extinction coefficient of the unionized form HA was small or zero at λ_{max} of the anion A^-. For such non-overlapping spectra, I may be calculated using the expression,

$$I = \frac{[A^-]}{[HA]} = \frac{e_A - e_{HA}}{e_{A^-} - e_A} \qquad (1)$$

where e_{A^-} is the extinction coefficient of the anion at its wavelength of maximum absorption λ_{A^-} max; e_{HA} is the extinction coefficient of the unionized amine at λ_{A^-} max; e_A is the extinction coefficient of a solution at λ_{A^-} max in which the amine is partially ionized.[15,39]

Since the same concentration in the cell was used for each measurement of I for a particular indicator, absorbance A, rather than the extinction coefficient e, was used in the above expression.

Most of the nucleotide bases studied in this work showed spectra comprising two or more transitions for both the ionized and unionized forms. For example, the spectrum of the neutral form of 2,8-dichloro-7-methyladenine (compound 23) could be reconstructed on a Dupont 310 Curve Resolver using a minimum of five Gaussian transitions; the ionized form required three such functions. With the exception of 1,7- and 1,9-dimethylguanine (20 and 21), the unionized and ionized spectra, though markedly different in shape from each other, overlapped to a large extent. In such overlapping spectra, the unionized form has a large absorption at λ_{A^-} max so that I cannot be evaluated accurately using the simple equation (1).

Although the ionization of the indicators used in this work is manifested by a large change in molar absorptivity, e, within a narrow solvent composition range, outside this range smaller changes were observed in e due entirely to effects of changing solvent composition on the e and λ_{max} values of the neutral molecule and the anion. These small changes, or medium effects, were noted as early as 1935 by Flexser, Hammett and Dingwall[39] when spectrophotometry rather than

colorimetry was first used to obtain I values. Failure to account for such changes may cause sizeable errors in I,[34,169] and consequently, erroneous pK_{HA} values, which may, in turn, lead to wrong assumptions about adherence to particular acidity functions.

Increasing basicity was achieved by maintaining the aqueous base concentration at 0.011 M and increasing the molar proportion of DMSO. The medium thus changes appreciably: 10 mole % DMSO is not equivalent to 90 mole % DMSO, just as 10% sulfuric acid is a considerably different medium than 90% sulfuric acid. As the mole fraction of DMSO is increased the wavelength maxima of both the unionized and ionized forms of all the indicators studied shift to the red. The λ_{max} of the neutral form usually shifts 1-2 nm and λ_{max} of the anion 3-5 nm over the region of 10-90% ionization, although shifts in the latter are as much as 10 nm in some cases. Furthermore the e_{max} of the unionized and ionized forms generally increase with increasing DMSO content. For complex spectra comprising several transitions, certain transitions may become better defined with increasing DMSO content or, alternatively, well-defined transitions become smeared into one broad band.

Several empirical methods have been devised for separating effects of the medium from the spectral changes accompanying ionization in non-overlapping spectra and are examined below in Section 2. Methods have also been developed for calculating values of I from overlapping spectra, as well as for compensating for medium changes. These methods are examined in Section 3.

2. Correction of Medium Effects in Non-Overlapping Spectra

(a) The Flexser-Hammett-Dingwall Method (FHD Method): Changes in λ_{max} with changing medium, or lateral shifts, are diagnosed by the lack of a single isosbestic point in the region of ionization. Lack of an isosbestic point means that e_{HA} and e_{A^-} are not constant in the region of ionization. Thus, measurements made at a single wavelength and used in equation (1) would give incorrect I values.

Such medium changes, consisting only of a lateral shift in λ_{max} with minimal change in the shape or height of the spectral curve, were corrected by Hammett et al.[39] by simply moving all the curves laterally to produce a single arbitrary isosbestic point, usually the point of intersection of the two curves closest to 50% ionization. However, it has been shown that measuring at the λ_{max} observed for each solution is equivalent to the above procedure since each curve shifts the exact amount necessary to move it back to the arbitrary common isosbestic point.[29,169]

(b) Corrections for Variations in e_{HA} and e_{A^-} : If e_{HA} and e_{A^-} also vary with the solvent composition one cannot use a constant value of e_{HA} in water and e_{A^-} in, say, 96 mole % basic DMSO to calculate I by means of equation (1) even though the indicator may be unionized in water and fully ionized in 96 mole % DMSO. Slopes of the plots of log I against solvent composition may depend considerably on the choice of e_{A^-} and e_{HA}, since errors in these have a large effect on values of I corresponding to very small or very large degrees of

ionization.[29,170] Using values of e_{BH^+} or e_B many H_o units removed from the region of ionization often gives non-linear plots.[171]

The effect of changing e_{A^-} and e_{HA} may be corrected by choosing reference curves for e_{A^-} and e_{HA} in solutions as close as possible to the range of ionization, yet sufficiently acidic or basic that the indicator is present in one form only. Values of e_{A^-} and e_{HA} at the boundaries of this estimated range are assumed to be constant in the calculation of I. Values of e_{A^-} and e_{HA} are usually taken to be the e values found in solutions two H_- units above and below the pK_{HA} of the indicator, respectively.[15,172]

(c) The Katritzky Method (The K Method): Katritzky has developed a straight-forward method for correcting small changes of both λ_{max} and e_{max} with changing acidic medium.[40] A suitable wavelength λ is chosen, usually the wavelength maximum of the cation or neutral molecule. The absorption at λ is plotted against H_o yielding a sigmoidal titration curve over the region of ionization. Measurements of absorption at λ are made in several solutions where the indicator is fully ionized (or unionized) to extend the shoulders of the titration curve until a linear change in e is obtained. It is assumed that variation of e_{BH^+} and e_B with the medium is linear in H_o throughout the region of ionization and that this variation is the same both here and at higher and lower acidities, respectively. Extrapolation of the linear arms of the titration curve, i.e., e_{BH^+} vs. H_o and e_B vs. H_o, to the region of ionization gives the precise values of e_{BH^+} and e_B in each solution where the indicator is partially ionized.

This method is easily adapted to basic solutions by plotting e against H_-^N [15] and extrapolating the linear arms of the titration curve, e_{A^-} vs. H_-^N and e_{HA} vs. H_-^N to the region of ionization. It is assumed that the H_- functions obeyed by the indicators studied were parallel to H_-^N. This seems to be a valid assumption in the light of the studies of Cox and Stewart.[12] Yates and McLelland[173] have demonstrated the parallelism between H_o[29] and other acidity functions established in acidic media.

(d) The Flexser-Hammett-Katritzky Method: In this work a combination of Hammett's[39] and Katritzky's[40] methods was used to correct for variations of λ_{max} and e with changing mole % DMSO. Measurements of e were made at λ_{A^-} max observed for each DMSO/water solution examined. Occasionally, in solutions where indicator was partially ionized, the spectrum of HA overlapped that of A^- to the extent that λ_{A^-} max could not be determined with certainty. It was observed experimentally, however, that λ_{A^-} max varied linearly with H_-^N for nearly all the indicators studied. Assuming that the rate of lateral shift of λ_{A^-} max is the same within and without the region of ionization, values of λ_{A^-} max where $e_{HA} = 0$ were plotted against H_-^N and the least squares straight line extrapolated to give λ_{A^-} max in the desired solution. Table I lists the indicators, the range of solvent composition over which λ_{A^-} max was plotted, and the correlation coefficients r of the plots of λ_{A^-} max against H_-^N. The correlation coefficient is usually 0.980 or better.

Values of e thus obtained were plotted against H_-^N yielding good

TABLE I. Plots of the Anionic Wavelength Maximum of the Indicators Against H_-^N.

No.	Compound	DMSO concentration range used (mole %)	Correlation coefficient
1	2-aminopyridine	96.8-99.6	0.988
2	2-amino-5-chloropyridine	93.4-99.6	0.988
3	2-amino-3,5-dichloro-pyridine	77.6-98.7	0.977
4	2-amino-3-nitropyridine	39.2-96.3	0.980
5	2-amino-5-nitropyridine	35.0-96.1	0.987
6	2-amino-3-chloro-5-nitropyridine	20.1-96.3	0.981
7	2-amino-3,5-dinitro-pyridine	7.8-49.3	0.984
8	4-aminopyridine	96.8-99.7	0.872
9	4-amino-3-nitropyridine	30.1-96.3	0.996
11	2-aminopyrimidine	87.4-99.6	0.982
12	2-amino-4-chloropyrimidine	68.3-96.8	0.984
13	2-amino-4,6-dichloro-pyrimidine	77.7-96.3	--[a]
14	2-amino-5-nitropyrimidine	55.1-86.0	0.9985
15	4-aminopyrimidine	80.5-99.65	0.993
16	4-amino-2-chloropyrimidine	68.0-96.2	0.996
17	4-amino-2,6-dichloro-pyrimidine	54.1-96.3	0.993
18	4-amino-2-chloro-5-nitro-pyrimidine	30.0-54.5	--[b]
19	2-amino-s-triazine	77.4-96.3	--[a]
20	1,7-dimethylguanine	48.8-96.1	0.999
21	1,9-dimethylguanine	48.8-96.2	0.991
23	2,8-dichloro-7-methyl-adenine	38.1-70.9	0.997
24	2-chloro-7-methyladenine	42.7-77.5	0.980
25	2-chloro-8-methoxy-7-methyl-adenine	60.8-96.2	--[a]
26	7-methyladenine	70.9-96.1	0.994

TABLE I. (continued)

No.	Compound	DMSO concentration range used (mole %)	Correlation coefficient
27	2,8-dichloro-9-methyl-adenine	53.5-96.3	0.987
28	2-chloro-9-methyladenine	72.1-96.7	0.930
29	2-chloro-8-methoxy-9-methyladenine	77.4-96.2	0.934
30	9-methyladenine	89.1-96.2	0.931
31	2,3-dihydro-1H-5-oxoimid-azo(1,2-c)pyrimidine hydrochloride	17.8-96.3	0.989
32	1-methylcytosine	88.5-96.3	0.796
33	1-methyl-5-bromocytosine	54.1-96.3	0.958
34	1-methylisocytosine	48.8-96.1	--[c]
35	3-methylcytosine	25.0-96.1	0.994
36	3-methylisocytosine	49.3-96.3	0.998
37	2',3'-O-benzylidene-5'-O-tritylcytidine	67.5-96.1	--[c]

[a] Wavelength maximum does not shift over solvent composition range examined.

[b] Wavelength maximum could not be measured due to rapid reaction of indicator with base.

[c] Wavelength maximum occurs at wavelengths shorter than 260 nm and was thus obscured by the absorption of DMSO itself.

sigmoidal titration curves in every case. The inflection point was estimated by eye and was taken to correspond to 50% ionization. To ensure that the values of e_{A^-} on the arm of the titration curve corresponded to values greater than 99% ionization, only values of e_{A^-} in solutions of H_-^N at least 3 units greater than H_-^N at half-ionization were used. (Plots of log I vs. mole % DMSO for the aminopyridines and pyrimidines rose less steeply than similar plots for the anilines and diphenylamines used to define the H_-^N function.) Extrapolation of the linear arm of the titration curve (e_{A^-} vs. H_-^N) gave the value of e_{A^-} in each solution where the indicator was partially ionized.

When using DMSO/water solutions e_{HA} may be directly determined in each solution by injecting a volume of water equal to the volume of base into the cell, or by omitting the injection of extra water altogether with an appropriate correction in e_{HA} due to the changed concentration. In practice, however, values of e_{HA} were smoothed out by plotting against H_-^N.

3. Calculation of the Ionization Ratios of Aminopyridines and Aminopyrimidines

The ionization ratio I was calculated for these compounds using the Flexser-Hammett-Katritzky method above (the FHK method). Good titration curves were obtained in all cases, of which the titration curve of 4-amino-2,6-dichloropyrimidine (Figure 1) is a representative example.

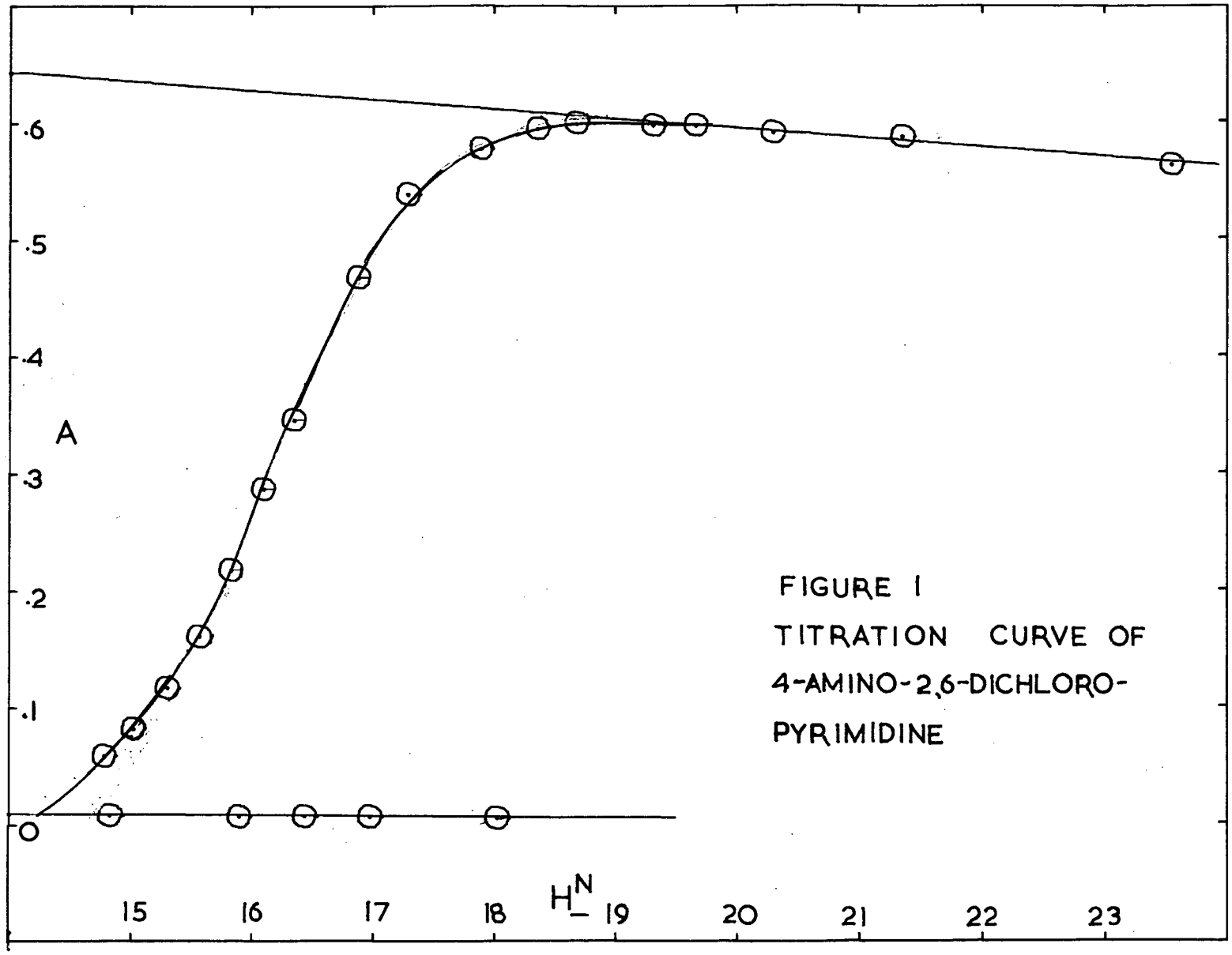


FIGURE 1
TITRATION CURVE OF
4-AMINO-2,6-DICHLORO-
PYRIMIDINE

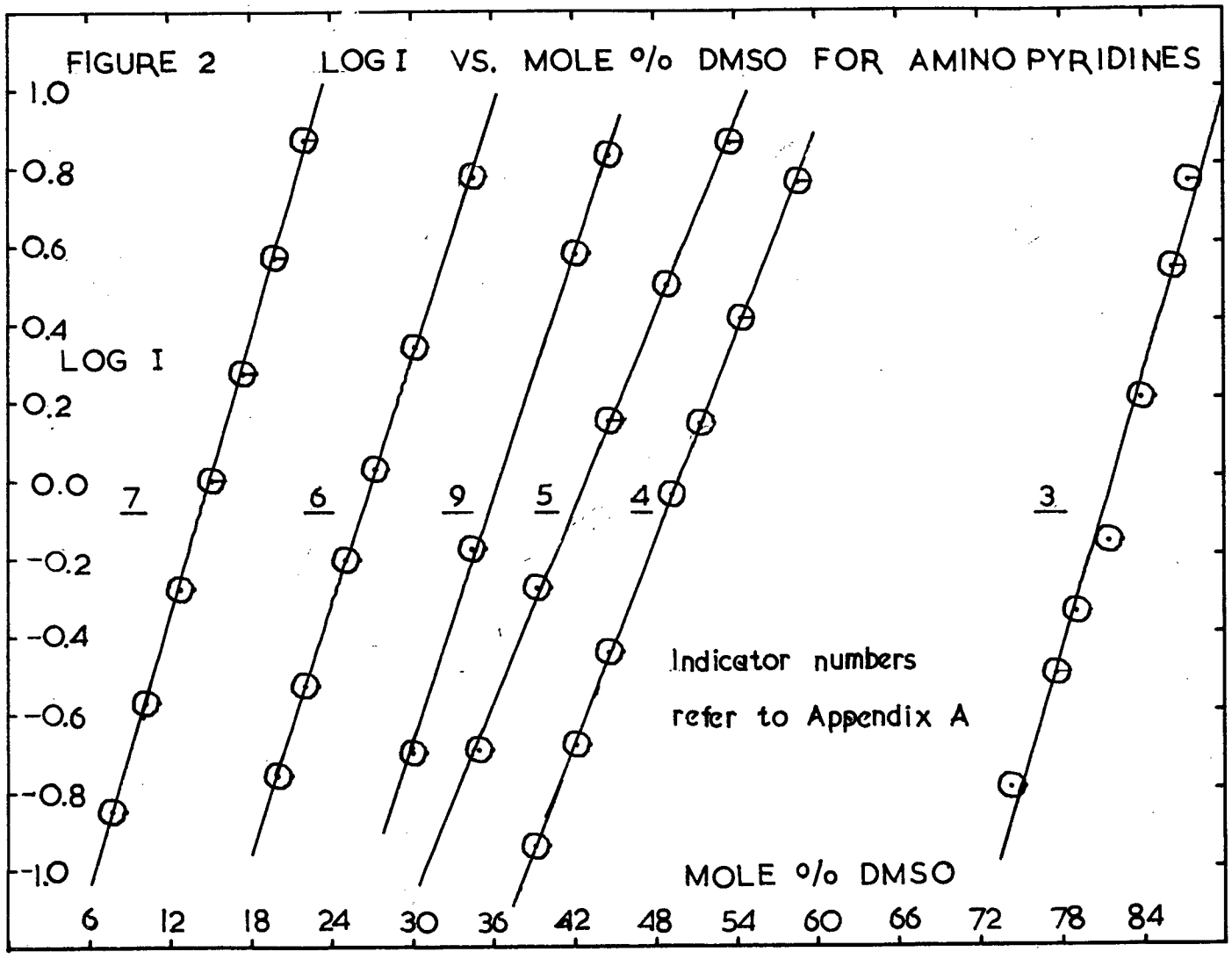


FIGURE 2 LOG I VS. MOLE % DMSO FOR AMINO PYRIDINES
LOG I
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
7
6
9
5
4
3
Indicator numbers
refer to Appendix A
MOLE % DMSO
6
12
18
24
30
36
42
48
54
60
66
72
78
84

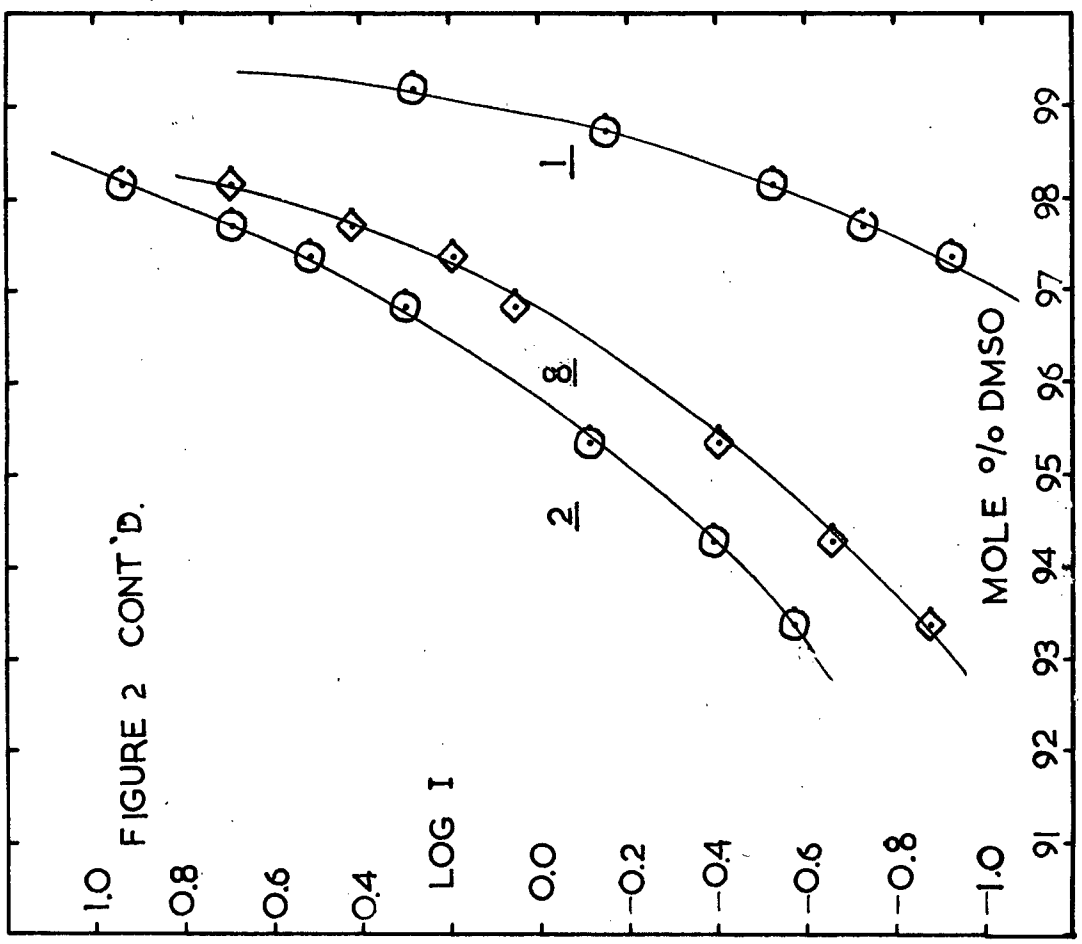
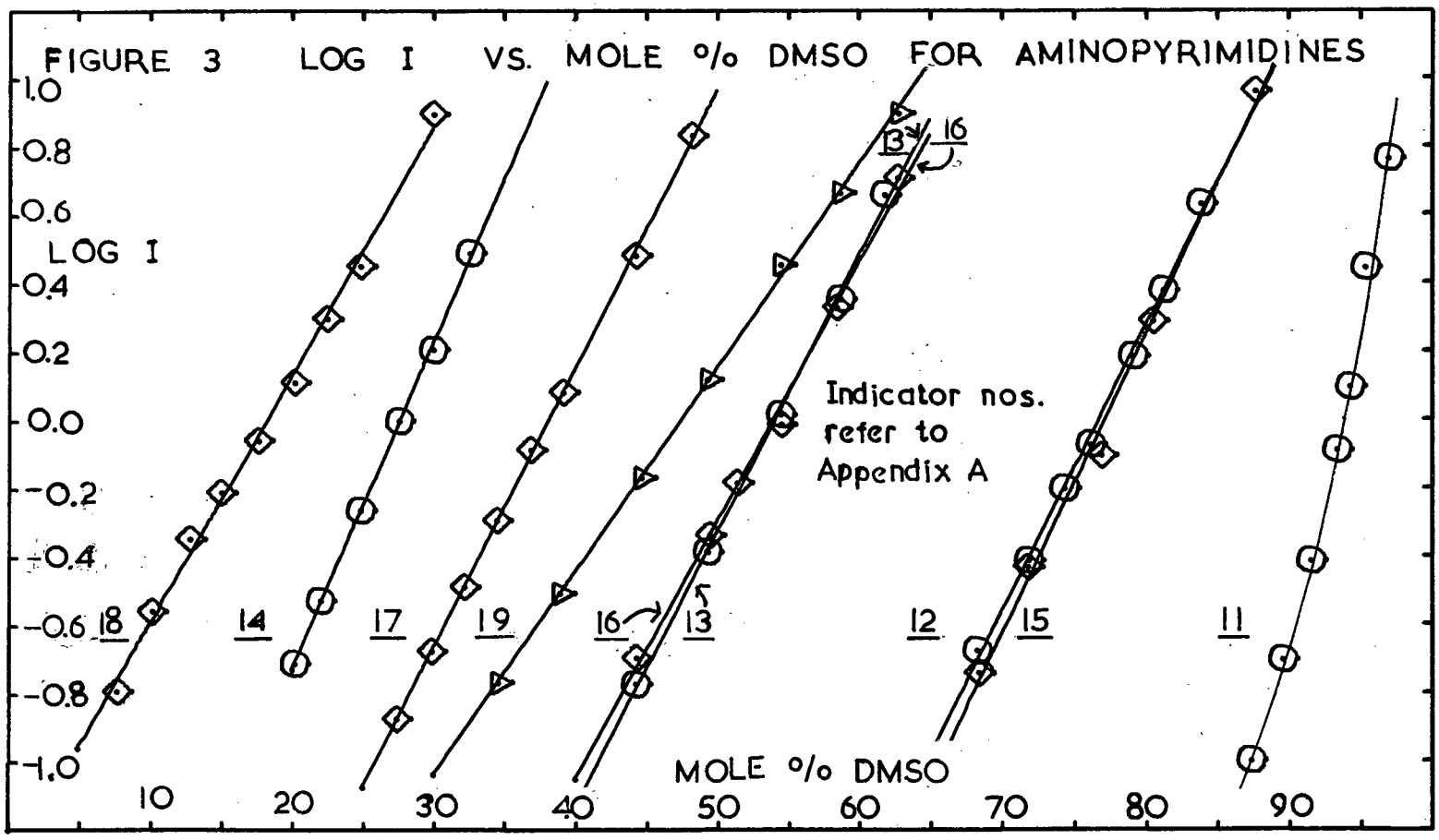


FIGURE 2 CONT'D.
LOG I
1.0
0.8
0.6
0.4
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
91
92
93
94
95
96
97
98
99
MOLE % DMSO
1
8
2


LEGEND :
2-aminopyrimidine
4-aminopyrimidine
2-amino-s-triazine
FIGURE 3 LOG I VS. MOLE % DMSO FOR AMINOPYRIMIDINES
LOG I
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
10
20
30
40
50
60
70
80
90
MOLE % DMSO
Indicator nos. refer to Appendix A
18
14
17
19
16
13
16
13
12
15
11

In the case of 2- and 4-aminopyridine and of 2-amino-5-chloropyridine, it was necessary to use potassium t-butoxide in 100 mole % DMSO to obtain e_{A^-}. It had to be assumed that e_{A^-} was invariant in the solutions used to cover the ionization ranges of these compounds, 93.4 to 99.2 mole % DMSO, but since the change in e_{A^-} is usually small for the aminopyridines, this assumption should lead to very small errors in I.

Appendix A contains the ionization ratio data for the aminopyridines and pyrimidines in DMSO/water/0.011 M TMAH, with details on minor difficulties that were encountered with individual indicators. Figure 2 contains the plots of log I against mole % DMSO for the aminopyridines and Figure 3 contains the same plots for the aminopyrimidines and for 2-amino-s-triazine.

4. Correction of Medium Effects in Overlapping Absorption Spectra

When the spectral change upon ionization is small, or when the spectra of the unionized and ionized forms of the indicator overlap so closely that the measurable spectral change upon ionization is small, I is very difficult to estimate accurately. All the nucleotide bases, with the exception of 1,7- and 1,9-dimethylguanine, exhibited overlapping spectra of their neutral and ionized forms, namely, fifteen of the thirty-five indicators studied for which meaningful I values could be estimated. For such difficult spectral changes, no one method of correcting medium effects appears to be entirely satisfactory and it is often useful to exploit the spectral peculiarities of an indicator to calculate I. Occasionally, the spectral change with the medium is

of the same order of magnitude as the change upon ionization, too small to permit an estimate of I. This was observed previously for anthraquinone[170] and in this work for 3,7-dimethylisoguanine.

Besides the Katritzky (and FHK) method, two other methods exist for determining I from complex spectral changes and which also compensate for spectral changes due to the medium. These are the method of Davis and Geissman[41] and the method developed in this work that employs the area under the spectral curve rather than the peak height at a particular wavelength to calculate I.

(a) The Davis and Geissman Method: When determining the pK_{BH^+} of substituted flavones, Davis and Geissman found no single wavelength at which there existed a regular relationship between e and I. They selected two wavelengths λ_B, where e_B was much greater than e_{BH^+}, and λ_{BH^+}, where e_{BH^+} was much greater than e_B, preferably the maxima of the ionized and unionized species. Plotting D (where $D = e_{\lambda_{BH^+}} - e_{\lambda_B}$) against H_o gave good sigmoidal titration curves with inflection points whose corresponding H_o values were taken to be equal to the pK_{BH^+} values of the indicators.

Alternatively, log I may be calculated directly using the following expression:[171]

$$I = \frac{\Delta D_{BH^+} - \Delta D}{\Delta D - \Delta D_B} \qquad (2)$$

where ΔD_B is D in acid solutions where the base is fully unionized, ΔD_{BH^+} is D in solutions where the base is fully ionized, ΔD is D where

the base is partially ionized.

It can be shown that in basic solutions,

$$I = \frac{\Delta D - \Delta D_{HA}}{\Delta D_{A^-} - \Delta D} \qquad (3)$$

where ΔD_{A^-} is D in solutions where the acid is fully ionized, ΔD_{HA} is D in solutions where the acid is fully unionized and ΔD is D in solutions where the acid is partially ionized.

This method has been criticized since the magnitude of I may depend on the choice of λ_{BH^+} and λ_B,[169] that is, where the boundaries of the ionization region are set, and that linear plots are obtained only if λ_{BH^+} and λ_B are well separated.[172] It was originally assumed that e_{BH^+} and e_B are invariant over the region of ionization and equal to the values of e_{BH^+} and e_B at the limits of ionization, as described in Section 2(b).

To correct for lateral shifts in λ_{BH^+} and λ_B and changes in e_{BH^+} and e_B within the ionization region, Farlow and Moodie[174] combined this method with that of Katritzky. D was plotted against H_A[172] in this case and the arms of the sigmoid curve extended until they were linear in H_A. Extrapolation of D to the ionization region gave ΔD_{BH^+} and ΔB_B at solvent compositions in this region.

(b) The Area Method: Values of I often depend on the choice of wavelength at which e_A, e_{HA} and e_{A^-} are measured, especially if no correction is made for the medium effect, as shown for p-nitrotoluene[175] and 6-bromo-2,4-dinitroaniline.[176] Where spectra are complex, or

overlap appreciably, the mean I values determined at several wavelengths have been used to calculate pK_a.[29,176-179] The ultimate method of averaging is to employ the area under the total spectral curve. Although this method has been applied by other workers[41,176], no critical study of the method has as yet been made.

(i) Derivation of the Method. For a given DMSO/water solution let

$$x + y = R \qquad (4)$$

where x is the molar concentration (M) of the unionized form of the indicator, y is the molar concentration (M) of the ionized form, R is the total concentration of the indicator in the cell.

Letting L equal the area under the unionized spectrum for R = 1 M and K equal the area under the fully ionized spectrum, again for R = 1 M, then

$$Lx + Ky = Z \qquad (5)$$

where Z is the area measured under the total spectrum at the given mole % DMSO.

From (4) and (5),

$$y = \frac{Z - LR}{K - L}$$

If P is the area observed under the spectrum of the unionized species at the given mole % DMSO and Q is the area of the fully ionized spectrum calculated for this solvent composition, then

$$L = P/R \quad \text{and} \quad K = Q/R \quad \text{and} \quad y = \frac{R(Z-P)}{(Q-P)}$$

Also,

$$x = (R-y) = \frac{R(Q-Z)}{(Q-P)}$$

Therefore,

$$I = \frac{[A^-]}{[HA]} = y/x = \frac{(Z-P)}{(Q-Z)} \qquad (6)$$

Equation (6) is equivalent to expression (1), $I = \frac{e_A - e_{HA}}{e_{A^-} - e_A}$.

The change in area upon ionization must be large and of much greater magnitude than changes with the medium for the method to be applicable. It was found experimentally in this work that best results were obtained if Q was much larger than P - at least 1.5 times as great if the results were to be meaningful. Values of Z close to P or Q could give I values considerably in error. Thus in plots of log I vs. mole % DMSO only values of log I between +0.8 and -0.8 were used.[29]

If the above requirement was met, good sigmoidal titration curves were obtained when Z in DMSO/water/0.011 M TMAH solutions was plotted against H_-^N for the compounds studied in this work. An estimate of H_-^N at half-ionization, $1/2H_-^N$, was made. Again only values of Q in

solutions with H_-^N 3 units greater than $1/2H_-^N$ were used in the extrapolation to the region of ionization. Values of P in this region were interpolated from a smoothed-out curve obtained by plotting the observed values of P against H_-^N. Using the measured values of Z, the extrapolated values of Q, and the interpolated values of P the ionization ratio was calculated using eqn. (6).

(ii) Difficulties Encountered Using the Area Method. Ideally the complete spectral curves for the ionized and unionized species should be accessible but since the nucleotide bases absorb at wavelengths shorter than 350 nm, both spectra become partially obscured on the short wavelength side of 260 nm by the absorption of DMSO itself. One is therefore limited to using a portion of the total spectrum. Ryabova[176] used the area extending from the anionic absorption maximum to "higher" wavelengths. Since the anionic and neutral spectra overlap, however, the anionic absorption maximum is not distinguishable in those solutions where the indicator is partially ionized. Davis and Geissman used the area between two arbitrarily selected wavelengths to calculate $\log^{\circ} I$ and found such values to be in good agreement with other methods.[41] Such values may depend on choice of wavelength.[180]

There are two alternative methods which were felt to be appropriate for choosing these wavelengths. The first is to use as much of the unobscured spectrum as possible by measuring the area from 260 nm to longer wavelengths. The second is to employ the area on either side of the isosbestic point, defined by the two curves closest to 50%

ionization.[39] For an indicator whose spectra are unobscured by DMSO absorbance, I values calculated from areas to the short wavelength side (left-hand side, L.H.S.) of the isosbestic point should be identical to those calculated from areas to the long wavelength side (right-hand side, R.H.S.) if I is independent of the choice of wavelength pair.

These two methods were investigated for two representative compounds: the imidazo-pyrimidone (indicator 31), used to anchor the H_-^P function determined in this work, and 2,8-dichloro-7-methyladenine (indicator 23).

The spectra of 31 were not obscured by DMSO absorption except for a small part of the short wavelength tail in each case. Log I was first estimated using Katritzky's method at 325 nm. From these values the curves at 2.88 mole % and 5.32 mole % DMSO were found to lie closest to 50% ionization. The isosbestic point was thus taken to be 307.5 nm. The areas from 265 nm to 307.5 nm, the L.H.S. of the isosbestic point, and the areas from 307.5 nm to the end of the long wavelength absorption, the R.H.S. of the isosbestic point, were then used to calculate I using equation (6). To the R.H.S. of 295 nm the difference in areas between neutral and anionic spectra appeared to be maximized so that the area from 295 nm to long wavelength absorption was also used to calculate I to see how I varies with arbitrary choice of wavelength pair. Unfortunately, the areas of the total neutral and anionic spectra (from 265 nm to longer wavelengths) were identical so that I could not be calculated from the total spectra for comparison.

TABLE II. Logarithms of Ionization Ratios Estimated for Indicator 31 Using the Katritzky and the Area Methods.

	Method of Estimation Used			
Mole % DMSO	Katritzky at 325 nm	Area (295 nm ⟶)	Area (265-307.5 nm)	Area (307.5 nm ⟶)
0.26	-0.46	--	-0.41	-0.72
1.42	-0.33	-0.59	-0.22	-0.49
2.88	-0.18	-0.32	-0.10	-0.26
5.32	0.05	-0.01	0.02	0.02
7.80	0.26	0.31	0.19	0.27
10.09	0.43	0.55	0.50	0.45
12.65	0.64	0.58	0.65	0.61
15.15	0.94	0.72	0.68	--

TABLE III. Least Squares Correlations of Various Estimated Log I Values with Mole % DMSO for Indicator 31.

Method of Estimation of Log I Values	Correlation Coefficient	Slope
Katritzky at 325 nm	0.998	0.090
Area (295 nm ⟶)	0.967	0.095
Area (265-307.5 nm)	0.984	0.074
Area (307.5 nm ⟶)	0.986	0.106

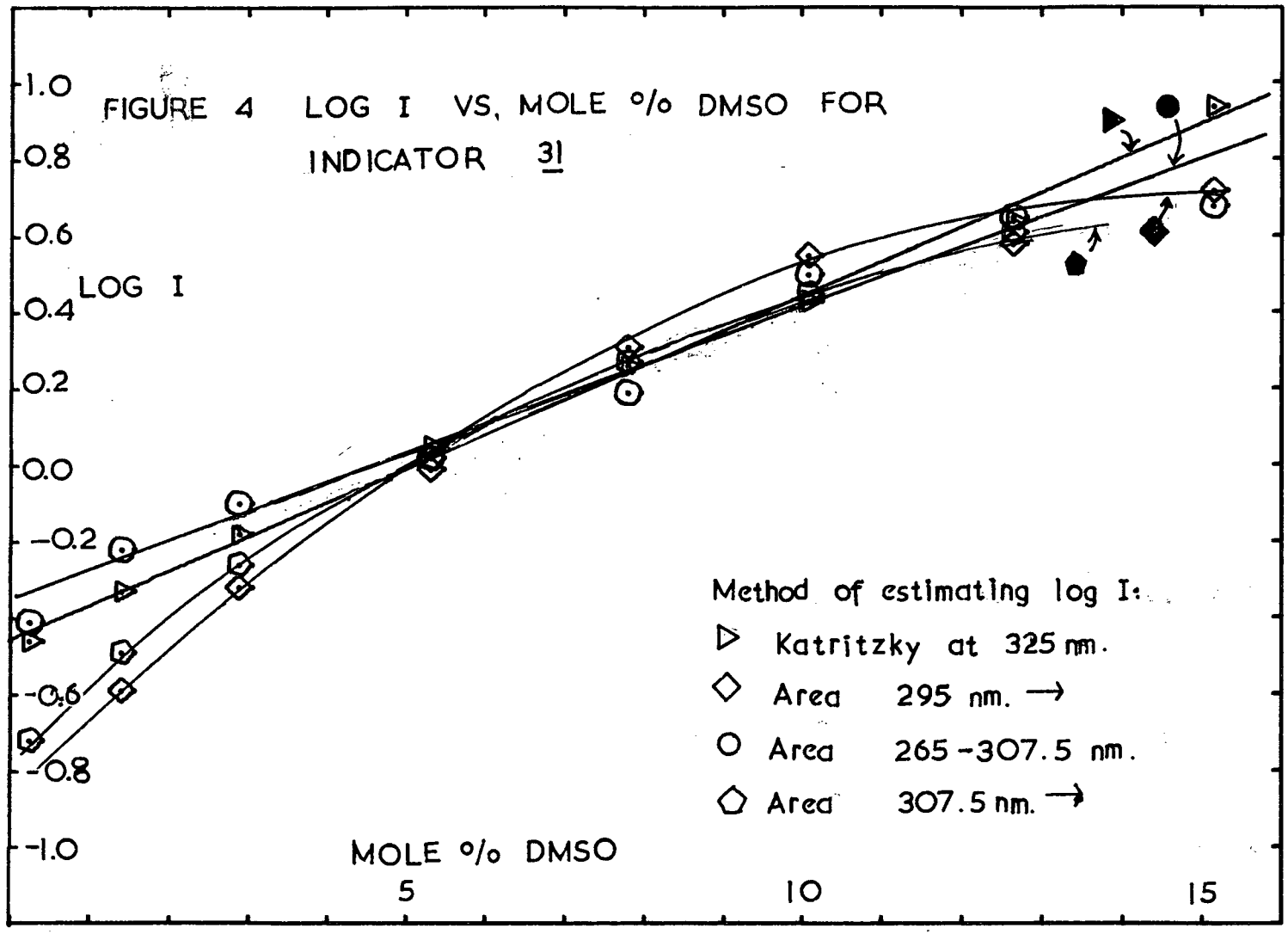


FIGURE 4 LOG I VS. MOLE % DMSO FOR INDICATOR 31
LOG I
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.6
-0.8
-1.0
MOLE % DMSO
5
10
15
Method of estimating log I:
Katritzky at 325 nm.
Area 295 nm. →
Area 265-307.5 nm.
Area 307.5 nm. →

Table II contains log I values obtained by the various methods for indicator 31. The values agree roughly with one another; agreement in the region of half-ionization is good but the values of log I determined by areas deviate from each other at the extrema. This is due entirely to the closeness of the values of Z, P and Q, so that the differences (Z-P) and (Q-Z) can be in considerable error at high and low degrees of ionization.

The log I values thus obtained were plotted against mole % DMSO. The correlation coefficient r and the slope of each straight line are given in Table III and the plots are shown in Figure 4. It is evident that the slopes are highly dependent on the values of log I at low and high degrees of ionization.

The neutral and anionic spectra of 2,8-dichloro-7-methyladenine, 23, were sufficiently different from one another that the problem of small values of (Z-P) and (Q-Z) did not arise. Using the Katritzky method at 300 nm the curves at 15.09 and 20.09 mole % DMSO were found to lie closest to 50% ionization. The isosbestic point is thus taken to be 270 nm. The area from 260 nm to 270 nm was too small for a meaningful calculation of I, but good results were obtained using the area to the R.H.S. of the isosbestic point, 270 nm to longer wavelengths, and the area of the unobscured spectrum, 260 nm to longer wavelengths. Table IV contains the log I values thus calculated. It is evident that there is good agreement between the log I values obtained by the two methods. The slopes of the plots against mole % DMSO are also in good agreement with one another: for areas to the R.H.S. of 260 nm the slope equals 0.094, and for areas to the R.H.S. of 270 nm, slope equals 0.088.

TABLE IV. Logarithms of the Ionization Ratios Estimated for 2,8-Dichloro-7-methyladenine Using the Katritzky and the Area Methods.

Mole % DMSO	Method of Estimation of Log I Values		
	Katritzky at 300 nm	Area (260 nm ⟶)	Area (270 nm ⟶)
10.17	-0.85	-0.74	-0.83
12.94	-0.61	-0.47	-0.52
15.09	-0.39	-0.40	-0.40
18.25	-0.05	0.00	-0.02
20.09	0.09	0.17	0.17
24.78	0.47	0.48	0.48
29.53	0.86	0.91	0.87

TABLE V. Logarithms of the Ionization Ratios Estimated for 2-Chloro-7-methyladenine Using the Katritzky and Area Methods.

Mole % DMSO	Method of Estimation of Log I Values		
	Katritzky at 300 nm	Area (260 nm ⟶)	Area (275 nm ⟶)
15.09	-0.84	-0.80	-0.85
17.48	-0.65	-0.61	-0.65
20.50	-0.40	-0.37	-0.36
22.08	-0.26	-0.22	-0.25
24.76	-0.07	-0.02	-0.05
29.85	0.41	0.52	0.44
34.00	0.68	0.70	0.66

Similar calculations were also made for 2-chloro-7-methyladenine, 24, which had a large difference in the neutral and anionic areas. Katritzky's method was used at 300 nm to establish the isosbestic point at 275 nm, and log I calculated using the areas to the R.H.S. of 260 nm and 275 nm. As can be seen from Table V the agreement between the two methods is excellent.

It thus appears from the above discussion that I calculated using the area method does not depend significantly on the choice of wavelength pair as long as the anionic area Q and the neutral area P are considerably different in magnitude. It was stated in (i) that, for best results, Q should be 1.5 times as large as P (or vice versa). Thus the area to the R.H.S. of the arbitrary isosbestic point[39] or the total area of the unobscured spectrum, usually the area to the R.H.S. of 260 nm, may be used. In determining log I for complex, overlapping spectra, the latter area was found to be most convenient to use.

Davis and Paabo[178] have pointed out the possibility of error in using overlapping transitions to calculate physical constants since the bands may not be equally affected by changing medium. The anionic spectrum of 1,9-dimethylguanine, 21, consists of two well-defined transitions, λ_{max} 275 nm and 311 nm. Using the area under the spectral curve of both transitions, I was calculated using the area to the R.H.S. of 260 nm. The values are in good agreement with the I values calculated using the FHK method at 311 nm. (Table VI). From this example it appears that the area method is independent of the number of bands comprising the total spectrum of the indicator.

TABLE VI. Logarithms of the Ionization Ratios Estimated for 1,9-Dimethylguanine Using the FHK and the Area Methods.

	Method of Estimation of Log I Values	
Mole % DMSO	FHK	Area (260 nm ⟶)
23.68	-0.91	-0.82
29.88	-0.40	-0.36
34.46	-0.05	-0.05
43.59	0.54	0.59

(c) Comparison of Methods Used to Correct Medium Effects In Complex or Overlapping Spectra: To estimate the uncertainties in log I values determined from overlapping spectra, four methods were applied to the spectra of the representative indicator, 2,8-dichloro-7-methyladenine, 23.

The Katritzky method was the first method examined. Three wavelengths, 295 nm, 300 nm, 305 nm, were chosen at which the anion had a significantly higher absorption than the neutral molecule. As was often the case with the overlapping spectra studied, this situation exists only at wavelengths where the anionic spectrum has a steeply rising slope. Thus log I is more subject to uncertainty than if measurements could be made at wavelengths where the anionic spectrum has a broad, flat portion. Excellent titration curves were obtained at all three wavelengths and Figure 5 shows that obtained by plotting $e_{300\ nm}$ against H_-^N.

The log I values obtained at 295, 300 and 305 nm are summarized in Table VII together with the average log I obtained at the three wavelengths for each solution. Table VIII contains the correlation coefficients of the least squares plot of log I against mole % DMSO and the slopes of the straight lines obtained. Agreement between the log I values obtained at all three wavelengths is reasonably good, and is excellent for 300 and 305 nm. Values at 295 nm differ at the extrema causing changes in slope (Table VIII). At 300 and 305 nm contributions from e_{HA} are nearly zero, whereas at 295 nm e_{HA} is fairly large. It appears then that the success of the Katritzky method does not depend on the choice of wavelength as long as e_{HA} is much smaller than e_{A^-}.

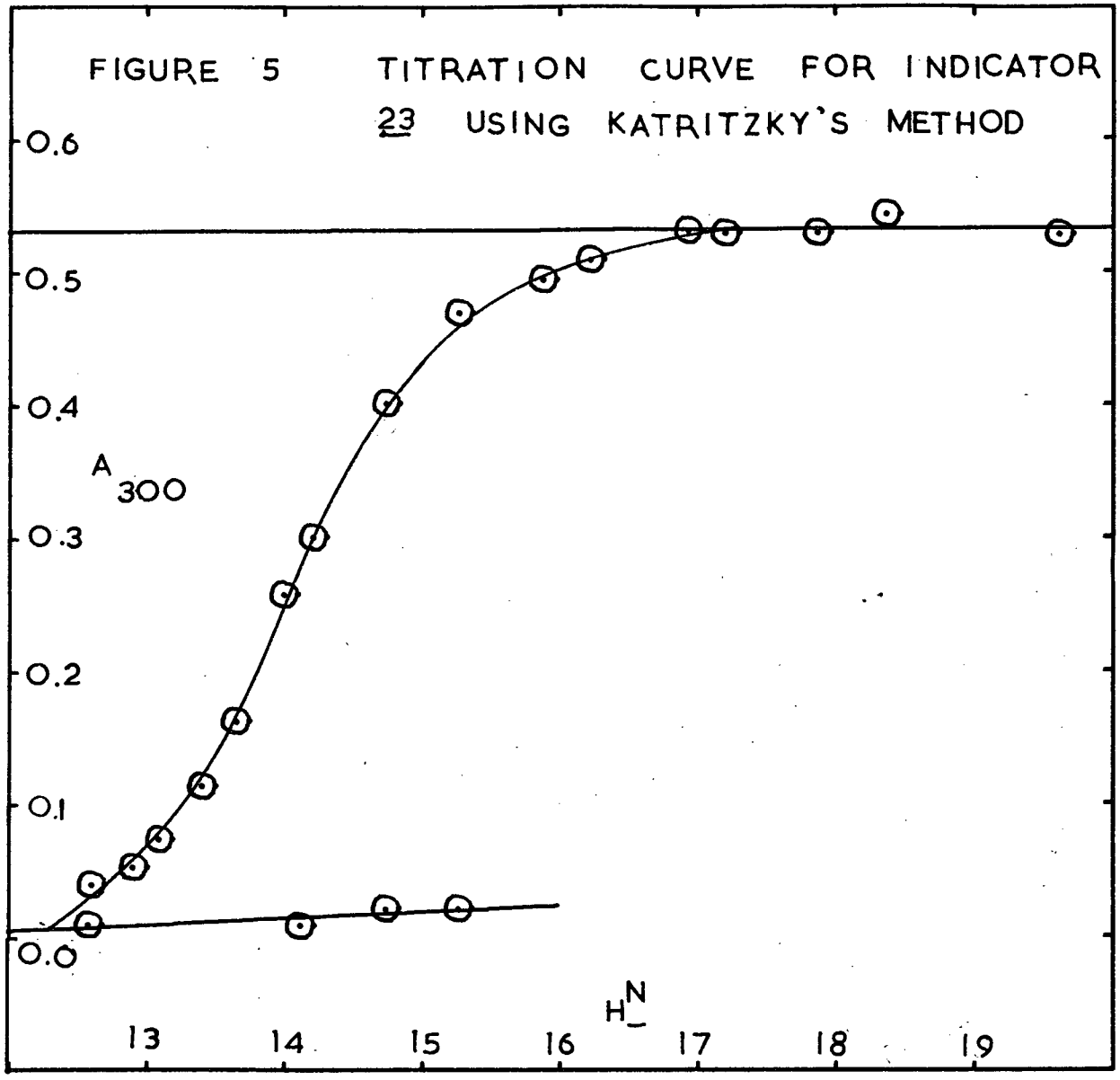


FIGURE 5 TITRATION CURVE FOR INDICATOR 23 USING KATRITZKY'S METHOD
0.6
0.5
0.4
A 300
0.3
0.2
0.1
0.0
13
14
15
16
17
18
19
H_ N

TABLE VII. Logarithms of the Ionization Ratios Estimated for 2,8-Dichloro-7-methyladenine Using the Katritzky, FHK, Davis and Geissman, and Area Methods.

	Method of Estimation of Log I Values						
Mole % DMSO	K. at 295 nm	K. at 300 nm	K. at 305 nm	Average of K. values	FHK	D.G.	Area (260 nm ⟶)
10.17	-0.77	-0.85	-0.82	-0.80	-0.92	-0.84	-0.74
12.94	-0.52	-0.61	-0.64	-0.57	-0.54	-0.54	-0.47
15.09	-0.28	-0.39	-0.34	-0.32	-0.31	-0.28	-0.40
18.25	0.05	-0.05	-0.04	0.01	0.05	-0.04	0.00
20.09	0.22	0.09	0.10	0.16	0.30	0.10	0.17
24.78	0.63	0.47	0.48	0.53	0.64	0.56	0.48
29.53	1.08	0.86	0.87	0.98	--	0.94	0.91

TABLE VIII. Least Squares Correlation of Various Log I Values Estimated for 2,8-Dichloro-7-methyladenine with Mole % DMSO.

Method of Estimation of Log I Values	Correlation Coefficient	Slope
Katritzky at 295 nm	0.999	0.099
Katritzky at 300 nm	0.998	0.089
Katritzky at 305 nm	0.997	0.089
Average of Katritzky Values	0.999	0.092
FHK	0.994	0.108
Davis and Geissman	0.998	0.090
Area (260 nm ⟶)	0.998	0.094

In this work, Katritzky's method was used only at wavelengths where e_{A^-} is large and e_{HA} is no greater than 10% of e_{A^-}.

The Flexser-Hammett-Katritzky method was also examined but good results were not expected since the spectra were of the overlapping type. Values of λ_{A^-} max between 38.1 and 70.9 mole % DMSO were plotted against H_-^N and a straight line obtained with correlation coefficient r 0.997, slope 0.85 and intercept 272.2 nm. Extrapolation of this plot to the region of ionization gave the estimated λ_{A^-} max for each solution.. Plots of e_A measured at these wavelengths and at λ_{A^-} max observed for the fully ionized spectra against H_-^N gave reasonably satisfactory titration curves (Figure 6), and log I values (Table VII) which agree well with those obtained by Katritzky's method at 295 nm. This agreement is no doubt due to sizeable contributions by e_{HA} at each λ_{A^-} max. The slope of the plot of log I vs. mole % DMSO is also higher than those obtained using other methods (Table VIII). Thus the FHK method is less satisfactory for calculating log I from overlapping spectra.

The Davis and Geissman method,[41] with Farlow and Moodie's modification[174] to correct for variation of e_{A^-} with the medium, was also used to calculate log I, using equation (3). The values of λ_{A^-} max 285 nm and λ_{HA} max 277 nm were those values of λ_{max} found at the borders of the region of ionization defined by the log I values calculated from Katritzky's method. A fairly satisfactory titration curve was obtained (Figure 7). Values of log I obtained (Table VII) and the value of the slope of log I vs. mole % DMSO (Table VIII) are in good agreement with other methods, particularly with those using the area method and the average log I's of the Katritzky method.

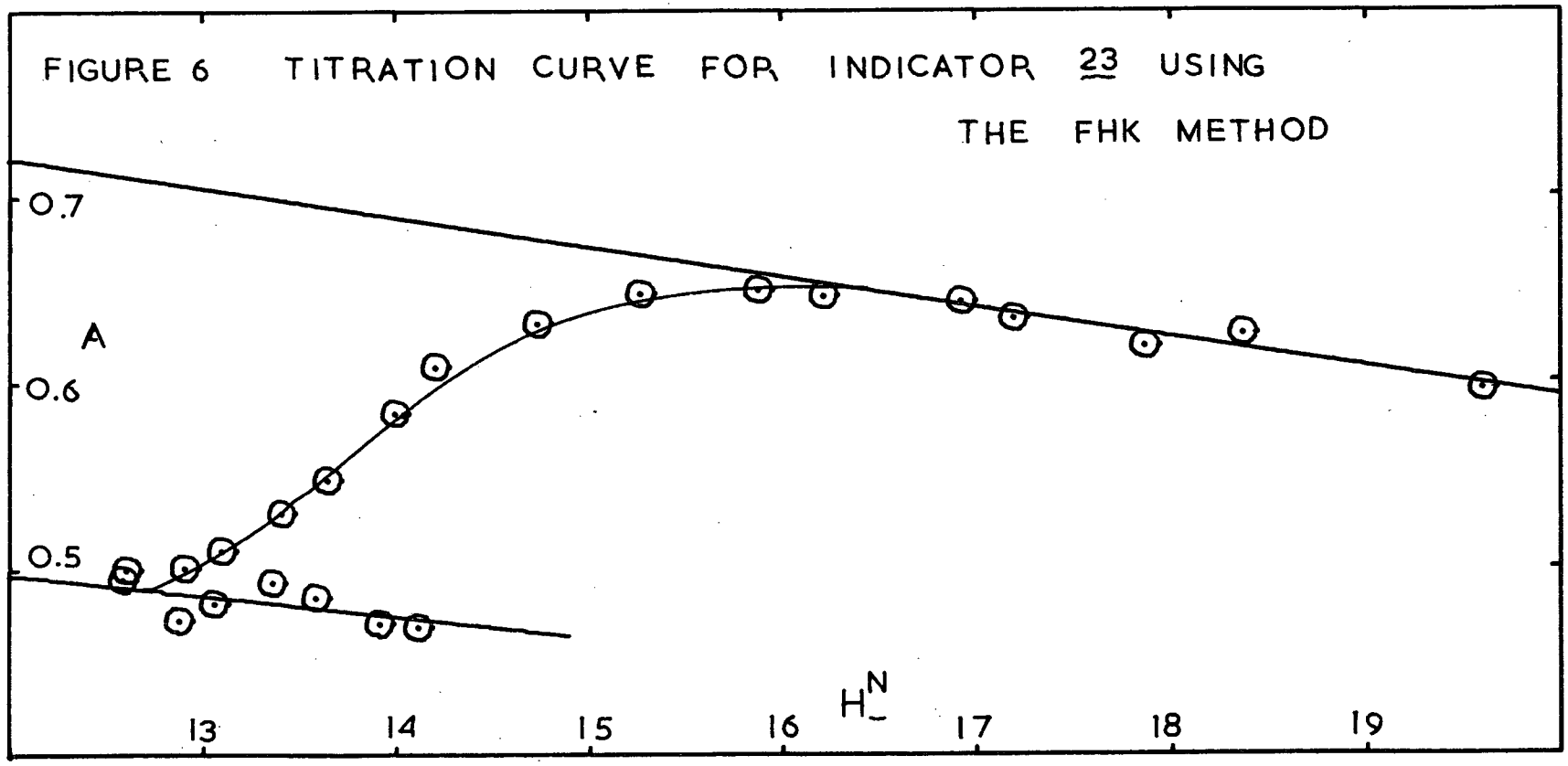


FIGURE 6 TITRATION CURVE FOR INDICATOR 23 USING THE FHK METHOD

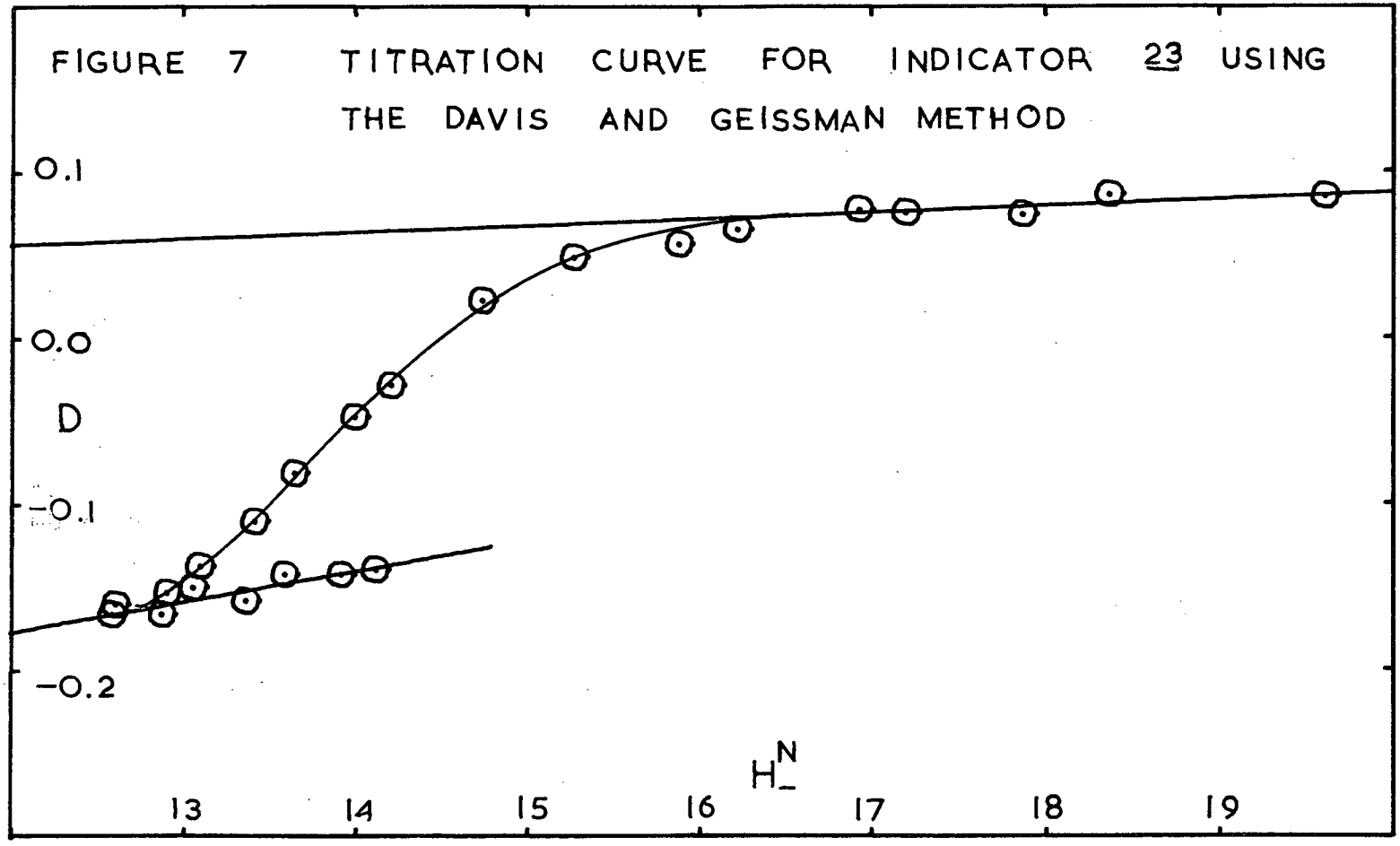


FIGURE 7 TITRATION CURVE FOR INDICATOR 23 USING THE DAVIS AND GEISSMAN METHOD
0.1
0.0
D
-0.1
-0.2
13
14
15
16
17
18
19
H_-^N

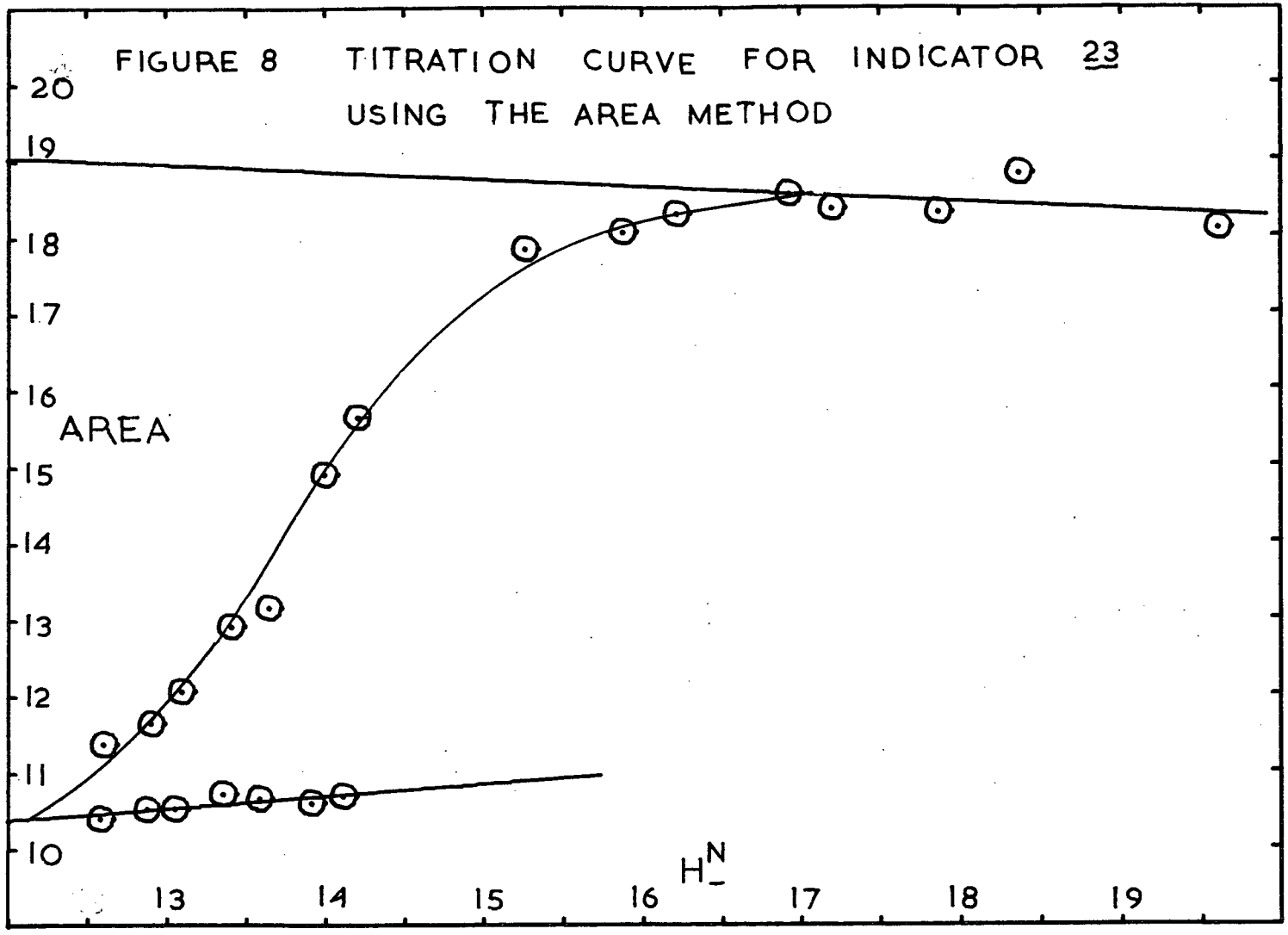


FIGURE 8 TITRATION CURVE FOR INDICATOR 23
USING THE AREA METHOD
AREA
20
19
18
17
16
15
14
13
12
11
10
13
14
15
16
17
18
19
H_-^N

The area method was discussed in detail for 2,8-dichloro-7-methyladenine in Section 4(b)(ii). A good sigmoidal titration curve was obtained by plotting Z vs. H_-^N (Figure 8). Values of log I and the slope of log I vs. mole % DMSO are in good agreement with other methods and particularly with the averaged results of Katritzky's method. This supports the premise that using areas to calculate I is equivalent to using average values of I obtained at several different wavelengths. The results are summarized in Tables VII and VIII.

Thus, the area method is as good a method for calculating log I from complex, overlapping spectra as is the Davis and Geissman method. It is independent of the choice of wavelength pair between which the area is measured and does not depend on the number of transitions incorporated into the spectral curve. Medium effects may be eliminated by an adaptation of the Katritzky method if the change in area with these is much smaller than the change in area upon ionization. However, if the results obtained are to be meaningful the change in area from P to Q must be large.

It was felt that the area method gave the best log I values for overlapping spectra. Katritzky's method also gave satisfactory results but is subject to more uncertainty since measurements must nearly always be made on a steeply sloping portion of the spectral curve. Both methods were used to calculate log I for overlapping spectra in this work.

5. Calculation of Ionization Ratios of the Nucleotide Bases

Nearly all of the nucleotide bases had complicated, overlapping spectra. For these indicators log I was determined using both

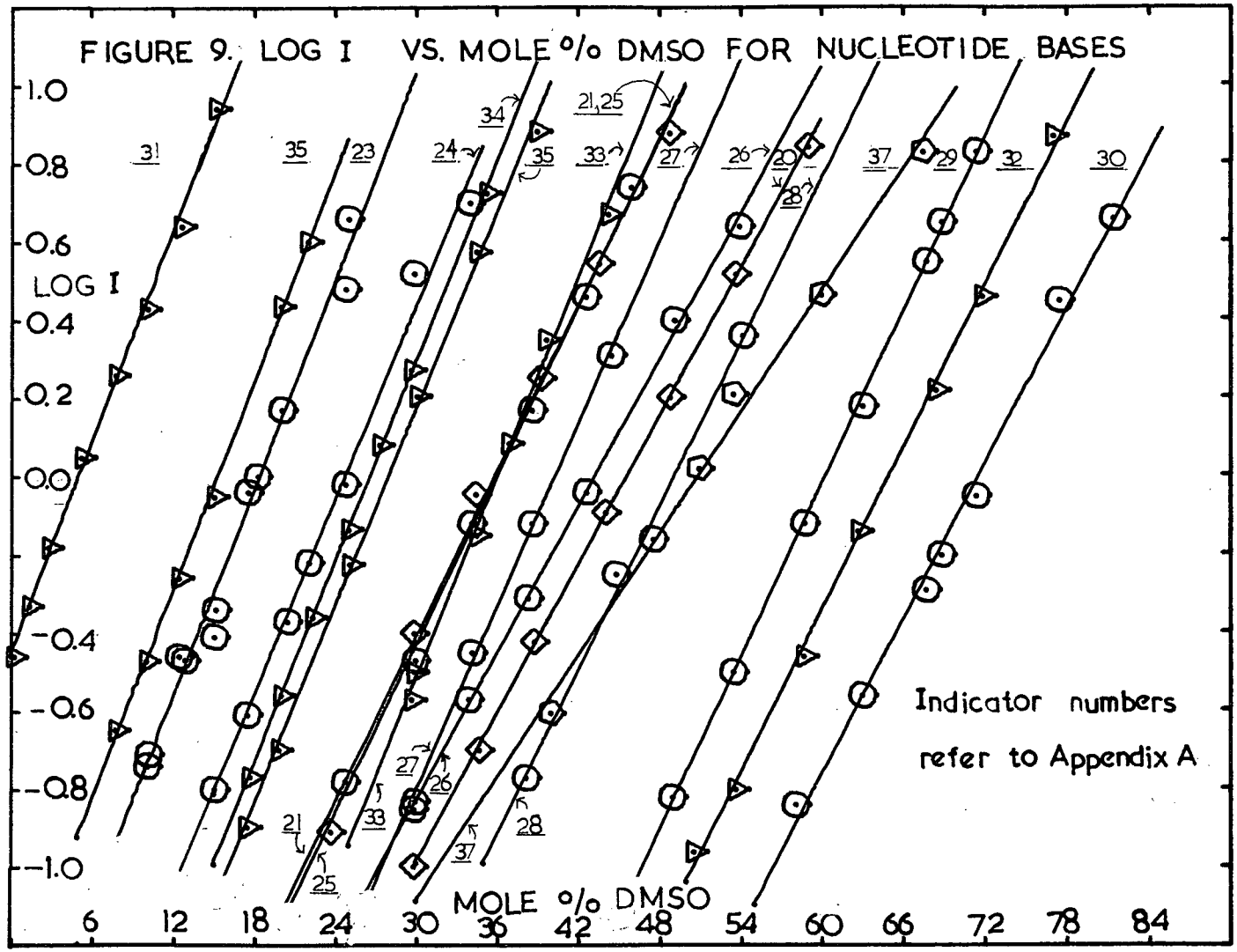


FIGURE 9. LOG I VS. MOLE °/o DMSO FOR NUCLEOTIDE BASES
LOG I
1.0
0.8
0.6
0.4
0.2
0.0
-0.4
-0.6
-0.8
-1.0
MOLE °/o DMSO
6
12
18
24
30
36
42
48
54
60
66
72
78
84
31
35
23
24
34
35
21,25
33
27
26
20
28
37
29
32
30
21
25
33
27
26
37
28
Indicator numbers refer to Appendix A
Legend:
aminopyrimidone
adenine
guanine
cytidine

Katritzky's method at an appropriate wavelength and the area method using the area under the spectral curve from 260 nm to the end of the long wavelength absorption. Areas were measured by means of a planimeter. In general, the agreement between the two methods is very good, although the log I values determined by the area method were slightly more positive than those determined using the Katritzky method. The indicator acid strength thus appears to be slightly greater when the area method is used.

Details of calculation for individual compounds are given in Appendix A together with indications of any minor difficulties encountered. Figure 9 contains the plots of log I against mole % DMSO for all the nucleotide bases.

B. Calculation of Acid Ionization Constants Using Two Extrapolative Procedures

The compounds studied in this work did not obey H_-^N.[15] Although plots of log I of the indicators against H_-^N were invariably linear, the slopes of these plots were always much less than unity. The pK_{HA} of a given indicator may be arrived at without the construction of an appropriate acidity function by using two extrapolative procedures: the Bunnett-Olson method[30] (the B.O. method) and the Marziano-Cimino-Passerini method[181] (the M.C.P. method). Both of these methods, originally derived for acid systems, have been adapted to basic DMSO/water systems by Cox and Stewart.[12]

1. Derivation of the Bunnett-Olsen Method (B.O. Method)

The protonating power of an acid medium varies for individual indicators, hence the existence of several H_o functions.[28] For a base B, protonation may be written

$$B + H^+ \rightleftharpoons BH^+$$

The logarithm of the equilibrium quotient is therefore

$$\log Q = \frac{[BH^+]}{[B][H^+]}$$

Log Q is a measure of the protonating power of the acid. In moderately concentrated mineral acids it is an experimental fact that log Q values of diverse bases are linearly related. Bunnett and Olsen have attempted to express the variable protonating power of a given solution in a standardized form suitable for use with all indicators. Since the log Q values for two bases are linear at acidities where they are both partially ionized one would like to have a standard indicator whose log Q values can be measured from 0-100% aqueous acid, for use as the horizontal coordinate against which to plot log Q of any base B. Of course no such indicator exists, but the H_o function for aromatic primary amines[29] can be taken as a good measure of the protonating power of sulfuric acid. Bunnett and Olsen used as horizontal coordinate a hypothetical amine, X, of pK_{BH^+} zero and which obeys H_o perfectly, that is, a plot of log I against $-H_o$ is linear with slope 1.00.

By definition,

$$H_o = pK_{XH^+} - \log \frac{[XH^+]}{[X]}$$

Thus for the hypothetical indicator X,

$$H_o = - \log \frac{[XH^+]}{[X]}$$

and $\log Q_X = -H_o - \log[H^+]$.

Consider a real indicator B. Since $\log Q_X$ and $\log Q_B$ will be linearly related,

$$\log \frac{[BH^+]}{[B]} - \log [H^+] = (1-\phi)(-H_o - \log [H^+]) + \text{constant}$$

As the acid solutions become more dilute H_o approaches the pH of the aqueous solution or $-(\log[H^+])$. In water the first term on the R.H.S. of the above equation will be zero and the L.H.S. is equal to the thermodynamic pK_{BH^+}, standard state water. The constant in the above equation is thus equal to pK_{BH^+}. Adding $(H_o + \log[H^+])$ to each side then gives

$$\log \frac{[BH^+]}{[B]} + H_o = \phi(H_o + \log[H^+]) + pK_{BH^+} \qquad (1)$$

The pK_{BH^+} of base B is then evaluated as the intercept of the straight line plot of the L.H.S. of (1) against $(H_o + \log[H^+])$. The slope ϕ is a measure of the deviation of log I from H_o.

The basis of the Bunnett-Olsen treatment is that the logarithm of the activity coefficient ratio of base B is a multiple of that of a standard Hammett base X. They have shown that sub-

$$\log\left(\frac{f_{XH^+}}{f_X}\cdot\frac{f_B}{f_{BH^+}}\right) = \phi\log\left(\frac{f_{XH^+}}{f_X\cdot f_{H^+}}\right)$$

tracting $(\log f_{H^+})$ to each side and rearranging gives,

$$\log\left(\frac{f_{BH^+}}{f_B\cdot f_{H^+}}\right) = (1-\phi)\log\left(\frac{f_{XH^+}}{f_X\cdot f_{H^+}}\right) \qquad (2)$$

Indicators obeying the same acidity function must have equal values of ϕ which is really a proportionality constant between the logarithms of the activity coefficient ratios. In practice, however, these values are almost always within 0.1-0.2 units of one another.

It was observed experimentally in this work that log I varies linearly with mole % DMSO up to 80 mole % DMSO. Table IX contains the correlation coefficients r of the linear plots, log I vs. mole % DMSO, and the slopes of these straight lines for the indicators with non-overlapping spectra. Table X contains the same data for indicators which exhibited overlapping spectra. Correlation coefficients and slopes are given for both methods used to calculate log I, namely the Katritzky and the area method. In all cases where the Katritzky or FHK method was used, only values of log I between +1.00 and -1.00 were used to establish the linear plot. Where the area method was used to calculate log I only values between +0.80 and -0.80 were used.

TABLE IX. Least Squares Correlation of Log I Values with Mole % DMSO for Spectrally Non-overlapping Indicators.

No.	Indicator	Range of DMSO concentration (mole %)	Correlation Coefficient	Slope
1	2-aminopyridine	97.4-99.2	non-linear	--
2	2-amino-5-chloropyridine	93.4-98.2	non-linear	--
3	2-amino-3,5-dichloropyridine	74.4-87.4	non-linear	--
4	2-amino-3-nitropyridine	39.2-58.7	0.9998	0.087
5	2-amino-5-nitropyridine	35.0-53.6	0.999	0.083
6	2-amino-3-chloro-5-nitro-pyridine	20.1-34.6	0.9998	0.106
7	2-amino-3,5-dinitropyridine	7.79-22.1	0.9996	0.120
8	4-aminopyridine	93.4-98.2	non-linear	--
9	4-amino-3-nitropyridine	30.1-44.65	0.993	0.104
11	2-aminopyrimidine	87.4-96.95	non-linear	--
12	2-amino-4-chloropyrimidine	68.3-83.9	0.999	0.084
13	2-amino-4,6-dichloropyrimidine	44.3-61.9	0.999	0.080
14	2-amino-5-nitropyrimidine	20.2-32.6	0.9997	0.096
15	4-aminopyrimidine	68.4-87.65	0.997	0.088
16	4-amino-2-chloropyrimidine	44.3-62.8	0.998	0.075
17	4-amino-2,6-dichloropyrimidine	27.4-48.2	0.9999	0.082
18	4-amino-2-chloro-5-nitro-pyrimidine	7.79-30.0	0.998	0.073
19	2-amino-s-triazine	34.6-62.8	0.9996	0.060
20	1,7-dimethylguanine	29.8-59.0	0.9999	0.063
21	1,9-dimethylguanine	23.7-48.75	0.998	0.071

TABLE X. Least Squares Correlation of Log I Values with Mole % DMSO for Spectrally Overlapping Indicators.

No.	Indicator	DMSO conc. range (mole %)	Log I Values Estimated by K. Method		Log I Values Estimated by Area Method	
			Correlation Coefficient	Slope	Correlation Coefficient	Slope
23	2,8-dichloro-7-methyladenine	10.2-29.7	0.998	0.087	0.992	0.089
24	2-chloro-7-methyladenine	15.1-34.0	0.999	0.082	0.996	0.083
25	2-chloro-8-methoxy-7-methyl-adenine	24.8-45.9	0.999	0.075	0.999	0.072
26	7-methyladenine	29.85-60.8	0.998	0.062	0.999	0.062
27	2,8-dichloro-9-methyladenine	30.0-49.0	0.9998	0.072	0.9999	0.073
28	2-chloro-9-methyladenine	35.1-59.05	0.998	0.069	0.996	0.069
29	2-chloro-8-methoxy-9-methyl-adenine	50.0-71.4	0.998	0.073	0.999	0.074
30	9-methyladenine	58.1-81.4	0.995	0.067	0.998	0.065
31	2,3-dihydro-1H-5-oxoimidazo-(1,2-c)pyrimidine hydrochloride	0.26-15.2	0.998	0.090	--[a]	
32	1-methylcytosine	50.55-81.7	0.999	0.069	0.998	0.050
33	1-methyl-5-bromocytosine	29.7-44.3	0.992	0.086	0.998	0.085
34	1-methylisocytosine	17.7-35.2	0.9998	0.086	0.996	0.076
35	3-methylcytosine	7.83-22.0	0.999	0.089	--[a]	
36	3-methylisocytosine	17.5-39.0	0.999	0.084	--[a]	
37	2',3'-O-benzylidene-5'-O-tritylcytidine	40.0-67.5	0.996	0.052	--[a]	

[a] Change in area upon ionization is too small to allow a meaningful estimation of the ionization ratio by this method.

As can be seen from the tables the correlation coefficients are uniformly excellent. Non-linear plots were observed only for indicators ionizing at very high mole % DMSO. These observations suggest that log I values of the indicators must be linear functions of one another as the DMSO content is varied.

Deprotonation of an acid HA obeying an acidity function H_- may be represented by,

$$HA + OH^- \rightleftharpoons A^- + H_2O$$

Whence,

$$K_b = \frac{a_{A^-} \cdot a_{H_2O}}{a_{HA} \cdot a_{OH^-}} = \frac{K_{HA}}{K_w} \qquad (3)$$

K_w is defined as the autoprotolysis constant of water $\left(\frac{a_{H^+} \cdot a_{OH^-}}{a_{H_2O}}\right)$.

Taking logarithms of both sides of equation (3) and rearranging yields equation (4) below.[12]

$$pK_{HA} + \log \frac{[A^-]}{[HA]} = pK_w + \log[OH^-] - \log a_{H_2O} - \log \left(\frac{f_{A^-}}{f_{HA} \cdot f_{OH^-}}\right) \qquad (4)$$

For the Hammett acid HA, the L.H.S. of (4) will be equal to H_- by definition. The first three terms on the R.H.S. of (4) will be equal for two acids HA and HS, whose regions of ionization overlap in basic

DMSO/water. HS is an acid not necessarily obeying H_-. The equivalent of the Bunnett-Olsen treatment in basic media would thus require, from equation (2), that

$$-\log \left(\frac{f_{S^-}}{f_{HS} \cdot f_{OH^-}} \right) = (\phi+1) \; -\log \left(\frac{f_{A^-}}{f_{HA} \cdot f_{OH^-}} \right) \quad (5)$$

If HA and HS both obey H_- then their activity coefficient ratios will cancel and the arbitrary parameter ϕ in the above equation will be zero. Thus, ϕ, as in the original B.O. treatment is a measure of how the activity coefficient ratio of an indicator HS deviates from H_-.

Applying relation (5), it can be shown that

$$H_- \; - \; \log \frac{[S^-]}{[HS]} \; = \; -\phi \left(H_- - pK_w - \log[OH^-] \; + \; \log a_{H_2O} \right) \; + \; pK_{HS} \quad (6)$$

The thermodynamic pK_{HA} of any acid, referred to standard state water, may thus be determined using equation (6), whether the acid obeys H_- or not. Plots of the L.H.S. of (6) against the first term on the R.H.S. give straight lines of slope $-\phi$ and intercept pK_{HS}.

The B.O. method is subject to two major sources of error. Uncertainty in the acidity function H_- is reflected in the pK_{HA} calculated. Furthermore, the method assumes that the plots using equation (6) are linear over the range of compositions from those in which measurements of log I are made down to pure water. Of course, any variation in the slope of this line owing to errors in log I will cause an error in the intercept - the farther one must extrapolate to water, the greater the error. It was of interest to determine the magnitude of uncertainty

in pK_{HA}'s calculated by the B.O. method when different methods were used to estimate log I.. The spread in values obtained is discussed in Section B(4).

2. The Marziano-Cimino-Passerini Method (M.C.P. method)

This method was first developed for acid systems as was the B.O. method. The basis of the two methods is the same: the logarithms of the activity coefficient ratios for two given bases, ionizing in the same acidity range, are multiples of one another. Thus, the M.C.P. method suggests that for two bases X and B protonating at the same solvent composition,

$$\log\left(\frac{f_B \cdot f_{H^+}}{f_{BH^+}}\right) = n_B \log\left(\frac{f_X \cdot f_{H^+}}{f_{XH^+}}\right)$$

If X is considered to be the standard Hammett base, then n_B is equal to the term $(1-\phi)$ of equation (2) in the B.O. method.

Consider a weaker base C with which base B overlaps. Applying the above assumption,

$$\log\left(\frac{f_C \cdot f_{H^+}}{f_{CH^+}}\right) = n_C \log\left(\frac{f_B \cdot f_{H^+}}{f_{BH^+}}\right) = n_B \cdot n_C \log\left(\frac{f_X \cdot f_{H^+}}{f_{XH^+}}\right)$$

This may be applied to weaker and weaker bases over the whole range of acidity. Thus the logarithm of the activity coefficient ratio of any base may be related back to that of the standard base X. The term

$$- \log\left(\frac{f_X \cdot f_{H^+}}{f_{XH^+}}\right)$$

may be calculated as a function of the whole acidity range for a given set of overlapping indicators, usually those used to set up the H_o acidity function. This function is defined as the M_c function[181] and it is equivalent to the negative of the horizontal coordinate used in the B.O. plots if X obeys H_o. Thus,

$$M_c = - \log\left(\frac{f_X \cdot f_{H^+}}{f_{XH^+}}\right) \equiv -(H_o + \log[H^+])$$

Rather than using a hypothetical amine as horizontal coordinate, the M.C.P. method uses the activity coefficient ratio of an arbitrary real indicator, say 4-nitroaniline. The M_c function of this real indicator was calculated using the set of primary nitroaniline indicators used to define H_o.[29] Thus, although the numerical values of H_o are not used in the subsequent plots to obtain pK_{BH^+} of a given base B, the same ionization ratio data used to construct H_o are used to obtain M_c. Any weakness inherent in this basic data will be reflected in both methods. The pK_{BH^+} of B may be calculated from the equation,[181]

$$-\log Q_B = n_B M_c - pK_{BH^+}$$

B need not be structurally similar to the indicators used to establish M_c. The values of pK_{BH^+} for several different structural types of indicators calculated by the M.C.P. method were found to be in good agreement with those calculated using the Hammett procedure and did not depend on the choice of standard base used to define M_c.

The M.C.P. method has also been adapted to basic DMSO/water systems by Cox and Stewart.[12] It requires that equation (5) be true for two acids HA and HS ionizing in the same medium. Given a standard acid HA* and an acid HS which overlaps with it, equation (4) may be rewritten,

$$pK_{HS} + \log \frac{[S^-]}{[HS]} = pK_w + \log[OH^-] - \log a_{H_2O} - m^* \log \left(\frac{f_{A^*}^-}{f_{HA^*} \cdot f_{OH^-}} \right) \quad (7)$$

The M_c function is then defined as the last term of equation (7). Using 2,4,4'-trinitrodiphenylamine, whose pK_{HA} is firmly established in water, as the standard indicator HA*, the M_c function in basic DMSO/water was calculated using the aromatic amines which define the H_-^N function.[15]

The pK_{HS} of any given acid, ionizing in these media, may then be calculated using the following equation

$$(pK_w + \log[OH^-] - \log a_{H_2O}) - \log \frac{[S^-]}{[HS]} = m^* M_c + pK_{HS} \quad (8)$$

The acid HS need not obey H_-^N nor be structurally similar to an aromatic amine. Plots of the L.H.S. of equation (8) against M_c give straight lines of slope m* and intercept pK_{HS}. Indicators obeying the same acidity function were shown to have very similar m* values, with a variation of less than $\pm$ 0.1 units.

The pK_{HA} values of 80 indicators were recalculated by Cox and Stewart using both the B.O. and M.C.P. methods. In general, good agreement was found between the pK_{HA}'s determined by each of the two

methods and with those found by Hammett procedure.[12]

3. Calculation of the Acidity Constants of the Indicators Using Extrapolative Procedures

The pK_{HA}'s of all the indicators studied in this work were calculated using both the B.O.[30] and the M.C.P.[181] methods.

In the B.O. procedure, the pK_{HA}'s of the indicators were found using equation (6). Calculations were made by means of least-squares line-fitting on a Hewlett-Packard calculator. The most accurately known H_- function is the H_-^N function of Dolman and Stewart,[15,18] established in DMSO/water/0.011 M TMAH using primary anilines and diphenylamines. It was used in all the Bunnett-Olsen calculations, although any other H_- function may be used and gives comparable results.[12] Activity coefficients of water in aqueous DMSO at 25° were those of Lam and Benoit,[182] except that values between 95-100 mole % DMSO were obtained by interpolation[12] since the necessary data are not available. The concentration of hydroxide ion was kept constant at 0.011 M for all measurements. The value of pK_w at 25° has been taken as 13.996.[12] Table XI contains the values of the pK_{HA}'s for the aminopyridines and pyrimidines determined by the B.O. method, as well as the number of points used in the plots of equation (6) and the ϕ values obtained. The correlation coefficients of the B.O. plots are not significant when ϕ is close to zero[183] and have been omitted since they do not give an indication of the goodness of fit obtained. Table XII contains the same data for the nucleotide bases (overlapping spectra). The pK_{HA}'a were found using the "best" log I values, that is those estimated using

TABLE XI. Slopes, Correlation Coefficients and Intercept pK_{HA} Values as Calculated by the B.O. and M.C.P. Methods for Indicators with Non-overlapping Spectra.

No.	Indicator	No. of Points	B.O. Method		M.C.P. Method		
			ϕ	pK_{HA}	Corr. Coeff.	m*	pK_{HA}
Pyridines:							
1	2-amino	5[a]	-0.150	23.50	0.992	0.924	23.48
2	2-amino-5-chloro	7	-0.184	21.74	0.999	0.905	21.88
3	2-amino-3,5-dichloro	7	+0.010	20.87	0.991	1.037	20.65
4	2-amino-3-nitro	7	-0.195	16.65	0.9998	0.887	16.76
5	2-amino-5-nitro	5	-0.272	15.69	0.9996	0.803	15.84
6	2-amino-3-chloro-5-nitro	6	-0.062	14.84	0.999	1.009	14.99
7	2-amino-3,5-dinitro	7	-0.087	13.75	0.999	1.143	13.87
8	4-amino	8	-0.156	22.26	0.990	0.927	22.33
9	4-amino-3-nitro	4	-0.068	15.82	0.999	1.046	16.08
Pyrimidines:							
11	2-amino	7	-0.353	19.78	0.992	0.885	21.24
12	2-amino-4-chloro*	7	-0.308	18.20	0.9997	0.715	18.07
13	2-amino-4,6-dichloro*	5	-0.268	16.61	0.998	0.809	16.77
14	2-amino-5-nitro	6	-0.179	14.60	0.999	0.881	14.72
15	4-amino*	5	-0.265	18.53	0.996	0.724	18.18
16	4-amino-2-chloro*	6	-0.322	16.34	0.995	0.741	16.45
17	4-amino-2,6-dichloro*	8	-0.328	15.07	0.9995	0.760	15.29
18	4-amino-2-chloro-5-nitro	9	-0.407	13.33	0.996	0.608	13.36
19	2-amino-s-triazine	7	-0.542	14.91	0.998	0.492	14.94
Guanines:							
20	1,7-dimethyl	7	-0.515	14.91	0.9998	0.547	15.06
21	1,9-dimethyl	6	-0.462	14.46	0.998	0.617	14.65

[a] Values of log I at 98.73 and 99.19 mole % DMSO were not included in the B.O. calculations.

TABLE XII. Slopes, Correlation Coefficients and Intercept pK_{HA} Values Calculated Using the B.O. and M.C.P. Methods for Indicators with Overlapping Spectra

No.	Indicator	Method of Estimating Log I Values	No. of Points	B.O. Method		M.C.P. Method		
				ϕ	pK_{HA}	Corr. Coeff.	m*	pK_{HA}
Adenines:								
23	2,8-dichloro-7-methyl(*)	Area	12	-0.259	13.61	0.987	0.793	13.69
24	2-chloro-7-methyl(*)	Area	7	-0.313	14.02	0.989	0.797	14.24
25	2-chloro-8-methoxy-7-methyl	Area	6	-0.421	14.62	0.996	0.629	14.70
26	7-methyl	Area	6	-0.539	14.67	0.998	0.511	14.78
27	2,8-dichloro-9-methyl(*)	Area	4	-0.374	15.10	0.997	0.704	15.27
28	2-chloro-9-methyl(*)	Area	4	-0.406	15.61	0.992	0.667	15.79
29	2-chloro-8-methoxy-9-methyl(*)	Area	7	-0.353	16.66	0.999	0.714	16.80
30	9-methyl	K	7	-0.479	16.74	0.996	0.554	16.73
31	2,3-dihydro-1H-5-oxo-imidazo(1,2-c)pyrimidine hydrochloride(*)	K	8	-0.246	12.42	0.998	0.802	12.50
Cytosines:								
32	1-methyl(*)	K	7	-0.411	16.69	0.999	0.641	16.77
33	1-methyl-5-bromo	Area	6	-0.294	15.03	0.997	0.799	15.25
35	3-methyl(*)	FHK	6	-0.226	13.37	0.999	0.800	13.42
Isocytosines:								
34	1-methyl(*)	K	7	-0.294	14.22	0.999	0.775	14.36
36	3-methyl(*)	FHK	6	-0.315	14.28	0.999	0.734	14.37
37	2',3'-O-benzylidene-5'-O-tritylcytidine	K	5	-0.624	14.75	0.990	0.405	14.78

the method best suited to eliminate medium effects in overlapping spectra.

In the M.C.P. procedure, the pK_{HA}'s of the indicators were found using equation (8). The values of pK_w and concentration of hydroxide ion are the same as above. Here, as in the above procedure, the molarity-based values of log a_{H_2O} tabulated by Cox and Stewart[12] were used. M_c was taken from the tables of Cox and Stewart.[12] Tables XI and XII contain the pK_{HA}'s of all the indicators, calculated by the M.C.P. method. The correlation coefficients obtained from the plots of equation (8) are generally good, 0.990 or better, and are included, as are the slopes of the lines, m*.

4. Estimation of the Uncertainty in the pK_{HA} Values

By far the greatest uncertainty in the pK_{HA} values must stem from errors in the estimation of log I. Table XI contains the pK_{HA}'s of the compounds which exhibit non-overlapping spectra. Thus examination of this table will give an estimate in the uncertainty in the pK_{HA}'s introduced by the extrapolative procedure alone, since only the FHK method was used to calculate log I.

The value of the pK_{HA}'s calculated by the two extrapolative procedures give very similar results, not surprisingly, since both methods depend on the same body of ionization data - that used to establish the H_-^N scale. The values are closely similar, as can be seen from Table XI, and the difference between the B.O. value and the M.C.P. value for a given indicator is usually no greater than 0.1-0.2 pK units, although those calculated for 2- and 4-aminopyrimidine differ by 1.46

and 0.35 units respectively. Both compounds are weak acids requiring a lengthy extrapolation to the standard state with increased uncertainty in the intercept pK_{HA}. However, close agreement is often observed for such weak acids as 4-aminopyridine: B.O. 22.26, M.C.P. 22.33.

The ionization data for the indicators in Table XI are considered to be accurate. However, the ionization ratio data for the indicators in Table XII are subject to much more uncertainty and it is expected that this uncertainty will be reflected in the pK_{HA} values. To estimate this calculations of pK_{HA} values for 2,8-dichloro-7-methyladenine, 23, and indicator 31, the anchor compound, were made using the log I values contained in Tables II, IV and VII.

Table XIII compares the values of pK_{HA} found using different parts of the spectral curve to calculate log I for indicator 31 by the area method. The values of pK_{HA} vary somewhat with the choice of wavelength pair but the spread in value is only 0.32 pK units. The correlation coefficients of the M.C.P. method are not good when log I is estimated by areas, but excellent when estimated by the Katritzky method. Also ϕ and m* appear to vary considerably with the method chosen to estimate log I. There is a correlation, however, between the size of the deviation of these values from the average and the size of the deviation of the slope of log I vs. mole % DMSO from the average (Table III). When the area from 265 nm to 307.5 nm was used to estimate log I, the slope of the plot of log I thus determined against mole % DMSO was 0.074, much lower than the average of the slopes similarly obtained using different parts of the curve or the Katritzky method to obtain log I. Table XIII reveals that the ϕ and m* values corresponding to this set of log I values are both much less than average.

TABLE XIII. Values of Intercept pK_{HA} Calculated by the B.O. and M.C.P. Methods for Indicator 31 Using Values of Log I Estimated by Several Methods.

Method of Estimating Log I Values	B.O. Method		M.C.P. Method		
	ϕ	pK_{HA}	Corr. Coeff.	m*	pK_{HA}
Katritzky at 325 nm	-0.246	12.42	0.998	0.802	12.50
Area (295 nm ⟶)	-0.199	12.53	0.955	0.858	12.62
Area (265-307.5 nm)	-0.348	12.35	0.984	0.705	12.42
Area (307.5 nm ⟶)	-0.075	12.59	0.982	0.996	12.67

TABLE XIV. Values of Intercept pK_{HA} Calculated by the B.O. and M.C.P. Methods for 2,8-Dichloro-7-methyladenine Using Values of Log I Estimated by Several Methods.

Method of Estimating Log I Values	B.O. Method		M.C.P. Method		
	ϕ	pK_{HA}	Corr. Coeff.	m*	pK_{HA}
Katritzky at 295 nm	-0.138	13.77	0.998	0.897	13.84
Katritzky at 300 nm	-0.237	13.72	0.997	0.797	13.79
Katritzky at 305 nm	-0.240	13.70	0.995	0.790	13.76
FHK	-0.041	13.92	0.992	1.010	14.02
Davis and Geissman	-0.221	13.69	0.998	0.813	13.76
Area (260 nm ⟶)	-0.261	13.61	0.987	0.756	13.66
Area (270 nm ⟶)	-0.146	13.80	0.973	0.885	13.87

Similar observations were made for 2,8-dichloro-7-methyladenine (Table XIV). From Table VIII the average slope of log I vs. mole % DMSO is 0.094. All values of slope lie close to this except when log I values obtained by the FHK method are used, for which the slope is 0.108. The pK_{HA} values for the FHK method are also higher than the others. If these pK_{HA}'s are included the spread in pK_{HA} values is 0.41 units; if they are omitted the spread is only 0.26 units. Similarly, the ϕ and m* values of the FHK log I values are quite different from the rest (Table XIV). It should be recalled, however, that the FHK method has been used at a wavelength where there was a large contribution from the neutral form of the indicator.

From Table XIV it appears that the log I values estimated by the area, Davis and Geissman, or Katritzky method give very similar pK_{HA} values. The FHK method, however, should not be used at wavelengths where the neutral form of the indicator absorbs to a large extent. The values of log I estimated in such cases yield greater uncertainty in the pK_{HA} values calculated, because log I at very high or low degrees of ionization are subject to greater uncertainty. This is reflected in the divergent slopes of the plots of such log I values against mole % DMSO, and consequently in the pK_{HA}'s determined by extrapolative procedures, since the slopes of these plots are also dependent on values of log I at high or low degrees of ionization. Furthermore, from Tables XIII and XIV, the pK_{HA} values determined from log I values estimated by the area method do not appear to depend on the choice of wavelengths between which the area change is measured.

Table XV compares the pK_{HA} values for the acids whose log I values were estimated by the area and Katritzky methods. The spread in values is usually 0.2-0.3 pK units, as was concluded for the representative compounds discussed above. The spread in values is 0.1 units greater than for the indicators exhibiting non-overlapping spectra, but is not large considering the difficulty in obtaining accurate log I values from overlapping spectra. This spread appears to hold for the entire solvent composition range and not merely for indicators ionizing in media close to water in composition, as do the representative indicators 31 and 23. The size of this spread seems to depend on the accuracy with which log I is determined.

From Table XV, the correlation coefficients of the M.C.P. method and the ϕ and m* values are generally very similar whether the log I values were estimated by the area or the Katritzky method. The values of ϕ and m*, however, may vary considerably if there is a large degree of uncertainty in log I at high or low degrees of ionization. This has been noted for indicator 31 when log I was estimated by areas. The change in area upon ionization was not large and this led to errors in log I beyond the region of half-ionization.

Discrepancies in ϕ and m* values are also noted for the compounds 1-methylisocytosine, 1-methylcytosine and 1-methyl-5-bromocytosine where there was difficulty in obtaining reasonable log I values (see Appendix A). A considerable difference (1.32 pK units) is observed for 1-methylcytosine between pK_{HA} values calculated using log I values estimated by the area and Katritzky methods, the pK_{HA} determined from area log I values being the lower of the two.

TABLE XV. Values of Intercept pK_{HA} Calculated by the B.O. and M.C.P. Methods for Indicators with Overlapping Spectra Using Log I Values Estimated by the Katritzky and Area Methods.

Indicator No.	B.O. Method				M.C.P. Method					
	K		Area		K			Area		
	ϕ	pK_{HA}	ϕ	pK_{HA}	Corr. Coeff.	m*	pK_{HA}	Corr. Coeff.	m*	pK_{HA}
21	-0.462	14.46	-0.474	14.38	0.998	0.617	14.65	0.9998	0.614	14.59
24	-0.319	14.06	-0.313	14.02	0.995	0.783	14.26	0.989	0.797	14.24
25	-0.416	14.57	-0.421	14.62	0.996	0.672	14.79	0.996	0.629	14.70
26	-0.530	14.82	-0.539	14.67	0.998	0.520	14.92	0.998	0.511	14.78
27	-0.430	14.97	-0.374	15.10	0.999	0.647	15.15	0.997	0.704	15.27
28	-0.431	15.51	-0.406	15.61	0.993	0.639	15.68	0.992	0.667	15.79
29	-0.358	16.63	-0.353	16.66	0.995	0.710	16.78	0.999	0.714	16.80
30	-0.479	16.74	-0.495	16.57	0.996	0.554	16.73	0.995	0.535	16.56
32	-0.411	16.69	-0.663	15.45	0.999	0.641	16.77	0.988	0.360	15.46
33	-0.292	15.24	-0.294	15.03	0.986	0.806	15.47	0.997	0.799	15.25
34	-0.294	14.22	-0.391	13.93	0.999	0.775	14.36	0.990	0.670	14.06

C. Calculation of Indicator pK_{HA}'s Using the Hammett Procedure. The H_-^P Acidity Function.

1. Selection of the Indicators Used to Establish the H_-^P Function

Cox and Stewart have proposed that a given set of indicators form an adequate acidity function set if their m* values lie within a range of $m^*_{av} \pm 0.100$.[12] Applying this criterion, the aminopyridines form such a set, $m^*_{av} = 0.962 \pm 0.084$, if 2-amino-5-nitropyridine and 2-amino-3,5-dinitropyridine are omitted. The 2- and 4-aminopyrimidines, with the exception of 4-amino-2-chloro-5-nitropyrimidine, form another set, $m^*_{av} = 0.788 \pm 0.097$. A fair acidity function set is defined by the nucleotide bases, $m^*_{av} = 0.729 \pm 0.112$, if 7- and 9-methyladenine and 1,7-dimethylguanine are omitted. Since the m^*_{av} values of the aminopyrimidines and nucleotide bases are close, a new set may be made by appropriate selection of both types of indicator. This set, $m^*_{av} = 0.745 \pm 0.104$, was used to define the H_-^P function where P represents "pyrimidine" and "purine". The indicators selected are marked in Tables XI and XII by an asterisk (*).

2. Anchoring the H_-^P Function

Indicator 31 is a secondary imidazo-pyrimidone whose pK_{HA} in water at 25° has been determined as 12.6.[68] Even though 31 is a secondary amine, a plot of log I against mole % DMSO is parallel to those of 3-methylcytosine and 2,8-dichloro-7-methyladenine, both primary amines whose ionization overlaps with that of 31. Dolman observed similar parallelism between primary and secondary aromatic amines.[15]

The fully ionized spectrum of 31 was determined in 3 M sodium hydroxide, λ_A- max 308 nm but measurements were made at 320 nm, in

order to maximize the spectral change upon ionization and to make the contribution of the neutral form small. After determining the unionized spectrum in water at pH 10, log I in 0.011 M TMAH at 25° was calculated to be -0.390. The pH of 0.011 M TMAH has been determined potentiometrically at 25° to be 12.04,[12] from which the pK_{HA} of 31 is 12.43. This value rather than that of Ueda and Fox[68] was used in subsequent calculations. It is in excellent agreement with the pK_{HA} values determined by the B.O. (12.42) and the M.C.P. (12.50) methods (Table XII).

Anchoring of the scale was also attempted in the following manner. At 25°, 2,8-dichloro-7-methyladenine is measurably ionized in 0.1 M TMAH, log I = -0.696 (by areas). The pH of 0.1 M TMAH is equal to 12.83, determined using 2,4-dinitrodiphenylamine, pK_{HA} 13.84[15], as indicator. The pK_{HA} of the adenine in water is calculated to be 13.53, in good agreement with the value 13.61 calculated by the B.O. method. Plots of log I vs. mole % DMSO in 0.011 M and 0.1 M TMAH are parallel to each other but the average Δ log I is 1.12 when log I is determined by areas in both cases. The difference in pH is 0.79, however (12.83-12.04). In view of the non-linear variation of H_- with increasing base concentration,[43] the first method of anchoring was considered more reliable and was used in this work.

3. Calculation of pK_{HA}'s Using the Overlap Procedure

The Hammett activity coefficient postulate was found to hold for all the indicators chosen to define H_-^P. Knowing the thermodynamic pK_{HA} of acid HA, the pK_{HA} of acid HS overlapping with HA can be calculated using the following equation,

$$pK_{HS} = pK_{HA} + \left(\log \frac{[A^-]}{[HA]} - \log \frac{[S^-]}{[HS]}\right) \qquad (9)$$

In practice, the solutions in which two given indicators were both measurably ionized differed slightly from each other in DMSO composition. If such was the case for HA and HS above, where HS is the weaker acid, then log I_{HS} was appropriately corrected by the quantity,

$$(\Delta\text{mole \% DMSO}) \times m_{HS}$$

where m_{HS} is the slope of the plot log I_{HS} vs. mole % DMSO.

Where HS overlapped with several stronger acids HA, HB, ..., HZ, the pK_{HA} given is the weighted average of the pK_{HA}'s determined using (9) with HA, HB, ..., HZ, individually. Thus,

$$K_{HS} = \frac{n_A K_A + n_B K_B + \ldots + n_Z K_Z}{n_A + n_B + \ldots + n_Z} \qquad (10)$$

In equation (10) K_A is the acidity constant evaluated for HS by overlapping it with acid HA alone and n_A is the number of solvent compositions at which a (Δlog I) could be obtained for the two compounds.

Table XVI lists the indicators used to establish H_-^P together with values of pK_{HA} determined by equation (9) and by the B.O. and M.C.P. methods. The agreement between all three values for any given indicator is good, but the M.C.P. values appear to lie closer to the acidity function (AF) values for the weaker acids than do the B.O. values. The average spread in all three values is usually 0.2-0.4 pK units

TABLE XVI. The pK_{HA} Values of the Indicators Used to Establish the H_-^P Function.

No.	Indicator	pK_{HA} (AF)	pK_{HA} (B.O.)	pK_{HA} (M.C.P.)
31	2,3-dihydro-1H-5-oxoimidazo-(1,2-c)pyrimidine hydrochloride	12.43	12.42	12.50
35	3-methylcytosine	13.36	13.37	13.42
23	2,8-dichloro-7-methyladenine	13.64	13.61	13.69
24	2-chloro-7-methyladenine	14.17	14.02	14.24
34	1-methylisocytosine	14.37	14.22	14.36
36	3-methylisocytosine	14.47	14.28	14.37
17	4-amino-2,6-dichloropyrimidine	15.34	15.07	15.29
27	2,8-dichloro-9-methyladenine	15.48	15.10	15.27
28	2-chloro-9-methyladenine	16.12	15.61	15.79
16	4-amino-2-chloropyrimidine	16.51	16.34	16.45
13	2-amino-4,6-dichloropyrimidine	16.55	16.61	16.77
29	2-chloro-8-methoxy-9-methyladenine	17.01	16.66	16.80
32	1-methylcytosine	17.34	16.63	16.69
12	2-amino-4-chloropyrimidine	18.25	18.20	18.07
15	4-aminopyrimidine	18.30	18.53	18.18

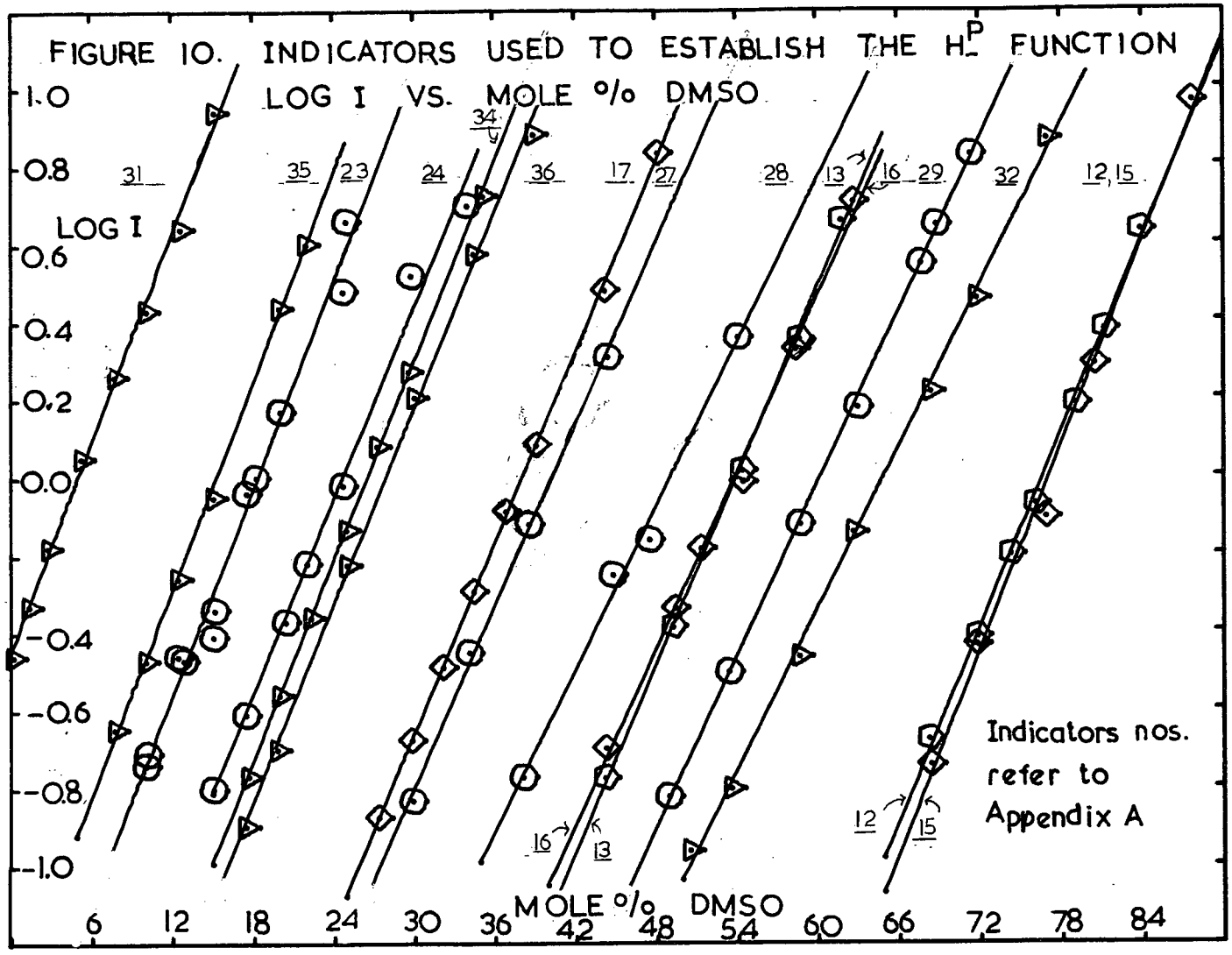


FIGURE 10. INDICATORS USED TO ESTABLISH THE H_-^P FUNCTION
LOG I VS. MOLE % DMSO
LOG I
1.0
0.8
0.6
0.4
0.2
0.0
-0.4
-0.6
-0.8
-1.0
31
35
23
24
34
36
17
27
28
13
16
29
32
12,15
16
13
12
15
6
12
18
24
30
36
42
48
54
60
66
72
78
84
MOLE % DMSO
Indicators nos. refer to Appendix A
Legend:
aminopyrimidones
adenines
4-aminopyrimidine
2-aminopyrimidine

and increases as the acid strength of the indicators decreases, that is, as the medium in which the degree of ionization is measured becomes farther removed from water. The largest differences occur for 2-chloro-9-methyladenine and 1-methylcytosine, 0.51 and 0.71 pK units, respectively. The m* values of these indicators, 0.667 and 0.641 respectively, are close to the limit of the range of m* values defined for the H_-^P function, $m^*_{av} = 0.745 \pm .100$ so that these two compounds may not "obey" H_-^P very well.

Figure 10 contains the plots of log I against mole % DMSO for the indicators used to establish the H_-^P function.

4. Calculation of H_-^P. Evaluation of Acidity Constants Using H_-^P

Using the AF pK_{HA}'s in Table XVI, H_-^P was calculated from the following equation,

$$H_- = pK_{HA} + \log \frac{[A^-]}{[HA]} \qquad (11)$$

The values of H_-^P for the system DMSO/water/0.011 TMAH are listed in Table XVII together with the indicators (by number) used to calculate H_-^P in a given solution. The value is usually an average of two or more indicators. Values in solutions greater than 58.7 mole % DMSO are less accurate since there is some uncertainty regarding the I values of 1-methylcytosine (32). Values in solutions of compositon greater than 74.4 mole % DMSO were determined using only one indicator and should be regarded as approximations.

The pK_{HA}'s of compounds have often been evaluated by assuming their

TABLE XVII. H_-^P Values for the Ternary System DMSO/water/0.011 M TMAH Determined Using the AF pK_{HA} Values of the Aminopyrimidines and Aminopurines in Table XVI.

Mole % DMSO	H_-^P Value	Indicator No.
0.3	11.98	31
1.4	12.11	31
2.9	12.26	31
5.4	12.49	31
7.8	12.70	31,35
10.1	12.89	31,35,23
12.5	13.11	31,35,23
15.1	13.32	31,35,23,24
17.5	13.58	23,24,34,36
18.25	13.64	23
20.0	13.80	35,23,24,34,36
22.0	13.96	35,24,34
24.8	14.13	23,24
25.0	14.25	23,34,36
27.3	14.45	34,17
29.85	14.66	23,34,36,17,27
32.2	14.85	17
34.0	14.94	24,27
34.5	15.05	36,17
35.2	15.10	34
36.9	15.25	17
38.2	15.35	28
39.0	15.38	36,17,27
44.3	15.80	17,27,28,16,13
47.6	15.96	28
48.2	16.17	17
49.5	16.19	16,13,29
50.6	16.38	32

TABLE XVII. (Continued)

Mole % DMSO	H_-^P Value	Indicator No.
51.4	16.33	16
54.5	16.53	28,16,13
58.7	16.88	16,13,29,32
61.9	17.21	13
62.8	17.20	16,29,32
67.7	17.56	29
68.4	17.58	29,32,12,15
71.9	17.84	29,32,12,15
74.4	18.05	12
76.2	18.18	12
77.0	18.20	32,15
79.1	18.44	12
80.5	18.59	15
81.7	18.63	12
83.9	18.89	12
87.6	19.27	15

adherence to a particular acidity function. If an indicator "obeys" H_-^P, say, then a plot of its log I values against H_-^P, according to (11) will give a straight line with slope 1.00 and pK_{HA} equal to H_-^P at half-ionization.

Table XVIII lists the aminopyrimidines and purines which were not used in the Hammett procedure, together with the slopes of plots of log I against H_-^P and the AF pK_{HA}'s, namely the H_-^P value of a solution in which the indicator is half-ionized. The slopes are by no means unity, but those indicators with slopes 0.9-1.1 had AF pK_{HA}'s in fair agreement (within 0.5 pK units) with the B.O. and M.C.P. values. Deviations were greater the farther the solutions in which the indicators ionized were removed from pure water. Other indicators with slopes less than 0.9 had AF pK_{HA}'s as much as 1.0 unit greater than the extrapolated values.

It was of interest to plot the log I values of the aminopyridines against H_-^N since the acidity function defined by these compounds would lie close to it. The m* value of the anilines and diphenylamines used to define H_-^N is 1.091[12] and that of the aminopyridines is 0.962. With two exceptions the slopes are all lower than unity and the AF pK_{HA} estimated for the aminopyridines is usually much greater than the extrapolated values, being as large as 1.6 units greater for 2-aminopyridine. It is noted that 2,2'-dipyridylamine has a slope of 0.85[15] for its plot of log I against H_-^N, in general agreement with those observed for the other aminopyridines (Table XIX).

TABLE XVIII. The pK_{HA} Values of Some Aminopyrimidines and Aminopurines Determined by Plotting Log I Values Against H_-^P.

No.	Indicator	Correlation Coefficient	Slope	pK_{HA} (Value of H_-^P at Half-ionization)	pK_{HA} (B.O.)	pK_{HA} (M.C.P.)
14	2-amino-5-nitropyrimidine	0.999	1.10	14.47	14.60	14.72
18	4-amino-2-chloro-5-nitropyrimidine	0.997	0.80	13.62	13.33	13.36
20	1,7-dimethylguanine	0.9997	0.82	15.88	14.91	15.06
21	1,9-dimethylguanine	0.999	0.90	15.13	14.46	14.65
25	2-chloro-8-methoxy-7-methyladenine	0.998	0.91	15.13	14.62	14.70
26	7-methyladenine	0.9999	0.80	15.72	14.67	14.78
30	9-methyladenine	0.998	0.86	17.85	16.74	16.73
33	1-methyl-5-bromocytosine	0.9985	1.11	15.15	15.03	15.25

TABLE XIX. The pK_{HA} Values of the Aminopyridines Determined by Plotting Log I Values Against H_{-}^{N}.

No.	Substituent	Correlation Coefficient	Slope	pK_{HA} (Value of H_{-}^{N} at Half-ionization)	pK_{HA} (B.O.)	pK_{HA} (M.C.P.)
1	2-amino	0.9990	0.81	25.07	23.50	23.48
2	2-amino-5-chloro	0.9996	0.87	23.37	21.74	21.88
3	2-amino-3,5-dichloro	0.9920	1.01	20.80	20.87	20.65
4	2-amino-3-nitro	0.9998	0.85	17.47	16.65	16.76
5	2-amino-5-nitro	0.9998	0.79	16.74	15.69	15.84
6	2-amino-3-chloro-5-nitro	0.9998	0.95	15.00	14.84	14.99
7	2-amino-3,5-dinitro	0.9995	1.07	13.64	13.75	13.87
8	4-amino	0.9966	0.88	23.69	22.26	22.33
9	4-amino-3-nitro	0.9994	0.95	16.05	15.82	16.08
	2,2'-dipyridylamine	--	0.85[15]	19.91[15]	18.56[12]	18.53[12]

From the above discussion it appears that the AF pK_{HA} of a given indicator may vary widely from those values obtained by the extrapolative procedures if it does not obey the given acidity function closely. In the discussions that follow the pK_{HA}'s of the indicators studied were taken to be those obtained by the B.O. procedure (Tables XI and XII) since the computations for the B.O. method are less tedious than for the M.C.P. method and the results of the two methods are comparable.

5. Comparison of the H_-^P Acidity Function to the H_-^N Function

As can be seen from Figure 11, the H_-^P acidity function, defined by aminopyrimidines and purines, rises much less steeply than the H_-^N function when plotted against mole % DMSO. Both functions converge at low mole % DMSO, as expected, since H_- is equivalent to pH in pure water.

The difference in the two functions in any water-DMSO mixture must be due to differences in the activity coefficient ratios of the indicator acids and those of their anions in the mixture. Thus,

$$H_-^N - H_-^P = -\log\left(\frac{f_{A^-} \cdot f_{HP}}{f_{P^-} \cdot f_{HA}}\right)$$

where subscripts A and P refer to aniline and aminopyrimidine-type acids respectively. For the left hand side of the above equation to be positive, the quotient on the right hand side must be less than unity. Either f_{A^-} being less than f_{P^-} or f_{HP} being less than f_{HA} could account for this difference.

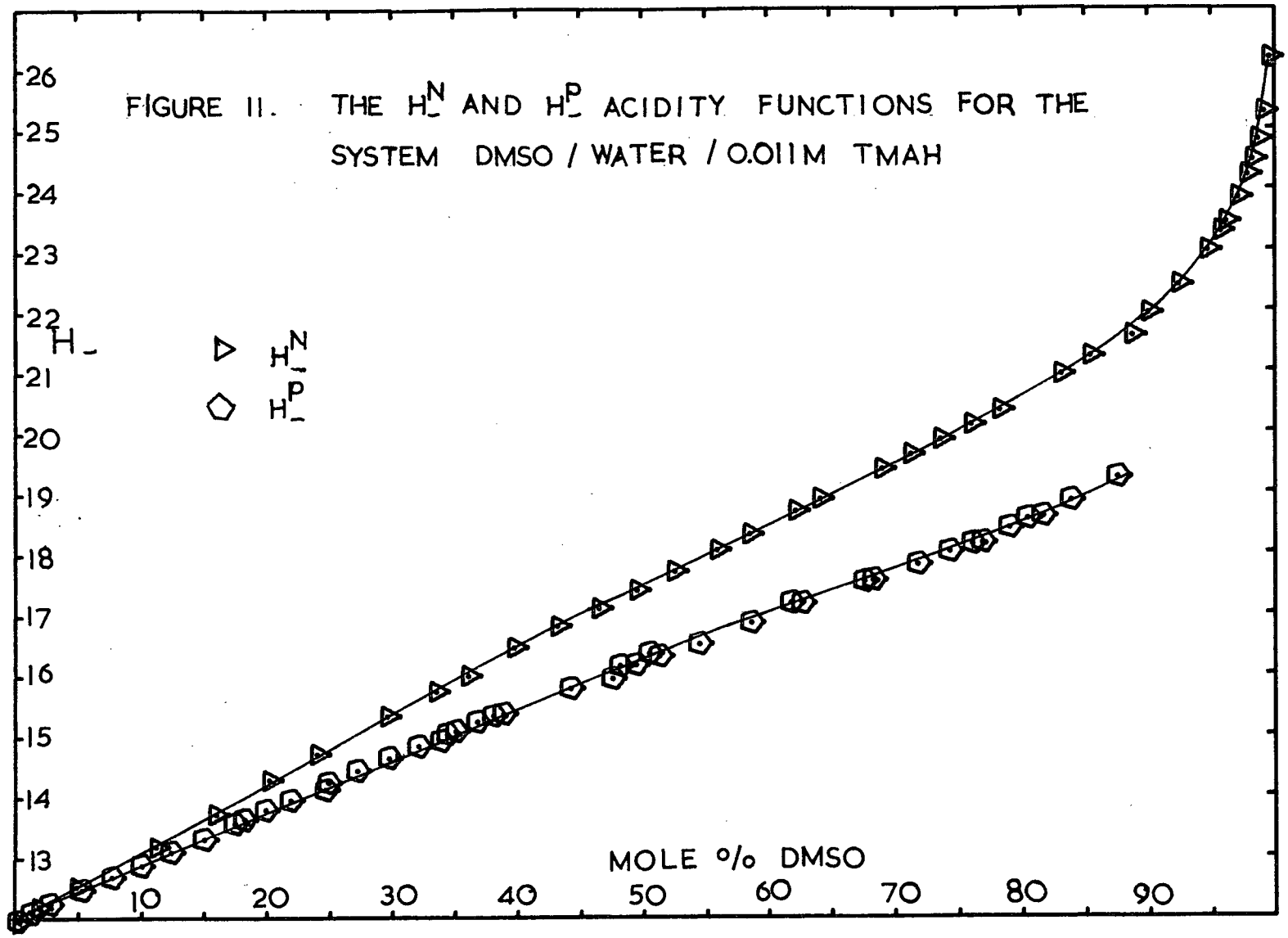
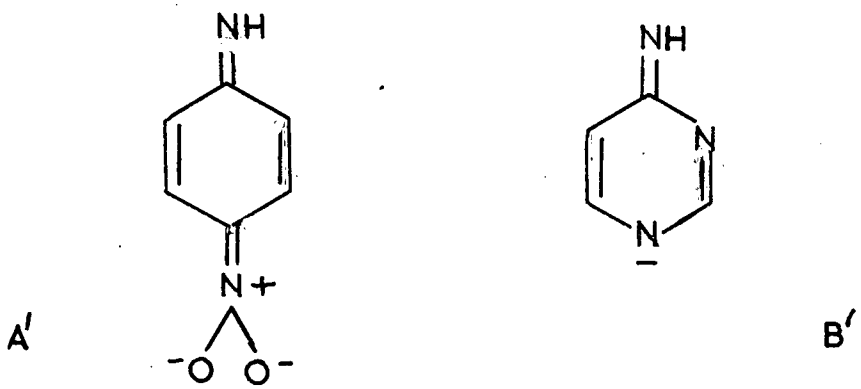


FIGURE II. THE H_-^N AND H_-^P ACIDITY FUNCTIONS FOR THE SYSTEM DMSO / WATER / 0.011M TMAH
H_-
H_-^N
H_-^P
MOLE % DMSO
26
25
24
23
22
21
20
19
18
17
16
15
14
13
10
20
30
40
50
60
70
80
90

Consider the two compounds 4-nitroaniline (A) and 4-aminopyrimidine (B) which ionize between 54.63-71.35[15] mole % DMSO and 68.43-80.52 mole % DMSO respectively. The B.O. pK_{HA}'s of these compounds are 18.11 for the aniline[12] and 18.53 for the aminopyrimidine. The regions of ionization of these two compounds overlap at DMSO compositions where H_-^N is greater than H_-^P.

The anilines and aminoheterocycles are expected to give anions with highly delocalized charge. A solvent change from water to DMSO does not destabilize anions of delocalized charge to nearly the same extent as occurs with anions of localized charge. Indeed, large polarizable, delocalized anions may even be stabilized by such a change in medium.[34] The activity coefficient of the delocalized type of anion should clearly be less than that of the localized type. In agreement with this notion the H_- function for phenols, which form anions in which the charge is largely localized on oxygen, is nearly flat when plotted against mole % DMSO.[18] The less steep rise of the H_-^P function might thus be explained if greater localization of anionic charge exists in the anion of 4-aminopyrimidine than in the anion of 4-nitroaniline; that is, if f_A^- be less than f_P^-. A para nitro group has a very large acid-strengthening effect on aniline acidity. The apparent substituent constant for the para nitro group, as calculated by Dolman and Stewart[15] was much greater than the usual σ^- value for a nitro group, $\sigma^- = 1.27$.[45] Dolman explained this large acid-strengthening effect by a large degree of dispersion of the anionic charge to the nitro group, resulting in increased delocalization of charge for the whole ion (A). It was found in this work that the apparent substituent constant of a 4-aza

group is equal to its normal σ_p value,[92] that is, it did not prove necessary to use σ^- constants for 2- or 4-aza substituents. This suggests that the aza substituent works primarily by induction and that there is less formal delocalization of charge by resonance, i.e. structures such as B' below are less important in the anion of 4-aminopyrimidine. Thus, it appears that the aminopyrimidine anion has, in fact, a more localized charge than the anilide ion leading to f_p- being greater than f_A-



For f_{HP} to be smaller than f_{HA} the solubility of aminopyrimidines and purines in DMSO should be very much greater than the solubility in water relative to the nitroanilines. It was observed experimentally in this work that the aminopurines in particular were nearly insoluble in water but reasonably soluble in DMSO. Unfortunately no data are available on the relative solubilities of anilines and aminopyrimidines and purines.

The steepness of the plot of the H_- function for a given class of aminoheterocycles against mole % DMSO may be estimated by the magnitude of Stewart and Cox's m* values.[12] It appears from consideration of the m* values for aminopyridines, m* = 0.962, pyrimidines, m* = 0.788, purines, m* = 0.729 and that of 2-amino-s-triazine, m* = 0.492, that

H_- decreases as the number of aza groups in the ring to which the amino group is attached increases.

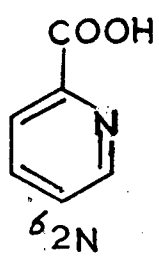
It is interesting to note that the presence of a carbonyl group, as in the cytosines, does not necessitate a new acidity function being used, although an oxygen atom might be expected to further delocalize the anionic charge. Nor does the presence of an imidazole ring require a new function for the aminopurines, within the limits of error assigned to m*. This points to the imidazole ring being much less important than the pyrimidine ring in stabilizing the purine anion.

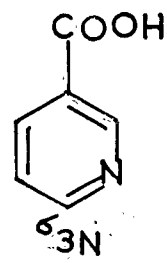
D. Correlation of Structure with Acidity for Aminopyridines and Aminopyrimidines

1. Correlation of pK_{HA} Values of Unsubstituted Aminopyridines and Aminopyrimidines with Aza Substituent Constants

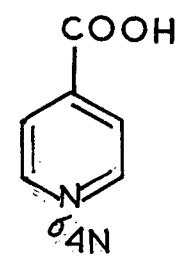
The heteroaromatic nucleus may be considered a substituted benzene, the endocyclic nitrogens being called aza substituents. One of the objects of this work was to determine the aza substituent constants, hereafter abbreviated σ_N, that correlate the acidities of the unsubstituted aminopyridines and pyrimidines and of 2-amino-s-triazine.

Primary substituent constants are determined from the ionization constants of the appropriately substituted benzoic acids.[4,184] Thus primary σ_N values can be calculated from dissociation constants of the pyridine carboxylic acids shown below.





nicotinic acid



isonicotinic acid

Such an evaluation was performed recently by Blanch.[185] In water at 22° these acids participate in the tautomeric equilibrium illustrated in Figure 12 and so the measured ionization constants K_{a1} and K_{a2} cannot be used directly to estimate σ_{4N}. Only K_N should be so used, and it may be shown that,[45,51]

$$K_N = \frac{K_{a1} \cdot K_{a2}}{K_2}$$

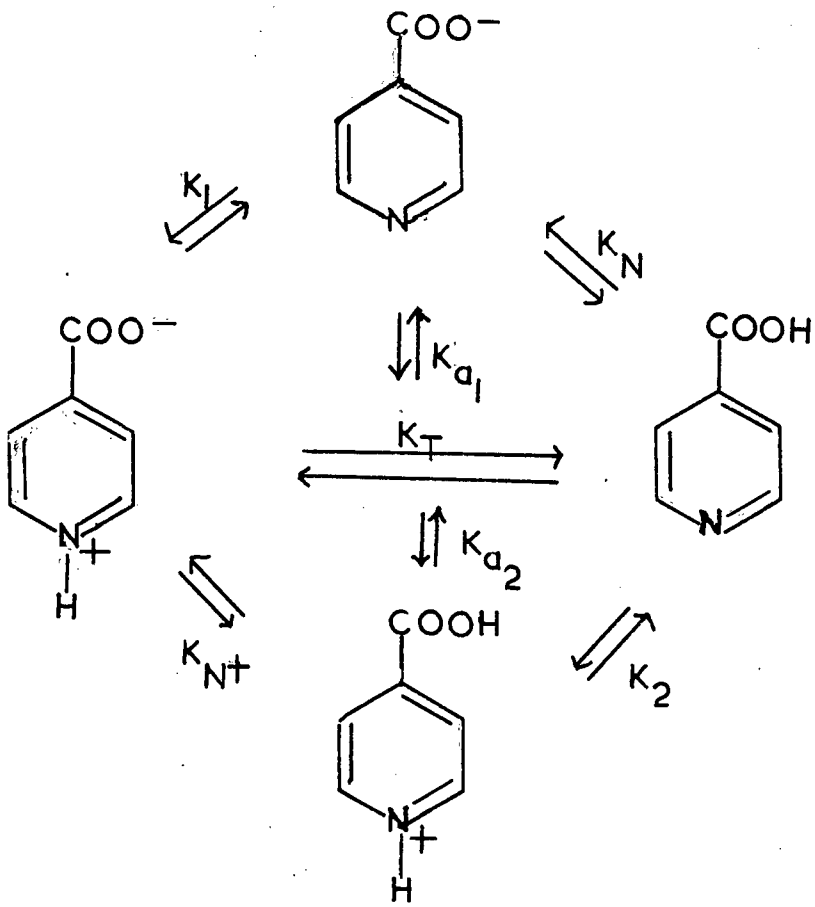
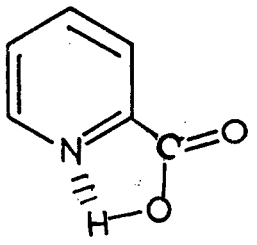


FIGURE 12. TAUTOMERIC EQUILIBRIA OF ISONICOTINIC ACID (REF. 45)

For evaluation of σ_{4N}, K_2 was taken to be the dissociation constant of the methyl ester of isonicotinic acid, i.e. the σ_p value of the -COOH group was taken to be equal to that of the $-COOCH_3$ group. The values obtained by Blanch[185] for σ_{2N}, σ_{3N} and σ_{4N} are listed in Table XX. The abnormally low value of σ_{2N} was explained by invoking intramolecular H-bonding between the COOH group and the endocyclic nitrogen[185,92,73] as illustrated below. The neutral molecule would thus be stabilized relative to the anion and the acidity would decrease.



When the ionization constants of the required benzoic acid cannot be evaluated, a substituent constant may be calculated from reaction rates, using a reaction whose ρ value is known. Such σ values are often called secondary substituent constants.[184] Because of the uncertainty in Blanch's σ_N values caused by the presence of tautomeric equilibria, the aza substituent constants were recalculated by Deady and Shanks[92] using the basic hydrolysis rates of methyl pyridine carboxylates in 85% methanol at 25°, and a ρ value of 2.26, established for the basic hydrolysis of methyl benzoates using the same reaction conditions. The values for the aza substituent were $\sigma_{2N} = 0.75$, $\sigma_{3N} = 0.65$ and $\sigma_{4N} = 0.96$. They are in good agreement with the σ_N values obtained previously using data for the basic hydrolysis of ethyl benzoates in 88% ethanol at 30°[51] (Table XX).

TABLE XX. Substituent Constants of the Aza Group.

Substituent Constant	2N	3N	4N	Ref.	Reaction
σ	0.08	0.45	0.76	185	ionization of pyridine carboxylates in water at 22°
σ	0.81	0.62	0.93	51	basic hydrolysis of ethyl pyridine carboxylates in aqueous ethanol at 30°
σ	0.75	0.65	0.96	92*	basic hydrolysis of methyl pyridine carboxylates in 85% methanol at 25°
σ^+	0.73	0.57	1.13	186	basic hydrolysis of pyridyldimethylcarbinyl chlorides in methanol at 25°
σ^-	0.58	0.76	1.26	187	Wolff-Kishner reduction of diarylketone hydrazones
σ^o	0.81	0.72	0.95	91	hydrolysis of methyl pyridine acetates in aqueous acetone at 25°
σ_R^o			0.24	188	intensity of IR stretching frequency at 1600 cm^{-1}

* σ_N values used in this work.

The additivity of aza substituent constants has been demonstrated by Deady and Shanks[93] using the basic hydrolysis rates of six methyl diazine carboxylates in 85% methanol at 10°. Excellent correlation with σ_N, previously determined,[92] was observed with a ρ value of 2.18, even if the aza groups were adjacent to each other. This additivity was further confirmed by Chan and Miller[189] using the nucleophilic substitution rates of chlorodiazines with p-nitrophenoxide ion. The additivity of aza substituent constants is not surprising because no steric interaction is possible between the aza substituent and the reacting side chain or another substituent on the nucleus.

Using Deady and Shanks' values of σ_{2N} and σ_{4N},[92] a plot of pK_{HA} was made against σ_N using the Bunnett-Olsen pK_{HA} values of 2- and 4-aminopyridine, 2- and 4-aminopyrimidine and 2-amino-s-triazine. The additivity of the σ_N values was assumed. Figure 13 illustrates the plot obtained. The plot gave a straight line with an excellent correlation coefficient, r = 0.9996, and with a ρ value of 4.99. The intercept or pK_o value (the pK_{HA} of aniline) was 27.2, in excellent agreement with the value of 27.3, estimated by Dolman and Stewart.[15]

Estimates of the values of the aza substituent constants for ortho, meta and para positions, σ_{2N}, σ_{3N} and σ_{4N}, respectively, have been made by earlier workers and are tabulated by Jaffé.[51] There has been some pessimism concerning the existence of a constant set of σ_N values owing to the large spread in σ_N values previously found. For example, values of σ_{4N} ranged from 0.20 in the nuclear quadrupole resonance frequencies of chloroazabenzenes to 1.6 in the basic hydrolysis of ethyl azabenzoates

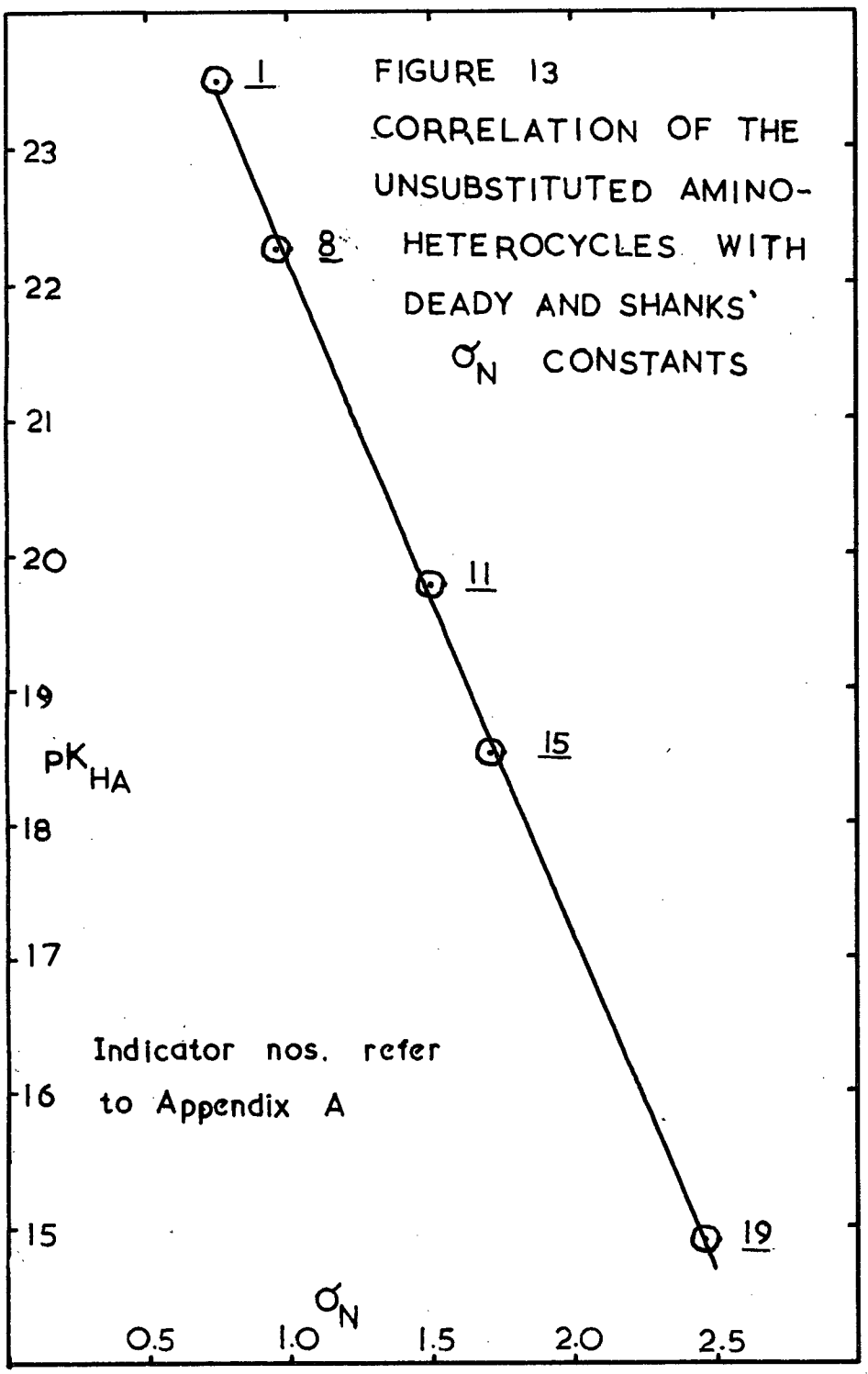


FIGURE 13
CORRELATION OF THE UNSUBSTITUTED AMINO-HETEROCYCLES WITH DEADY AND SHANKS' σ_N CONSTANTS

in 88% dioxane.[51] However, in comparing literature σ_m values to his newly derived σ_m^+ constants, Brown[47] found that large discrepancies between the values for a given substituent frequently occurred when σ_m was a secondary substituent constant. This may account for some of the variability observed for the aza constant, since many were estimated using reaction rates.[51]

It has been suggested that these discrepancies in σ_N arise because σ_N may depend on the extent of solvation of the heteroaromatic nitrogen atom in a given reaction.[186] Deady and Shanks have suggested that σ_{2N} may be solvation dependent in DMSO/water mixtures.[94] They remeasured the basic hydrolysis rates of methyl pyridine carboxylates in 60% and 80% aqueous DMSO (by volume) at 25°. (original conditions-85% methanol).[92] The values of σ_{3N} and σ_{4N} were within 0.04 of those originally determined, but the values of σ_{2N}, 0.60 and 0.64, respectively, were about 0.10 units lower than the original value of 0.75. Brown has suggested that discrepancies of ± 0.1 may be expected for substituent constants determined in mixed solvents.[184] Thus the σ_{2N} values determined in aqueous DMSO may be within experimental error of the value determined in 85% methanol. No such solvation dependency was observed for the pK_{HA}'s of the aminoheterocycles studied in this work since their pK_{HA}'s could be well-correlated with Deady and Shanks' original σ_N values.

The σ value of a substituent may vary if the entropy (of activation or reaction) is much different from those for other members of the series of compounds studied. A constant entropy change is sometimes considered a requirement for the Hammett equation to be obeyed,[46,4] although this view has been criticized by Johnson.[45] Such a variation

has been reported for σ_N^+ by Fischer et al.,[186] when determining σ_N^+ values from the basic hydrolysis rates of pyridyldimethylcarbinyl chlorides in methanol at 25.5° (Brown's ρ value of -4.82[47] for the hydrolysis of cumyl chlorides in methanol at 25° was used to calculate the σ_N^+ constants, Table XX). The values of σ_N^+ and σ_N should be close together for a -R substituent like the aza group. Agreement between σ_{2N}^+ and σ_{2N}, and σ_{3N}^+ and σ_{3N} is good, but σ_{4N}^+ (1.13) is much greater than σ_{4N} (0.96). Calculation of $\Delta S^\dagger$ for the hydrolysis of all three aza derivatives revealed that $\Delta S^\dagger$ for the 4-substituted compound is much more negative than for the others. Brown has attributed the anomalous σ value for the charged group $-NMe_3^+$ to a different $\Delta S^\dagger$ from other members of the cumyl chloride series, since a charged substituent might be expected to have a marked effect on the ordering of the solvent molecules surrounding it.[47c] Fischer attributed the altered $\Delta S^\dagger$ of the 4-aza derivative to a greater degree of solvation, which increases the electron-withdrawing ability of the 4-aza group and hence the value of σ_{4N}^+ over σ_{4N}:

No such discrepancy was observed for 4-aminopyridine or 4-aminopyrimidine when the pK_{HA}'s determined in this work were plotted against the original σ_{4N} values.[92] Thus in the solvent mixtures employed in this work, the aza substituent constant appears to be independent of solvation whether it is located ortho or para to the reacting side chain.

Can the aminopyridines and aminopyrimidines studied in this work really be regarded as substituted aza-anilines? If the ρ values for the acid ionization of anilines and aminoheterocycles are identical, it would suggest that the aza substituent can be regarded as a "normal" ring

substituent and that the aminopyridines and pyrimidines are really substituted anilines.

Dolman found a ρ value of 4.07[15] for the acid ionization of substituted diphenylamines. By plotting the pK_{HA} values of diphenylamines and anilines against those of the correspondingly substituted phenols, he obtained separate but parallel lines for the diphenylamines and anilines. Therefore the ρ value for the acid ionization of anilines may also be taken to be 4.07.

The diphenylamine pK_{HA}'s plotted by Dolman were determined using the Hammett overlap procedure. Using the same diphenylamines, but with the pK_{HA} values calculated according to the Bunnett-Olsen method of Cox and Stewart[12] a new plot was made of pK_{HA} against σ. A straight line was obtained with correlation coefficient r = 0.994 and ρ value 3.95. A plot was also made using only meta and para substituted anilines without nitro substituents, namely 3,4,5-trichloroaniline, 3,5-dichloroaniline, 3-cyanoaniline, 3-chloroaniline and 3,4-dichloroaniline. Plotting the B.O. pK_{HA}'s of these anilines against σ gave a straight line of correlation coefficient r = 0.970 and ρ value 3.92. Although the correlation coefficient is poor, the slope is identical to that obtained using the diphenylamines. The ρ values are, however, lower than that obtained with the aminoheterocycles, ρ = 4.99.

A ρ value determined using only a few substituted compounds from a plot spanning a narrow range of either pK_{HA} or σ values is subject to error.[190] The ρ value for diphenylamines was established using six compounds spanning a range of 3.0 pK units and that of the anilines using five compounds spanning 2.5 pK units, whereas the plot of the

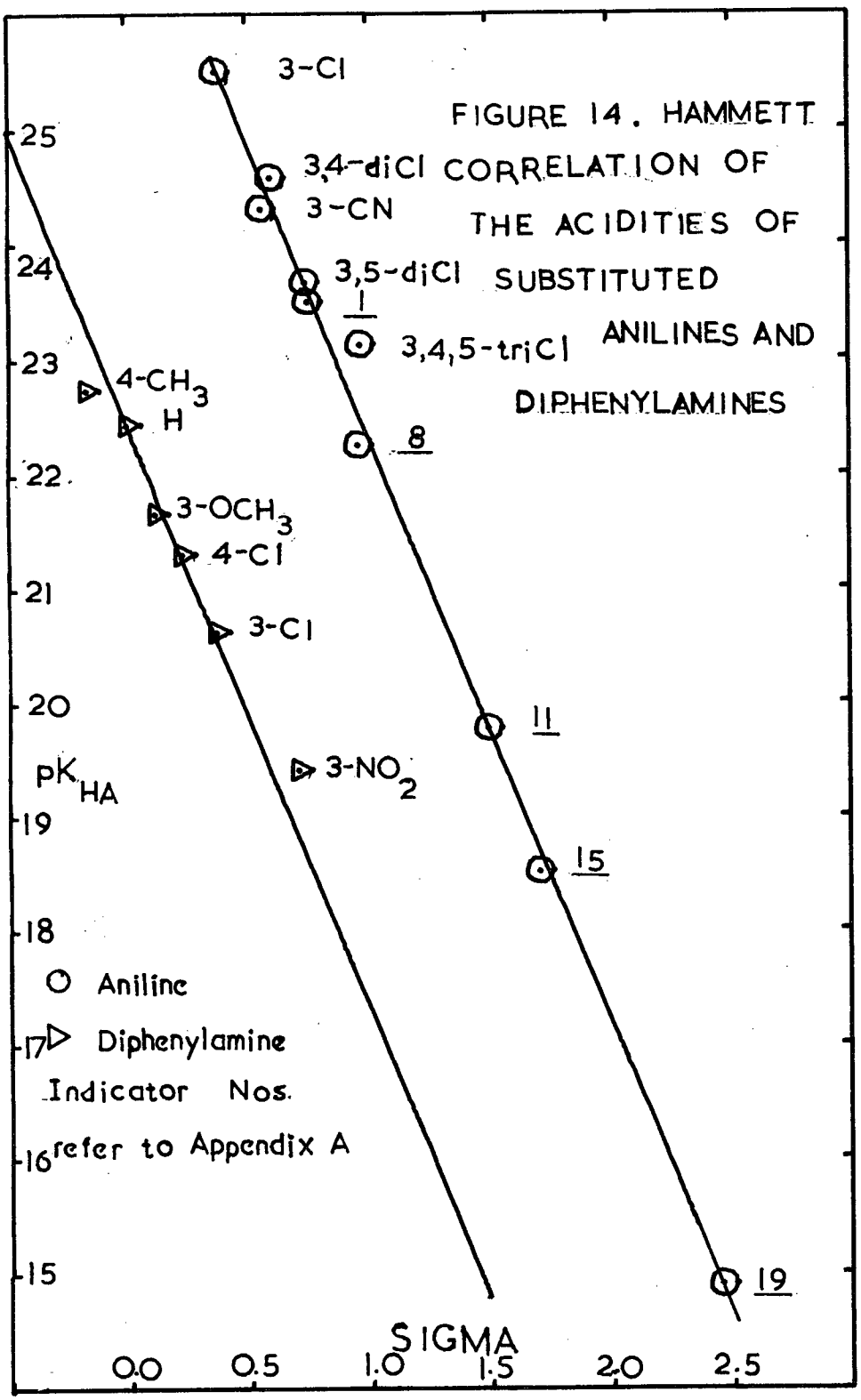


FIGURE 14. HAMMETT CORRELATION OF THE ACIDITIES OF SUBSTITUTED ANILINES AND DIPHENYLAMINES

five aminoheterocycles spanned a range of nearly 9 pK_{HA} units. The B.O. pK_{HA} values of the five substituted anilines actually fall close to the extrapolated plot of pK_{HA} against σ_N for the aminoheterocycles. A straight line was obtained when the pK_{HA}'s of these anilines and the five heterocycles were plotted together against σ (correlation coefficient r = 0.997 and intercept pK_o = 27.4, Figure 14). The ρ value of 5.10 obtained from this plot is close to the ρ of 4.99 established using only the aminoheterocycles, and the pK_o (the pK_{HA} of aniline) is close both to Dolman and Stewart's value of 27.3[15] and to the pK_o of the previous plot, pK_o = 27.2. A line of slope 5.10 may also be drawn through the points for the diphenylamines accommodating most of the points. This suggests that the sensitivity of anilines and diphenylamines to deprotonation is greater than previously believed.

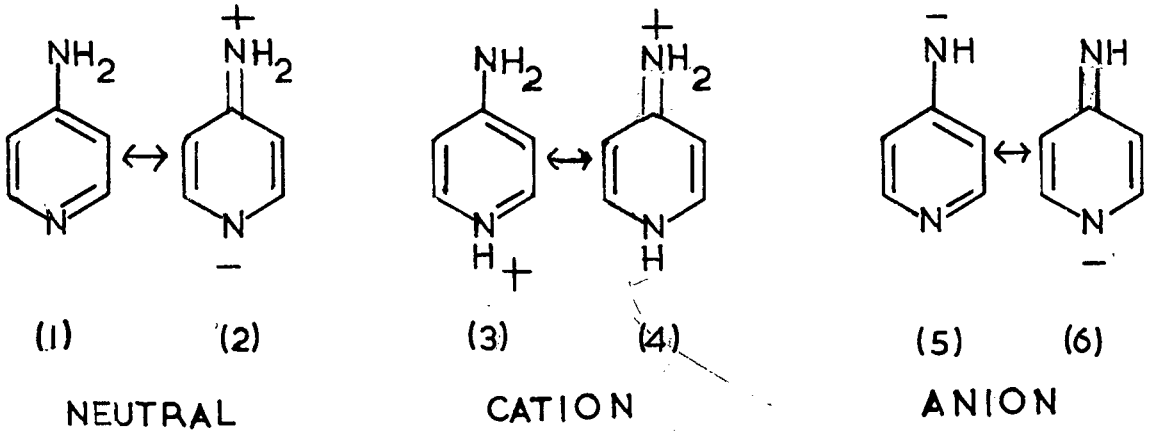
The fact that the points for the aminoheterocycles and for the anilines fall on the same straight line when pK_{HA} is plotted against σ supports the assumptions that the former compounds may be treated as substituted anilines and that Deady and Shanks' σ_N values are the correct σ_N values for the aza substituent. Introduction of an aza group into the benzene nucleus does not change the sensitivity of the amino group to deprotonation.

The pK_{HA} of the very weak acid, 3-aminopyridine may thus be estimated using Deady and Shanks' value for σ_{3N}, 0.65.[92] From the Hammett plot of the anilines and aminoheterocycles, ρ is 5.10 and pK_o is 27.4. Thus, the pK_{HA} of 3-aminopyridine is estimated to be 24.1.

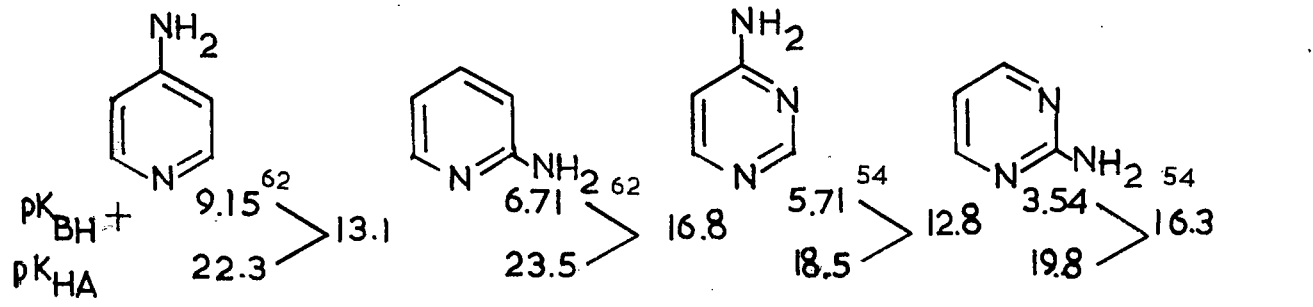
The ρ value of 5.10 is much greater than the value for the ionization of anilinium ions, ρ = 2.81[191] or 2.89,[192] and of comparable magnitude

to ρ values calculated for the protonation of pyridines, $\rho^{o} = 6.01$ [60] or $\rho = 5.77$.[51] This greater sensitivity of anilines to deprotonation has been explained by increased delocalization of amino group charge into the aromatic ring in the anion, whereas in the anilinium ion no such delocalization is possible.[15]

Introduction of a nitro group ortho or para to the amino group greatly increases this delocalization of charge in the anilide ion. The pK_{HA} of 4-nitroaniline, for example, does not fall on the Hammett plot established using the anilines and aminoheterocycles when $\sigma = 0.78$[45] or $\sigma^{-} = 1.27$[45] is used for the nitro substituent constant. Using the B.O. pK_{HA} of 18.11[12] for 4-nitroaniline, the apparent substituent constant for the para nitro group is 1.82 in the ionization of anilines. The acidities of the aminoheterocycles, however, are well correlated by normal σ constants and do not require the use of Szmant and Harmuth's σ_{N}^{-} constants.[187] This suggests that delocalization of anionic charge in the aminoheterocycles is not nearly as great as in nitroanilines and that resonance structures, such as (6) shown below for the anion of 4-aminopyridine cannot be much more important in the anion than similar structures in the neutral molecule.



This is in contrast to the accepted explanation of the high basicity of 4-aminopyridine. Its high basicity is explained by the stabilization of the cation by resonance forms (4) above; similar forms in the neutral molecule are less stable owing to charge separation.[54] Furthermore, the greater acidity and basicity of 4-aminopyridine relative to 2-aminopyridine can be explained by the importance of forms (4) and (6) in the cation and anion respectively. Forms such as (2) in 2-aminopyridine do not involve as large a charge separation as they do in 4-aminopyridine, whereas in the anion and cation no charge separation is involved. Hence, both the anion and cation of 4-aminopyridine will be relatively more stabilized with reference to the neutral molecule than will the anion and cation of 2-aminopyridine. This could account for the higher acidity and basicity of 4-aminopyridine relative to 2-aminopyridine. The greater acidity and basicity of 4-aminopyrimidine relative to 2-aminopyrimidine may be similarly explained.



It will be recalled that the H_-^P function for aminopyrimidines and the H_- function for the aminopyridines, as indicated by the average of the m* values, rose less steeply than did the H_-^N function when these were plotted against mole % DMSO. This less steep rise of H_-^P could be explained by less extensive delocalization of anionic charge in the aminopyrimidines than in the anilines, usually a nitroaniline, ionizing at the same medium composition. This was taken as evidence for the

relative unimportance of resonance structure (6) in the aminopyrimidine anion.

The evidence in this work suggests that the aza group in the 2 or 4 position exerts its electrical effect on exocyclic amino group acidity primarily by induction rather than by resonance. This may be demonstrated by examining the relative contributions of inductive and resonance effects to σ_{4N}. To a first approximation,[45]

$$\sigma_I = \sigma_{3N}$$

$$\sigma_R = \sigma_{4N} - \sigma_{3N}$$

Using Deady and Shanks' σ_N^o [91] and σ_N [92] values (Table XX) the resonance contributions to σ_{4N} and σ_{4N}^o were found to be 0.31 and 0.23 respectively, in agreement with $\sigma_R^o = 0.24$, as estimated by Katritzky from the intensity of IR stretching bands for amines at 1600 cm^{-1}.[188]

The azonium group, or the protonated aza group, exerts its electrical effect primarily by induction rather than by resonance because of its positive charge. Using the mean σ constants for σ_m = 2.09 and for σ_p = 2.34,[185] the resonance contribution to σ_p for the azonium group is estimated to be 0.25. This value is nearly identical to σ_R^o for a 4-aza substituent and not much smaller than the resonance contribution of 0.31 to σ_{4N}.

In contrast, the resonance contribution to the substituent constant of the para nitro group is estimated as 1.11, using σ_p^- = 1.82 as calculated in this work and σ_m = 0.71,[45] very much greater than the corresponding resonance contribution in σ_{4N}.

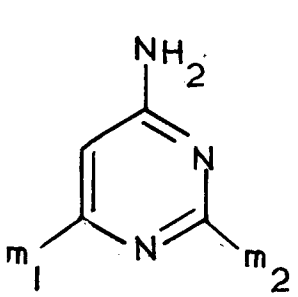
2. Transmission of Substituent Effects Through the Heterocyclic Nucleus

(a) Evidence from the Acidities of Aminoheterocycles: The evaluation of σ_N by Deady and Shanks[92] was based on the assumption that transmission of substituent effects through a heterocyclic nucleus is equal to that through the benzene nucleus. Johnson[45] has shown that an equality between the ρ values of a benzene and a corresponding heterocyclic series is a necessary consequence of the additivity of substituent effects. In other words if the aza groups do not perturb the electrical effect of other substituents on the same nucleus, the total substituent effect will be the sum of the aza σ values plus the sum of σ values of the other substituents. If such additivity does not hold for a given reaction, then ρ for the six-membered heterocycles studied will not be equal to the ρ value for the ionization of the substituted anilines. Thus a substituted pyridine or pyrimidine could not be treated as an aniline containing aza and other substituents. Jaffé[51] has given examples of reactions where the ρ values of a pyridyl series are both greater and less than in the corresponding benzene series. However, one series whose ρ value is 0.6 as large as that of the benzene series consists only of three compounds so that the ρ value found may not be accurate.

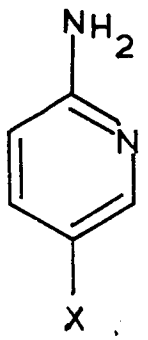
On the other hand Deady and Shanks demonstrated the additivity of σ_N constants using the basic hydrolysis rates of methyl diazine carboxylates.[93] The substituent effects of two or more aza groups in a given nucleus are additive even when the aza groups are adjacent to each other.

It was shown in this work that the pK_{HA}'s of unsubstituted aminoheterocycles are well correlated, using Deady and Shanks' σ_N [92] values and assuming the additivity of aza substituent effects. Furthermore the ρ value of these aminoheterocycles is equal to the ρ value of substituted anilines. Thus the electrical effect of a given endocyclic nitrogen is unaffected by the presence of other nitrogens in the ring. In the pyrimidine and triazine systems, the aza groups are meta to each other. In these systems perturbation of the effects of one aza group by another would occur by induction but not by resonance. Thus the present work suggests no perturbation of aza substituent effects occurs by induction. Deady and Shanks' observation that the effects of two aza groups ortho to each other are independent of each other suggests that aza groups will not perturb each other's effects by induction or resonance, since both -I and -R effects would be felt ortho to an aza group.

If another kind of substituent, such as a halogen, lies ortho or para to an aza group, the powerfully -I, -R endocyclic nitrogen atom may alter the σ value of the substituent. Jaffé has pointed out the severe complications that are possible if the electrical effects of a substituent X are perturbed by the presence of an aza group.[51] Thus positions m_1 and m_2, meta to the amino group in 4-aminopyrimidine (1) are not equivalent to each other, nor to the meta position in aniline, since m_1 is ortho to one nitrogen and para to another and m_2 is ortho to two nitrogens.

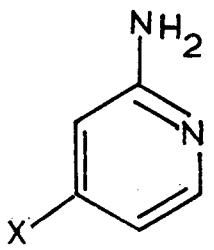


(1)



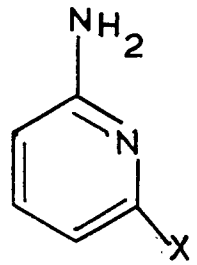
$\sigma_{\beta,p}$

(2)



$\sigma_{\gamma,m}$

(3)



$\sigma_{\alpha,m}$

(4)

In 2-amino-5-X-pyridine (2) the X group is meta (β) to the aza group and para to the amino group and one may thus refer to its substituent constant as a $\sigma_{\beta,p}$ constant. The σ constants for the two meta positions in 2-amino-4-X- (3) and 2-amino-6-X-pyridine (4) may thus be labelled $\sigma_{\gamma,m}$ and $\sigma_{\alpha,m}$, respectively. If the chloro group is not affected by the aza group it will, of course, be correlated by σ_p in 2-amino-5-X-pyridine and by σ_m in both of the meta-substituted pyridines with $\rho = 5.10$. However, if the X group is affected by the aza group then $\sigma_{\beta,p}$ will not be equal to σ_p and $\sigma_{\alpha,m}$ will be different from $\sigma_{\gamma,m}$ and not equal to the σ_m value for X. A correlation using Hammett's sigma constants would give a ρ value different from 5.10.

To test the effect of aza groups on other ring substituents, the pK_{HA}'s of substituted 2-amino and 4-aminopyrimidines were plotted against Hammett's substituent constants.[45] (Sufficient data were not available to treat the aminopyridines in the same way.) All compounds containing substituents ortho to the amino function were omitted from the correlations as were those containing nitro groups ortho or para to the amino group, since the nitro substituent constant varied with the number of other electronegative substituents on the ring.[15] Thus only compounds containing chloro substituents were considered.

If aza groups do not perturb the electrical effects of halogen groups, then the ρ values obtained for the 2-aminopyrimidines and the 4-aminopyrimidines, plotted separately, should lie close to ρ = 5.10. (In the correlations, the aza groups were considered "constant substituents" according to Jaffé's constant substituent concept.[46]) Each plot contained only three points so that the ρ values obtained are probably somewhat uncertain.[190]

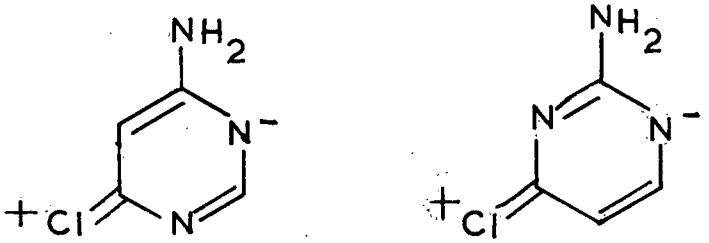
For the 2-aminopyrimidines a straight line was obtained r = 1.00, ρ = 4.28. For the 4-aminopyrimidines a straight line was also obtained, r = 0.988, ρ = 4.68. Judging by these lowered ρ values, the effect of the halogen group does appear to be somewhat damped by the presence of aza groups.

In 2-aminopyrimidine the para position is meta to the aza groups and the meta positions are equivalent, ortho to one aza group and para to the other. In 4-aminopyrimidine no para substitution is possible. The C-2 meta position lies ortho to both aza groups and the C-6 meta position is equivalent to the meta position in 2-aminopyrimidine, namely ortho to one aza group and para to the other.

Substituting a chloro group at C-2 in 4-aminopyrimidine increases the acidity (decreases the pK_{HA}) by 2.19 pK units. Substituting a meta chloro group in 2-aminopyrimidine increases the acidity by only 1.58 units; a second meta chloro group increases the acidity a further 1.59 units. The electrical effects of these meta chloro groups are additive as expected since their location relative to the aza groups is the same. Introduction of a second chloro group at C-6 in 4-amino-2-chloropyrimidine increases the acidity by 1.27 units only, much less than a

group at C-2, but nearly equivalent to the effect of a meta chloro group in 2-aminopyrimidine as expected since its location relative to the two aza groups is the same.

Thus it appears that a (-I,+R) substituent such as the chloro group has its electrical effects more perturbed by a 4-aza group than by an 2-aza group. A 4-aza group may encourage electron-donation from the chloro group by resonance structures such as those below, thereby decreasing its total electron-withdrawing effect and thus its σ_m value. The -R effects rather than the -I effects of an aza group appear to be more effective at altering the electrical effects of a +R substituent such as the chloro group. If the -I effects predominated, one would expect the σ_m value of the C-2 chloro group in 4-aminopyrimidine to be much less than that observed, since it lies ortho to two aza groups.



Another approach may be used to test the independence of ring substituents and aza groups. If the halogen and aza substituent constants are additive, the points for the substituted aminopyrimidines and pyridines should fall close to the straight line obtained by plotting pK_{HA} vs. σ using the anilines and unsubstituted aminoheterocycles.

All compounds containing substituents ortho to the amino function were omitted from the correlations as were those containing nitro groups; only compounds containing chloro substituents were considered.

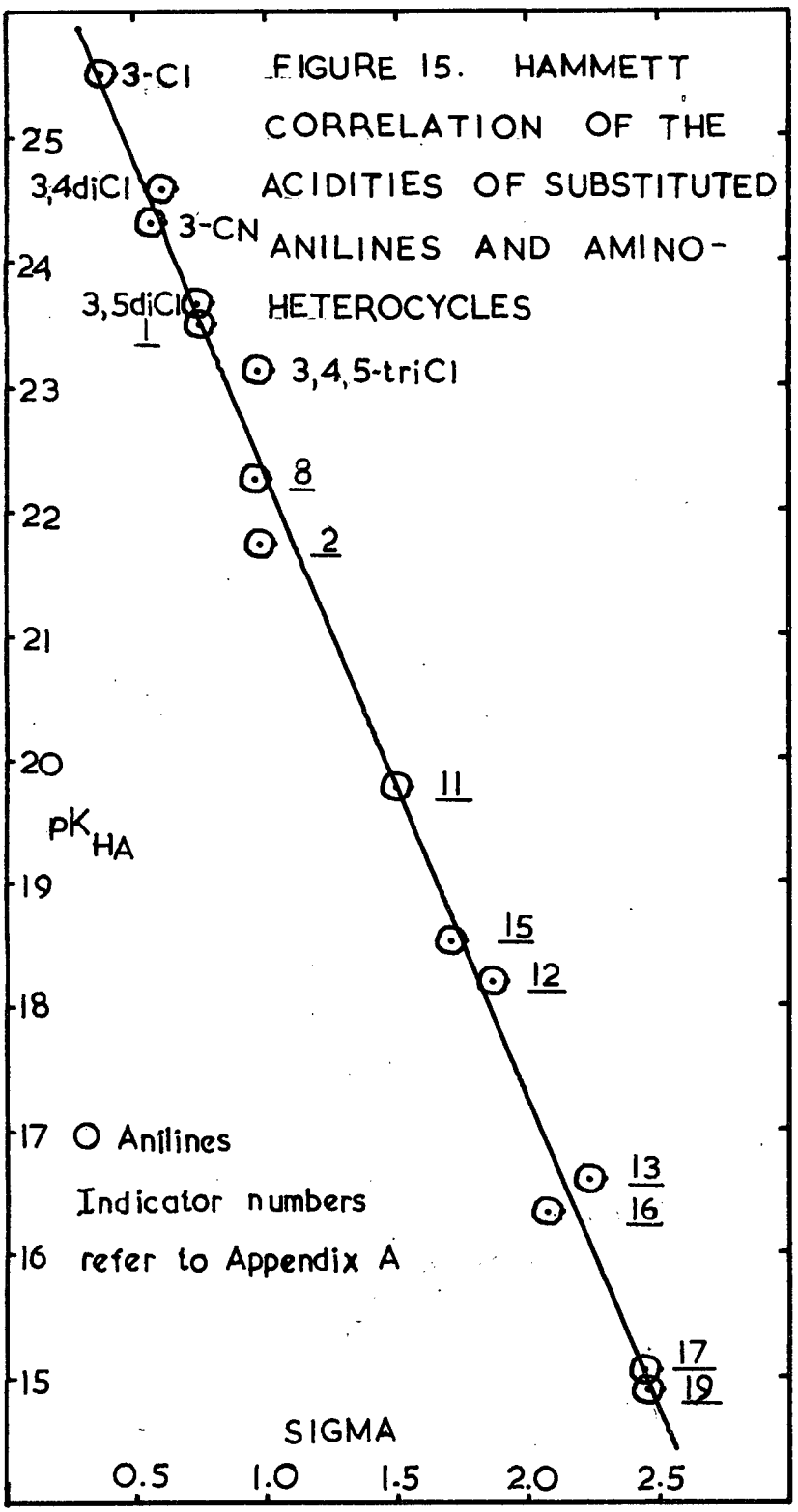


FIGURE 15. HAMMETT CORRELATION OF THE ACIDITIES OF SUBSTITUTED ANILINES AND AMINO-HETEROCYCLES

Values of σ_{2N} and σ_{4N} were those of Deady and Shanks.[92] Values of σ_m = 0.37[45] and $\sigma\rho$ = 0.23[45] were used for the chloro group depending on its location relative to the amino group regardless of the location relative to the aza group.

As can be seen from Figure 15 the points for the substituted aminoheterocycles are well accommodated by the straight line ρ = 5.10, previously established for the anilines and aminoheterocycles. A straight line drawn through all the points had correlation coefficient, r = 0.995, ρ = 5.00, and pK_o = 27.3. This ρ value is in excellent agreement with the value obtained using unsubstituted aminoheterocycles. These facts suggest that the perturbation of the chloro group by an aza group is small and that the electrical effects of halogen and aza substituents are additive, or independent of each other, to a first approximation.

(b) Evidence from the Basicities of Aminoheterocycles: To further investigate the transmission of substituent effects through a heterocyclic nucleus relative to a benzene nucleus, the basicities of aminopyridines and pyrimidines were examined, using pK_{BH^+} values available in the literature. In the previous sections the aza group was regarded as a substituent in a benzene ring; the reacting side chain was the exocyclic amino group. Since protonation of aminoheterocycles occurs exclusively on the endocyclic nitrogen atom,[54-57] the aza group must now be considered to be the reaction site with the amino group as a substituent.

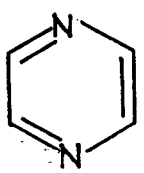
The protonation of pyridines in water at 25° was found by Jaffé to have a ρ value of 5.77[51] and by Russian workers, a value of 5.70.[190] These values are comparable to the reaction constants for electrophilic substitution reactions at a ring carbon. The pK_{BH^+} of a substituted pyridine is given by the equation,

$$pK_{BH^+} = pK_o - \sigma\rho = 5.21 - (5.77)\sigma \qquad (1)$$

The effective substituent constant $\bar{\sigma}$ in the reaction under consideration is given by

$$\bar{\sigma} = \frac{pK_o - pK_{BH^+}}{\rho} \qquad (2)$$

In equation (1), the ρ value of 5.77[51] was used and pK_o, the pK_{BH^+} of pyridine itself, was taken to be 5.21.[60] The pK_{BH^+} values of three aminopyridines studied in this work were calculated using equation (1) and the values obtained compared to those experimentally observed. The pK_{BH^+} values of pyrazine and of unsubstituted pyrimidine were also calculated using Deady and Shanks' σ_N values. The results are tabulated below.



pyrazine

TABLE XXI. The pK_{BH^+} Values of Some Selected Heterocyclic Compounds.

Compound	pK_{BH^+} calc.	pK_{BH^+} found	T
4-aminopyridine	9.01	9.17[60];9.15[62]	25°
3-aminopyridine	6.13	6.04[60];6.01[62]	25°
4-amino-3-nitropyridine	4.92	5.05[62]	25°
pyrazine	-0.03	0.7[58]	--
pyrimidine	1.76	1.30[57];1.31[55a]	20°

For both pyrazine and pyrimidine a statistical correction of log 2 was added to the calculated pK_{BH^+}, since both compounds have twice as many sites available for protonation as pyridine.

The agreement between the values of pK_{BH^+} calculated for the aminopyridines is good, within ± 0.15 pK unit. The agreement for pyrazine and pyrimidine, especially pyrazine, is not as good.

The protonation reaction of pyridines was reinvestigated by Fischer et al.[60] to verify the high ρ value and to examine resonance interactions between the -R aza group and other +R or -R substituents on the pyridine nucleus. They plotted the pK_{BH^+} values of six meta-substituted pyridines against σ^o, obtaining a straight line with ρ^o value 6.01 in fairly good agreement with Jaffé's value of 5.77. (Wepster[50] has suggested that σ^o constants be used to establish ρ values since σ^o does not incorporate minor resonance contributions included in the original Hammett σ values.) For meta substituents a good correlation

is obtained using σ_m constants, implying that there is little interaction between these substituents and an aza group. However, +R substituents, such as the amino and methoxy groups, lying para to the aza group appear to interact with it to the extent of requiring σ rather than σ^o values for correlation. The apparent substituent constants for the amino group and methoxy group were -0.65[60] and -0.23[60] respectively. (σ_p for the amino group is -0.66[45] and for the methoxy group -0.27[45]). This interaction is not so pronounced as to require σ^+ for correlation, as in the case of protonation of pyridine-N-oxides.[193] A σ^+ constant for the amino group might have been expected, since greater through-resonance between the amino group and the aza group in the cation was used by Albert[54] to explain the high basicity of 4-aminopyridine.

An effective σ constant was calculated for the para chloro group by Fischer.[60] It will be recalled that an aza group appears to decrease the acid-strengthening effects of a chloro group if they are para to one another. This interaction, although weak, occurs via a resonance interaction between the +R chloro substituent and the -R aza group. A very slight interaction was also observed by Fischer.[60] A para chloro group was better correlated using $\sigma_p = 0.23$ [5] rather than $\sigma_\rho^o = 0.27$[60] in the protonation of substituted pyridines.

The pK_{BH^+} of 4-aminopyridine calculated using Hammett's σ constants in equation (1) was in good agreement with the experimental value. Using Hammett's σ_p constants for the chloro and methoxy group the pK_{BH^+} values of 4-chloropyridine and 4-methoxypyridine were calculated according to equation (1) to give pK_{BH^+} 3.88 and 6.77, respectively, in good agreement with the experimental values, 3.83 and 6.58.[60] Thus no

resonance interaction between the aza group and a +R substituent is evident from the protonation of pyridines; at least it is no greater than the resonance contribution incorporated into Hammett's original σ constants.

For a nitro group para to the pyridine aza group, Fischer has calculated an apparent σ_p value of 0.63, close to the value of σ_I = 0.64[45] rather than to the Hammett σ_p value of 0.78.[45] Fischer has interpreted this as a reduction of the σ_R contribution to the apparent substituent constant of the nitro group. One might then expect a similar reduction in the -R 4-aza substituent, in pyrazine. Using equation (2) and substituting ρ^o = 6.01,[60] pK_o = 5.21 and pK_{BH^+} pyrazine 0.7,[58] the apparent σ_{4N} value of the aza substituent is estimated to be 0.75, less than the σ_{4N} value of 0.96.[92] This could also be interpreted as a reduction of the σ_R contribution to σ_{4N}. Fischer argues that an aza group may enhance the σ_R effect of a +R substituent, but inhibit the σ_R effect of a -R substituent.

Using equation (1) the pK_{BH^+} of 4-nitropyridine and 4-cyanopyridine were calculated using Hammett's σ constants.[45] The calculated value of 4-nitropyridine was 0.71 as opposed to the observed value of 1.39[60] and for 4-cyanopyridine the values were 1.40 and 1.86.[60] These values and the values for pyrazine (Table XXI) are in the direction one would expect if the σ_R value of a -R substituent were reduced. Why an aza group would have such an effect on a -R substituent is not clear.

To further examine the interaction between an aza group and a +R substituent lying para to it, the basicities of some 4-aminopyrimidines were calculated using equation (1) and compared to those observed

experimentally. 4-Aminopyrimidines may be considered pyridines with a 4-amino and 3-aza substituent since they protonate at N_1.[54]

The pK_{BH}^+ values of four 4-aminopyrimidines substituted at C_5 and of 5-aminopyrimidine were calculated using Hammett σ_m values,[45] $\sigma_{3N} = 0.65$,[92] and $\rho = 5.77$. The results are tabulated below.

TABLE XXII. The pK_{BH}^+ Values of Some Selected Aminopyrimidines.

Compound	pK_{BH}^+ calc.	pK_{BH}^+ found	T
4-aminopyrimidine	5.27	5.71[54]	20°
4-amino-5-nitropyrimidine	1.17	1.98[194]	20°
4-amino-5-bromopyrimidine	3.02	3.97[195]	not stated
4-amino-5-cyanopyrimidine	2.04	2.54[196]	20°
5-aminopyrimidine	2.68	2.83[53];2.51[73]	20°;25°

The agreement of the calculated pK_{BH}^+ values with those found experimentally is fair, the latter being 0.5-1.0 pK units higher than predicted. The C_5 substituents lie meta to both aza groups so that little perturbation of their electrical effects by resonance is expected. Similarly the σ value of the 3-aza group is unlikely to be affected since it lies meta to N_1. In accordance with this the pK_{BH}^+ of 5-aminopyrimidine (with the amino group meta to both aza groups) is close to the experimental values, after a statistical correction. Thus

the results may be interpreted as an increase in the +R effect of the amino group by the aza group para to it, since such an interaction would be expected to increase the basicity.

It is noted that most of the pK_{BH^+} values were measured at 20° whereas the calculated values are those at 25°. Essery and Schofield[197] measured the temperature variation of substituted 4-aminopyridines and found that the pK_{BH^+} value varies inversely with temperature. From 5.4° to 35° the average change in pK_{BH^+} is about 0.8 pK units. Assuming that the pK_{BH^+} dependence is similar for 4-aminopyrimidines it is possible that some of the discrepancy may be explained by the change in pK_{BH^+} with temperature.

The transmission of the effect of the positive charge to the substituent in pyrimidinium ions is through what is essentially a benzene nucleus rather than through a heterocycle. In the 4-aminopyrimidines transmission of effects does go through a heteroatom but the pK_{BH^+}'s calculated using the same ρ value as for the protonation of pyridines gave values in fair agreement with those found experimentally. Plotting the pK_{BH^+}'s of the 4-aminopyrimidines against Hammett's σ constants gave a straight line with correlation coefficient $r = 0.992$ and $\rho = 5.40$. This latter value is probably identical to the ρ value of 5.77 for the pyridines, since the plot of the aminopyrimidines involved only four points.[190] These facts then suggest that transmission of effects through a benzene and heterocyclic ring are identical.

The conclusion reached above could be further tested by examining the basicities of some 5-substituted 2-aminopyrimidines. In these compounds the substituents lie meta to both aza groups so that no resonance interaction between them is possible. Thus the basicities

would reflect the transmission of effects through a heterocyclic nucleus. The amino group lies ortho to the two aza groups, but the basicities may be calculated by assuming that 2-aminopyrimidines are really 3-aza-substituted 2-aminopyridines.

The pK_{BH}^+'s of 2-aminopyridines may be calculated using the "constant-ortho-substituent concept".[46] (The ρ value for the protonation of the 2-aminopyridines is assumed to be equal to that of the protonation of meta and para substituted pyridines if the ortho amino group has no proximity effect on the aza group.) Calculations were made for the following 2-aminopyridines using $\rho = 5.77$ and $pK_o = 6.71$[62] in equation (1) and are tabulated below.

TABLE XXIII. The pK_{BH}^+ Values of Some 2-Aminopyridines

Compound	pK_{BH}^+ calc.	pK_{BH}^+ found	T
2-aminopyridine	--	6.71[62];6.86[53]	25°;20°
2-amino-5-chloropyridine	4.58	4.71[199];4.83[73]	25°;not stated
2-amino-3,5-dichloropyridine	2.45	2.80[198]	20°
2-amino-5-nitropyridine	2.61	2.80[62];2.83[198]	25°;20°
2-amino-3-nitropyridine	2.61	2.38[78,198]; 2.42[62]	20°;25°
2-amino-5-cyanopyridine	3.48	3.46[198]	20°

As can be seen the agreement is good: the calculated values are within 0.15-0.4 pK units of those experimentally observed. Thus the 2-amino group appears to have no proximity effect on the aza group.

Calculations were then made for five C-5-substituted 2-aminopyrimidines, using $\sigma_{3N} = 0.65$[92] and assuming that these compounds were 2-aminopyridines. A statistical correction was required because of the two equivalent protonation sites. A factor of log 2 was added to each pK_{BH^+} calculated. The results are tabulated below.

TABLE XXIV. The pK_{BH^+} Values of Some 2-Aminopyrimidines.

Compound	pK_{BH^+} calc.	pK_{BH^+} found	T
2-aminopyrimidine	3.26	3.54[53]; 3.45[73]	20°
2-amino-5-chloropyrimidine	1.13	1.73[196]	20°
2-amino-5-nitropyrimidine	-0.83	0.35[73]	20°
2-amino-5-bromopyrimidine	1.01	1.95[73]	20°
2-amino-5-cyanopyrimidine	0.03	0.66[196]	20°
2-amino-5-iodopyrimidine	1.24	2.23[196]	20°

The agreement between the calculated and observed values is as good as for the 4-aminopyrimidines but again the calculated values are about 0.5-1.2 pK units lower than those observed experimentally suggesting resonance interaction between the amino and aza groups. It is conceivable that a -R group, such as NO_2 at C_5, para to the amino group may

increase electron-donation into the ring and thus increase the basicity. In accordance with this, the basicity of 2-amino-5-nitropyrimidine is much higher than predicted. A similar increase in basicity over that predicted was not observed for the corresponding 2-aminopyridine, 2-amino-5-nitropyridine, however. Thus the lower values of the calculated basicities for 2- and 4-aminopyrimidines lie in the direction expected if there were a resonance interaction between the amino group and the 2- or 4-aza groups.

In summary, it appears that the transmission of substituent effects through benzene and unsubstituted azine nuclei are equal since the effects of two or more aza groups on the acidities of unsubstituted aminoheterocycles are additive.[45] Although there is some evidence from the acidities of substituted aminoheterocycles to suggest that an aza group may interact with a +R substituent lying para to it, to a first approximation the aza groups do not perturb the effects of chloro substituents on the ring by either induction or resonance. From the basicities of pyridines there appears to be little resonance interaction between an aza group and a +R substituent since these are well-correlated using Hammett's σ constants rather than σ^+ constants. However, the aza group may inhibit the resonance effects of a -R substituent lying para to it.[60] From an examination of the basicities of 5-substituted 2- and 4-aminopyrimidines one can conclude that the transmission of substituent effects through a substituted pyrimidine and benzene ring are approximately equal, although the basicities of the pyrimidines lie in the direction one would expect if there were a small resonance interaction between the +R amino group and the aza groups. Hence the

basicities of substituted aminoheterocycles support the conclusion drawn from the acidities of aminoheterocycles: to a first approximation the transmission of substituent effects through benzene and azine nuclei are equal, whether the rings are substituted or not. Aza groups do not perturb the effects of other substituents on the ring by induction but they may interact with other +R or -R substituents to a small degree. Aza groups may thus be treated as "normal" ring substituents with σ_N values those of Deady and Shanks.[92]

The basicity of 2-amino-s-triazine (19) has not been experimentally determined, but may be estimated by assuming that it is a substituted pyridine (i.e., it protonates at N_5) and that the transmission of substituent effects through the triazine nucleus is the same as through a benzene nucleus. Using equation (1) the basicity is estimated to be 1.52.

3. The Effect of Nitro Substituents on the Acidity of Aminopyridines and Aminopyrimidines

(a) Comparison of the Effect of Nitro Groups on Amino Group Acidity in Anilines and Aminoheterocycles: A commonly used concept in heterocyclic chemistry is that insertion of a nitrogen atom into the aromatic ring has the same effect as introducing a nitro group into the ring.[49,58] And, indeed, a comparison of the published substituent constants of aza and nitro groups (Table XXV) shows that they are similar in magnitude, except for the high σ^+ constant for the 4-aza group.[186] In general, the meta constants for the nitro group are somewhat greater and the para constants slightly less than the correspond-

ing constants for the aza group.

TABLE XXV. Substituent Constants of Aza and Nitro Groups.

	Aza σ values				Nitro σ values			
	2N	3N	4N	ref.	ortho	meta	para	ref.
σ	0.75	0.65	0.96	92	0.80	0.71	0.78	45
σ^+	0.73	0.57	1.13	186	--	0.67	0.79	45
σ^-	0.58	0.76	1.26	187	--	--	1.27	45
σ^o	0.81	0.72	0.95	91	--	0.70	0.82	60

One of the objects of the present research was to compare the effects of aza and nitro groups on the acidities of anilines. For this comparison, the pK_{HA} values of the nitroanilines considered were those recalculated by Cox[12] using the Bunnett-Olsen method. The effects of aza and nitro groups are very different. The electrical effects of aza groups on aniline acidity were shown in this work to be additive whereas those of the nitro groups are not.[15]

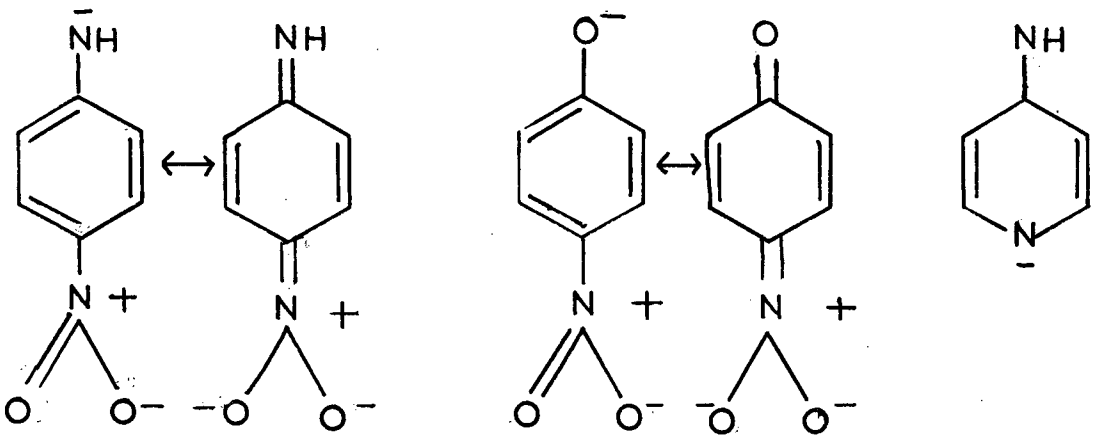
The effects of a <u>para</u> nitro or 4-aza group on the acidity of aniline are large. Using the value of 27.4 for the pK_{HA} of aniline (Section D(1)), the pK_{HA} of 4-aminopyridine, 22.26 (Table XI), and the pK_{HA} of 4-nitroaniline, 18.11,[12] a 4-aza group appears to increase the acidity of aniline by 5.1 pK units, much less than the increase of 9.3 units caused by a para nitro group. Whereas σ_p for the nitro group in phenols and anilinium ions is 1.27,[45] the apparent σ_p constant in

anilide ions is 1.82 (re-evaluated in Section D(1) using the B.O. value of 18.11[12] for 4-nitroaniline and ρ = 5.10). Such abnormally large values are due to increased resonance between the nitro and amino groups in the anilide ion with respect to the neutral molecule.[50] Using the equation,[45]

$$\sigma_R = \sigma_p - \sigma_m$$

the resonance contributions to the nitro substituent constants may be estimated. Thus σ_R for the Hammett σ_p constant 0.78[45] is 0.07, for σ_p^- (phenols, 1.27[45]) 0.56 and for σ_p^- (anilines) 1.11. The resonance contribution to σ_{4N}, 0.96[92], which correlates the acidities of the aminoheterocycles, is only 0.31.

The greater importance of through-resonance in the nitroanilide ion (1) over that in the nitrophenolate ion (2) was attributed to the smaller electronegativity of nitrogen than oxygen, so that the negative charge resides to a greater extent in the nitro substituent in the anilide ion than in the phenolate ion.[15]



(1) (2) (3)

The greater acid-strengthening effect of a para nitro group relative to a 4-aza group must be due to the greater ability of the nitro group to stabilize a negative charge by resonance. The nitro group possesses two oxygen atoms which can delocalize the increased negative charge. Structures such as (3), illustrated for the 4-aminopyridine anion cannot be important in delocalizing charge by resonance since the acidities of the aminoheterocycles were well-correlated using normal σ_N constants.

Ortho-nitro groups also have a great effect on the acidity of anilines. Using the pK_{HA} of aniline, 27.4, and the pK_{HA} of 2-nitroaniline 17.54[12] it appears that an ortho nitro group increases the acidity by 9.9 units, slightly more than a para nitro group. A similar effect was noted for the ionization of anilinium ions (pK_{BH^+} 4-nitroaniline 1.00, pK_{BH^+} 2-nitroaniline -0.26[15]) and for the ionization of diprotonated 2-aminopyridines ($pK_{BH_2^{++}}$ 2-amino-5-nitropyridine -12.1 and $pK_{BH_2^{++}}$ 2-amino-3-nitropyridine -12.4[62]). An ortho aza group increases the acidity of aniline by only 3.9 units (the pK_{HA} of 2-aminopyridine is 23.50). A 2-aza group must exert its electrical effect primarily by induction, with the resonance effect being less than that of a 4-aza group. The powerful acid-strengthening effect of an ortho nitro group must be due both to inductive and resonance effects, comparable to the corresponding effects of a para nitro group.

The first nitro group introduced into the aniline ring may drastically alter the resonance structure of the anilide ion such that most of the negative charge is located on the nitro group rather than on the amino nitrogen.[9,15] Introduction of subsequent nitro groups causes little increase in the delocalization of charge because a single

nitro group delocalizes the charge by resonance so effectively. Thus, the effects of several nitro groups on the same ring are not additive whereas the effects of aza groups within the same ring have been demonstrated previously in Section D(1) to be additive.

Using the value 27.4 for the pK_{HA} of aniline, 23.50 for 2-aminopyridine, 22.26 for 4-aminopyridine, 18.11 for 4-nitroaniline, and 17.54 for 2-nitroaniline, the pK_{HA}'s of several polysubstituted nitro- and aza-anilines were calculated, assuming additivity of substituents, and are tabulated below.

TABLE XXVI. The pK_{HA} Values of Some Nitroanilines and Aminoheterocycles.

Compound	pK_{HA} calc.	pK_{HA} found
2-aminopyrimidine	19.6	19.8*
4-aminopyrimidine	18.4	18.5*
2-amino-s-triazine	14.5	14.9*
2,4-dinitroaniline	8.2	15.0[15b]
2,4,6-trinitroaniline	-1.7	12.2[15b]

* This work

As can be seen above, the calculated values for the aminoheterocycles give excellent results, but the values calculated for the nitroanilines are grossly in error.

(b) Effect of Nitro Groups on the Acidity of Aminoheterocycles: It is interesting to examine the effect of a nitro group, ortho or para to the amino group, upon the acidity of an aminoheterocycle. It is observed that the acid-strengthening effects of such a nitro group are large but not as great as on the acidity of aniline.

Substitution of a nitro group para to the amino group in 2-aminopyridine (pK_{HA} 23.48) increases the acidity by 7.8 pK units (the pK_{HA} of 2-amino-5-nitropyridine is 15.69). This increase in acidity, though sizeable, is not as great as in the anilines where a para nitro group causes a 9.9 unit increase, owing, presumably, to the presence in the pyridine compound of the -I,-R aza group which can also accommodate part of the anion's negative charge. The increase is even less in aminopyrimidines where there are already two aza groups; a para nitro group increases the acidity of 2-aminopyrimidine (pK_{HA} 19.78) by only 5.2 units (the pK_{HA} of 2-amino-5-nitropyrimidine is 14.60).

The increase in acidity upon introduction of an ortho nitro group is less than the effect of a para nitro group in the aminoheterocycles, in contrast to the relative effects observed in anilines, phenols and anilinium ions. An ortho nitro group increases the acidity of 2-aminopyridine by 6.8 units, (pK_{HA} of 2-amino-3-nitropyridine is 16.65) and the acidity of 4-aminopyridine (pK_{HA} 22.26) by 6.4 units (the pK_{HA} of 4-amino-3-nitropyridine is 15.82). The effect of an ortho nitro group is even less in the aminopyrimidines: an ortho nitro group increases the acidity of 4-amino-2-chloropyrimidine (pK_{HA} 16.34) by only 3.0 units (the pK_{HA} of 4-amino-2-chloro-5-nitropyrimidine is 13.33).

As observed in the anilines and diphenylamines,[15] the effect of two nitro groups within the same ring is not additive in the aminopyridines.

A pK_{HA} of 8.84 can be calculated for 2-amino-3,5-dinitropyridine, based on pK_{HA} values of 23.50 for 2-aminopyridine, 15.69 for 2-amino-5-nitropyridine and 16.65 for 2-amino-3-nitropyridine. It is in fact 13.75. This observation of non-additivity as well as the large acid-strengthening effect of a nitro group, ortho or para to the amino group, suggests that there is a large through-resonance between it and the amino group. A large amount of the anionic charge is delocalized onto the nitro group although some will reside on the -I,-R aza groups. The aza groups are evidently much less effective than a single nitro group at delocalizing anionic charge by resonance.

4. Effect of Ortho Substituents on Amino Group Acidity of Aminoheterocycles

The Hammett equation is not generally obeyed by ortho-substituted benzenes.[4,45] Since ortho substituents lie close to the reaction centre, proximity effects, which vary with the given reaction, will contribute to the observed ortho substituent effect. Bulky ortho groups, for example, may force the reacting side chain out of the plane of the aromatic ring. If the side chain is in resonance with the ring, the neutral or ionic form (in an acid-base equilibrium) may be destabilized, thus altering the ionization constant. This effect is known as steric inhibition of resonance. Bulky groups may also interfere with the solvation of, or with the approach of a reagent to, the reaction site. Furthermore, hydrogen-bonding is sometimes possible between the reacting side chain and a given ortho substituent.

Attempts have been made to separate and evaluate the electronic and steric factors contributing to ortho substituent constants,[200,201]

but no one set of ortho σ values exists which can correlate all available rate data of ortho-substituted compounds.[4,45,49]

Charton has shown that in general the electrical effects of ortho substituents are linearly related to those of para substituents for reactions occurring at an exocyclic reaction site.[201b,202] Using several sets of apparent σ_o constants, Charton plotted them against σ_p, σ_m or σ_I for the same substituents. Correlation was best with σ_p except for 2-substituted pyridines which correlate best with σ_m[190,202] suggesting that correlation of $\overline{\sigma_o}$ with σ_m may be generally true for endocyclic reaction sites, although Katritzky observed a good correlation by setting σ_o^+ equal to σ_p^+ in the hydrogen exchange of monosubstituted benzenes in sulfuric acid.[203]

Some workers have assumed that in the absence of proximity effects the electrical effects of ortho and para substituents are equal and good correlations have been obtained by setting σ_o equal to σ_p. Sbarbati[204] found that the rates of piperidino-dechlorination of 2,6-disubstituted chlorobenzenes correlated with σ_p if the ortho substituents were of van der Waal's radius less than 1.9 Å, below which point steric effects were considered negligible. Spinelli[205] obtained good correlation of the rates of piperidino-debromination of 2-bromo-3-R-5-nitrothiophens for several substituents, R, by setting $\sigma_o = \sigma_p^-$. Only the bulky bromo group with van der Waal's radius greater than 1.9 Å failed to correlate. Charton's work, however, suggests that the observation of equality of σ_o and σ_p is purely coincidental and that no conclusion should be drawn about the relative σ_I and σ_R contributions to an apparent σ_o constant even if correlation is observed by setting $\sigma_o = \sigma_p$.[201,202]

In this work the ortho substituents used were chloro and nitro groups, both of which have been correlated with σ_p[204] or σ_p^-[205]. It was of interest to examine whether the substituent effects of these compounds were constant in the acid ionization of anilines and amino-heterocycles and whether the apparent σ_o values were correlated with σ_p (or σ_p^-).

The apparent ortho substituent constant was calculated for compounds containing ortho chloro and nitro substituents by using the B.O. pK_{HA} values calculated in this work (Table XI) or by Cox and Stewart[12] in the equation below,

$$\Sigma\bar{\sigma} = \frac{27.4 - pK_{HA}}{5.10} \qquad (1)$$

The additivity of ortho, meta and para substituent constants was assumed. Indicators containing a nitro group para to the amino group were omitted in view of the variability of $\bar{\sigma}_p$ of this group when other electronegative groups are present in the ring, especially aza and other nitro groups.[15] For 2-amino-3,5-dichloropyridine, pK_{HA} 20.9, which contains a 2-aza, para chloro and ortho chloro group, $\Sigma\bar{\sigma}$ from equation (1) is 1.27. Using the value 0.75[92] for the 2-aza group and 0.23[45] for the para chloro group, $\bar{\sigma}_o$ for the 3-chloro group is calculated to be 0.29. Table XXVII contains the apparent σ_o values, $\bar{\sigma}_o$, for the chloro groups in the acid ionization of anilines and aminoheterocycles and Table XXVIII contains the same data for ortho nitro groups.

From Table XXVII it is apparent that the effect of ortho chloro substituents on the acidity of anilines and aminoheterocycles is not

TABLE XXVII. Apparent Ortho Chloro Substituent Constants in the Acid Ionization of Anilines and Aminoheterocycles.

Indicator	pK_{HA}	$\bar{\sigma}_o$
2,3-dichloroaniline	22.03[12]	0.69
2,4-dichloroaniline	22.64[12]	0.71
2,5-dichloroaniline	22.84[12]	0.53
2,6-dichloroaniline	22.61[12]	0.94 (value for two o-Cl groups)
2,3,5,6-tetrachloroaniline	19.47[12]	0.81 (value for two o-Cl groups)
2-amino-3,5-dichloropyridine	20.87	0.29

TABLE XXVIII. Apparent Ortho Nitro Substituent Constants in the Acid Ionization of Anilines and Aminoheterocycles

Indicator	pK_{HA}	$\bar{\sigma}_o$
4-chloro-2-nitroaniline	16.62[12]	1.89
2-nitroaniline	17.54[12]	1.94
2-nitrodiphenylamine	17.92[12]	1.86
2-amino-3-nitropyridine	16.65	1.36
4-amino-3-nitropyridine	15.82	1.31
4-amino-2-chloro-5-nitro-pyrimidine	13.33	0.69

constant, unlike the situation with anilinium ions.[49] Hence no correlation would be expected by setting σ_o equal to σ_p. (The electrical effects of given meta and para substituents on the acidity of anilines and aminopyridines and pyrimidines were shown to be equal in Section D(1).) The values of $\bar{\sigma}_o$ for the first two anilines in Table XXVII lie close to the value of 0.67 for $\bar{\sigma}_o$ in the protonation of anilines,[49] which at first might suggest that the effect of an ortho chloro group on the acidity of anilines is similar to the effect on the acidity of anilinium ions. However, the effect of two ortho chloro substituents on the acidity of anilines, assuming σ_o = 0.67, is obviously not additive from the apparent value of two ortho chloro groups in 2,6-dichloro and 2,3,5,6-tetrachloroaniline. Furthermore the ortho value of 0.29 in 2-amino-3,5-dichloropyridine is much lower than in the chloro anilines, no doubt due to the presence of the 2-aza group. The electrical effect of the ortho chloro group at C_3 may not be felt as greatly by the amino group because of competition with the nearby strongly -I,-R aza substituent.

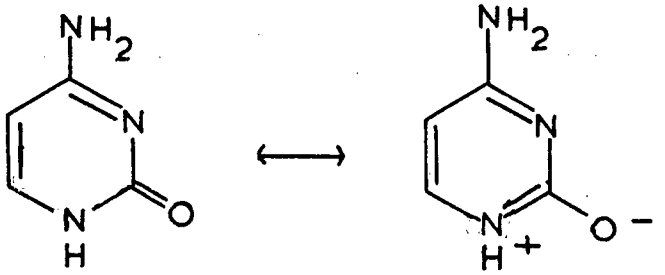
From Table XXVIII it can be seen that the effect of an ortho nitro group on aniline acidity is somewhat greater than its effect on aniline basicity, i.e. $\bar{\sigma}_o$ = 1.72[49] in the latter case and 1.9 in the former. These values do not differ markedly from the value of $\bar{\sigma}_p$ of 1.82 calculated in Section D(1) for the effect of a para nitro group on the acidities of anilines. The $\bar{\sigma}_o$ value for the nitro group in the two aminopyridines is less than that found in the nitroanilines. This is in accord with the conclusion that the effect on amino group acidity

of introducing a nitro group into a heterocyclic ring is less than that of introducing it into a benzene ring. A single ortho or para nitro group is able to accommodate most of the negative charge of the anilide ion by increased through-resonance with the amino group - hence, the very large σ_p value of this group in the acidity of aniline. In the aminopyridines the aza group already accommodates a portion of this negative charge so that introduction of the nitro group does not have as great an effect. In fact the apparent ortho constant of the nitro group in aminopyridines approaches 1.27, the σ_p^- value for the nitro group.[45] In the aminopyrimidine, the presence of two aza groups have reduced $\bar{\sigma}_o$ of the nitro group to 0.69, not markedly different from the Hammett σ_p value of 0.78.[45]

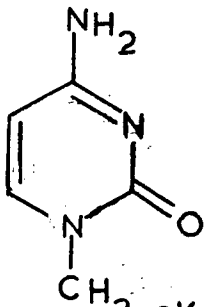
E. Correlation of Structure with Acidity in the Nucleotide Bases

1. Cytosine Derivatives

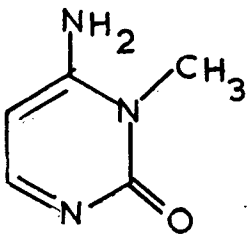
The cytosines and isocytosines examined in this work are 4- and 2-aminopyrimidones, respectively. These compounds are not strictly aromatic (although stabilized by a minor zwitterionic contribution, as illustrated for cytosine (I)) because they adopt a cyclic amide form in aqueous solution.[54,55] The acidities of these compounds are much greater than the corresponding unsubstituted aminopyrimidines. Since the -I,-R effects of the aza groups in both types of compound are probably comparable, the greater acidity must be due to the presence of the carbonyl group in the ring. (Methylation of the aza group may have a slight acid-weakening effect due to the +I properties of the methyl



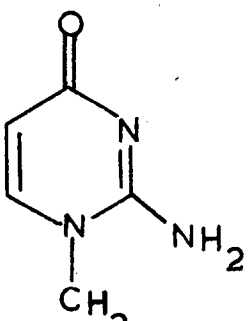
I pK_{BH^+} 4.58[54]
pK_{HA} 12.15[54]



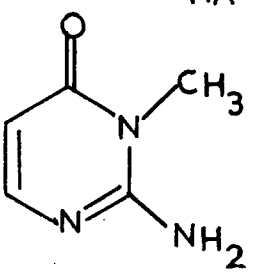
32 pK_{BH^+} 4.55[68]
pK_{HA} 16.69



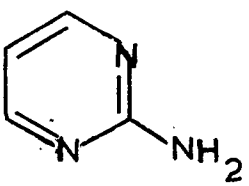
35 pK_{BH^+} 7.38[68]
pK_{HA} 13.37



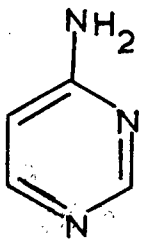
34 pK_{BH^+} 4.0[69]
pK_{HA} 14.22



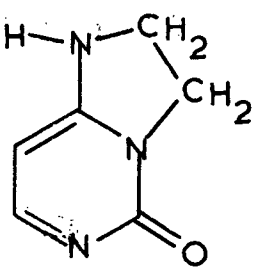
36 pK_{BH^+} 4.2[69]
pK_{HA} 14.28



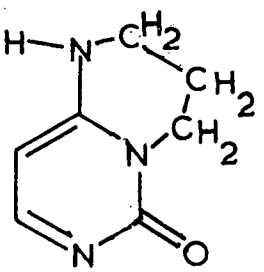
11 pK_{BH^+} 3.54[54]
pK_{HA} 19.78



15 pK_{BH^+} 5.71[54]
pK_{HA} 18.53



31 pK_{BH^+} 6.99[68]
pK_{HA} 12.42



II pK_{BH^+} 7.97[68]
pK_{HA} ca. 14[68]

group.)*

Deprotonation occurs at the amino group which lies "meta" to the carbonyl group so that both the -I,-R effects of the carbonyl group are felt. The increase in acidity of 1-methylcytosine, pK_{HA} 16.69, over 4-aminopyrimidine, pK_{HA} 18.53, is 1.84 pK units. However, when an aza group lying ortho to the ionizing amino group is methylated the acidity is even more enhanced. For 3-methylcytosine, pK_{HA} 13.37, the increase in acidity is 5.16 units. The increase of 1-methylisocytosine, pK_{HA} 14.22, over the acidity of 2-aminopyrimidine, pK_{HA} 19.78, is 5.56 units and for 3-methylisocytosine, pK_{HA} 14.28, 5.50 units. An explanation of this effect is put forward in Section (b).**

(a) Effect of Electron-Withdrawing Substituents on the Acidity of 1-Methylcytosine: The pK_{HA} of cytosine, 12.15,[54] corresponds to the loss of the proton at N_1 in the predominant neutral species. To prevent this latter dissociation from interfering with the determination of the acid ionization of the exocyclic amino group, the labile proton was replaced by a methyl group in the present work. Methylated

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* Protonation of these compounds occurs at the endocyclic nitrogens which lie "ortho" and "para" to the carbonyl group. 1-Methylcytosine (32), pK_{BH^+} 4.55[68], 1-methylisocytosine (34), pK_{BH^+} 4.0[69], and 3-methylisocytosine (36), pK_{BH^+} 4.2[69], which protonate "ortho" to the amino group, as does 2-aminopyrimidine (11), pK_{BH^+} 3.54,[54] all have greater basicities than 11. Similarly the pK_{BH^+} 7.38[68] of 3-methylcytosine (35), is greater than the basicity of 4-aminopyrimidine (15), pK_{BH^+} 5.71.[54]

** In the Dimroth reaction aminopyrimidines possessing a methylated aza group ortho to the exocyclic amino group rearrange in alkali to the methylamino derivative.[55] 3-Methylcytosine does not undergo rearrangement in alkali.[206] Thus the spectral changes observed in this work are not due to the Dimroth reaction taking place.

nucleotide bases may also be used as models for nucleosides. Thus the pK_{HA} of 1-methylcytosine provides an estimate of the pK_{HA} of cytidine as well as of cytosine itself. It appears that the electronic effects of a methylated aza group are very similar to the unmethylated group since the basicities of cytosine pK_{BH^+} 4.58[54] and 1-methylcytosine, pK_{BH^+} 4.55[68] are nearly identical. Both compounds protonate at N_3, or "meta" to N_1, the aza group in question.

Electron-withdrawing groups ortho to the amino group produce the expected increase in acidity and the effects of these substituents is comparable to the effects of ortho chloro and ortho nitro substituents on the acidity of aminopyridines. The pK_{HA} of 1-methyl-5-nitrocytosine is 10.55,[67] an increase of 6.14 pK units over 1-methylcytosine pK_{HA} 16.69. An ortho nitro group increases the acidity of 2-aminopyridine by 6.85 units and of 4-aminopyridine by 6.44 units. However, an ortho group increases the acidity of 4-amino-2-chloropyrimidine by only 3.0 units; the smaller decrease was attributed to the presence of a second aza group and possibly the C_2 electron-withdrawing chloro group (Section D(3)). The electron-withdrawing carbonyl function and the second aza group in 1-methylcytosine do not appear to reduce the effect of an ortho nitro group.

An ortho bromo group increases the acidity of 1-methylcytosine by 1.66 units (pK_{HA} of 1-methyl-5-bromocytosine is 15.03), but the ortho chloro group increases the acidity of 2-amino-5-chloropyridine, pK_{HA} 21.74, in which electron-withdrawing groups are already present, by only 0.87 units (pK_{HA} of 2-amino-3,5-dichloropyridine is 20.87). The larger size of the bromine atom may increase steric interaction between it and the

amino group, thus destabilizing the neutral molecule and increasing the acidity. Sbarbati[204] and Spinelli[205] were unable to fit ortho-bromo-substituted compounds to their linear free energy correlations for ortho substituted compounds and attributed this to bromine's large van der Waal's radius.

(b) Effect of Aza Group Methylation on the Acidity of Amino-pyrimidones: The structures of the cytosines and isocytosines studied in this work are closely similar and on this basis one might expect their acidities to be nearly identical. In fact, the pK_{HA} of 3-methylcytosine (35) was determined in this work to be 13.37, the pK_{HA}'s of 1- and 3-methylisocytosine (34) and (36) to be 14.22 and 14.28, respectively,* and the pK_{HA} of 1-methylcytosine (32) to be 16.69. The pK_{HA} value of 32 used in this discussion was calculated by the B.O. method from ionization ratios determined by Katritzky's method. This compound showed a large discrepancy between I values (and hence pK_{HA} values) estimated by different methods. Using I values estimated by the area method, the B.O. pK_{HA} is 15.45, but this value is also significantly greater than the pK_{HA} values of the other aminopyrimidones.

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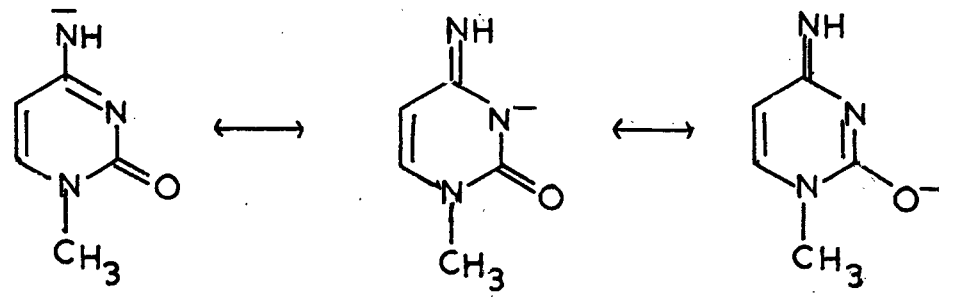
* The pK_{HA}'s of the isocytosines 34 and 36 have been estimated by Hirata[69] to be 12.4 and 12.5 respectively, much lower than the values obtained in this work. For the anion of 3-methylisocytosine (36) the anionic spectrum in 96.1 mole % DMSO showed λ_{max} 318nm,log e_{max} 3.84 and for 1-methylisocytosine (34) in 63.4 mole % DMSO sh285nm, log e_{max} 3.25. Hirata reports for 34, λ_{max} 258nm,log e_{max} 3.59 and for 36, λ_{max} 287-8nm,log e_{max} 3.89 at pH 14 for both. However, the neutral spectra for these compounds in 50 mole % DMSO are: 34 λ_{max} 258nm, log e_{max} 3.67 and 36, λ_{max} 288nm,log e_{max} 3.88. Thus the spectral changes Hirata has assigned to ionization are probably small changes due to a solvent effect.

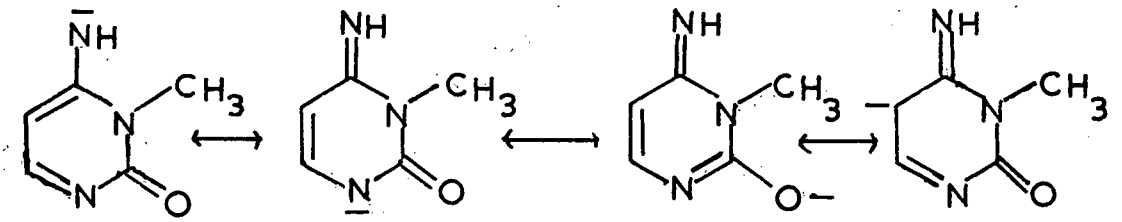
That these differences in acidity are real may be illustrated by determining the mole % DMSO of the solution in which each compound is half-ionized. From the plots of log I vs. mole % DMSO, the solvent compositions are, thus, for 3-methylcytosine 15.4 mole %, 1-methylisocytosine 26.6 mole %, 3-methylisocytosine 27.9 mole %, and 1-methylcytosine 65.0 mole % (using I estimated by Katritzky's method; 68.3 using I estimated by the area method). The large difference in acidity between 1- and 3-methylcytosine was observed by Fox.[68] He estimated the pK_{HA} of the latter compound as being between 13-14, in agreement with the value 13.37 found in this work. The spectral curve of 1-methylcytosine corresponding to the neutral species was the same in 6 M sodium hydroxide as in water, pH 7-14 and its pK_{HA} was estimated to be greater than 14.[68]

The differences in acidity are not readily explicable in terms of resonance stabilization of the anions. The anions of the four compounds are illustrated on page 184. On the basis of the extent of delocalization of anionic charge one might expect the acidities to be 35 ∿ 36 > 32 ∿ 34 since 35' and 36' both have one additional atom to which the negative charge can be dispersed by resonance. The additional structure, in each case, has the negative charge located on carbon and such structures, of course, will make the smallest contribution to the character of the resonance hybrids. In fact, the acidities follow the order ∿ 35 > 34 = 36 > 32.

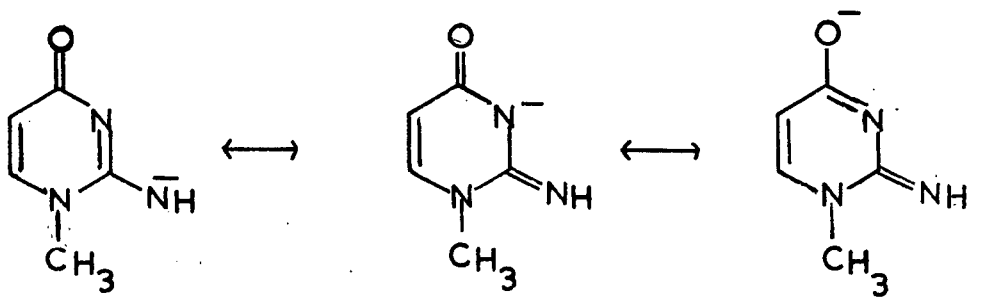
The greater acidity of 3-methylcytosine relative to 1- and 3-methylisocytosine can be explained by the location of the amino group relative to the endocyclic nitrogens. In the first compound the amino



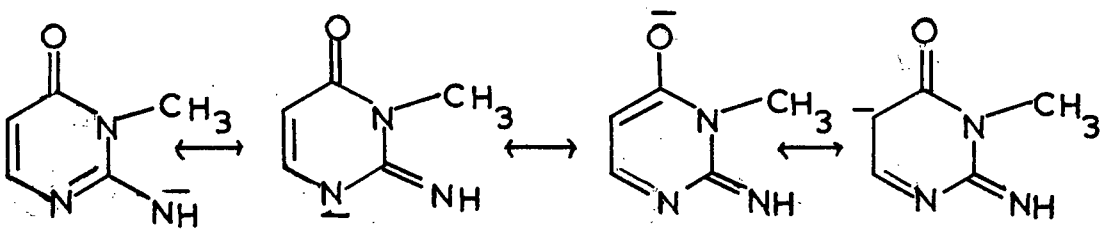
32′



35′



34′



36′

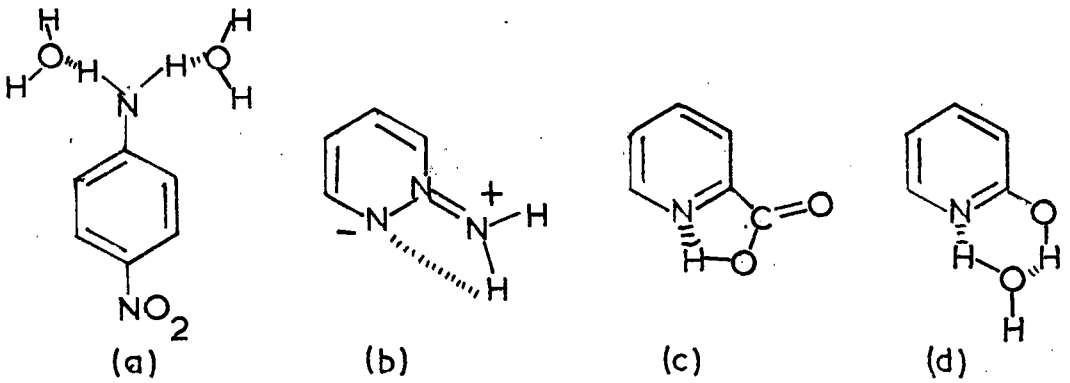
group lies ortho to one aza group and para to the other and in the latter two compounds ortho to both aza groups. From Deady and Shanks' aza substituent constants,[92] a 2-aza group, σ = 0.75, is less electron-withdrawing than a 4-aza group, σ = 0.96. Since the effects of aza groups have been shown to be additive, it is obvious that the effect of two 2-aza groups on the exocyclic amino group will be less than the combined effect of one 2-aza and one 4-aza group. Thus, 2-amino-pyrimidine (11) is a weaker acid than 4-aminopyrimidine (15) by 1.25 pK units and this difference is comparable to the difference of 0.91 pK units between 3-methylcytosine (35) and 3-methylisocytosine (36).

From the location of its amino group relative to the endocyclic nitrogens, one might expect the acidity of 1-methylcytosine (32) to be equal to that of 35 but it is the weakest of all the four acids, 32 and 34-36. The only structural difference between 32 and the other acids is that it does not possess a methyl substituent on the aza group adjacent to the carbon bearing the exocyclic amino group. The higher acidity of 35, 34 and 36 appears, thus, to be due to an interaction between the methyl and the amino group.

Small increases in the acidities of ortho-alkylated-4-nitroanilines over that of unsubstituted 4-nitroaniline were noted by Dolman.[15b] One ortho methyl group decreased the pK_{HA} by 0.1 unit, two ortho methyl groups by 0.2 units and two ortho t-butyl groups by more than 1.0 unit. On the basis of the inductive effects of alkyl groups, the alkylated anilines would be expected to be slightly weaker acids than 4-nitro-aniline. A destabilization of the neutral molecule due to steric hindrance of solution was felt to be the cause of this increase.

Anilines containing electron-withdrawing groups were thought to be solvated as illustrated below. Alkylation at the ortho position would interfere with such solvation, destabilizing the neutral molecule relative to the anion and thus increasing the acidity.

The interaction between the exocyclic amino group and an "ortho" aza group may be important in the neutral molecule of aminoheterocycles. There is evidence from dipole moment studies of interaction between the amino group protons and the lone electron pair of the aza group in the diazine (b) below.[207] Hydrogen-bonding between the hydroxyl group proton and the aza group in pyridine-2-carboxylic acids (c) was thought to be the reason for the weak acidity of these compounds.[185] The 2-hydroxypyridines may hydrogen-bond with a molecule of water forming a stable six-membered ring (d).[185*] Any disruption of the powerful interaction



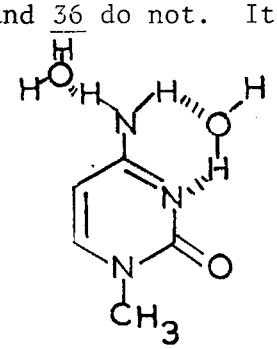
(a) (b) (c) (d)

of the amino and "ortho" aza groups, such as would be caused by methylation of the aza group, could destabilize the neutral molecule and cause a sizeable increase in acidity.

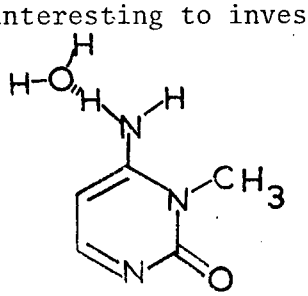
In 1-methylcytosine interaction between the amino and ortho aza group is unhindered. The interaction may involve a molecule of water such that the amino group, the aza group and the water molecule form a

* However, 2-hydroxypyridine is mainly 2-pyridone in aqueous solution.[215]

hydrogen-bonded six-membered ring as illustrated below in (e). In 3-methylcytosine such an interaction would be hindered because of the methyl group at N_3 (f) so that the neutral molecule of 3-methylcytosine is destabilized relative to the neutral form of 1-methylcytosine., This could explain the relatively greater acidity of 3-methylcytosine. In isocytosine interactions between the amino group hydrogens and the two aza groups are possible. In 1- and 3-methylisocytosine one such interaction is disrupted because of the presence of the methyl group at N_1 and N_3 respectively. The effect of one such disruption must destabilize the neutral molecules of 34 and 36 to a large extent since these compounds are stronger acids than 1-methylcytosine, even though the latter compound possesses an aza group para to the amino group which 34 and 36 do not. It would be interesting to investigate the order



(e)



(f)

of acidity of these compounds in anhydrous DMSO to determine whether or not a molecule of water is important in this interaction.

The greater acidity of the secondary cytosine 31 which was used to anchor the H_-^P scale may also be explained by absence of interaction between the protons of the amino group and the vicinal aza group. Because of the inductive effect of the methylene groups one would expect its acidity to be less than that of 3-methylcytosine, but in fact

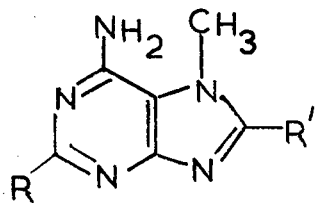
it is about one pK unit greater. An increase in acidity of about 0.5 pK units was observed by Dolman[15b] in anilines in which the exocyclic amino group bore one alkyl group. He suggested that the alkyl substituent destroys a potential hydrogen-bonding site and thus prevents solvation by a molecule of water. This destabilizes the neutral molecule and increases the acidity. The anchor compound has only one amino proton available for solvation by a water molecule whereas 3-methylcytosine has two (f) even though solvation of one amino proton will be restricted by the methyl group at N-3. In 31 interaction between the amino proton and the "ortho" aza group is impossible and solvation is only possible by one molecule of water. Thus its neutral form is destabilized with respect to that of 3-methylcytosine, accounting for its greater acidity. When the alkyl ring contains three methylene units rather than two as in 31, the inductive effect of the alkyl ring overcomes the acid-strengthening effect due to solvation differences and the pK_{HA} of II lies closer to that of 3-methylcytosine.[68]

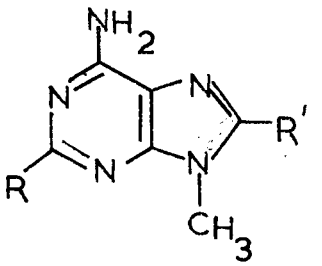
2. Adenine and Guanine Derivatives

(a) Effect on Adenine Amino Group Acidity of C_2 and C_8 Substituents: Table XXIX lists the pK_{HA} values of the substituted 7- and 9-methyladenines determined in this work. A chloro group, whether at C_2 or C_8, exerts an expected acid-strengthening effect. A methoxy group at C_8 exerts an acid-weakening effect suggesting that its +R effect must be felt by the exocyclic amino group.

Using Hammett's σ_m value of 0.37[45] for the chloro group at C_2 and the σ_p values of 0.23 and -0.27 for the C_8 chloro and methoxy groups

TABLE XXIX. The pK_{HA} Values of Substituted Adenines.



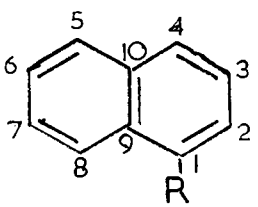


Substituent	pK_{HA} 7-methyladenine	pK_{HA} 9-methyladenine
R=R'=H	14.67	16.74
R=Cl, R'=OCH_3	14.62	16.66
R=Cl, R'=H	14.02	15.61
R=R'=Cl	13.61	15.10

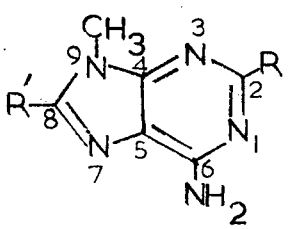
respectively, a plot of pK_{HA} vs. σ was made using the pK_{HA} values in Table XXIX. A good correlation was found for the 7-methyladenines, $r = 0.994$ and $\rho = 1.86$ and a reasonable correlation for the 9-methyladenines, $r = 0.989$ and $\rho = 2.94$. Since both plots were made using only four derivatives whose pK_{HA} values ranged over 1.06 and 1.64 pK units, respectively, there may be some uncertainty in the ρ values of these plots.[190] Nonetheless, it appears that both ρ values are much less than the ρ of 5.10 calculated in this work for the acid ionization of substituted anilines and aminoheterocycles, and that the ρ value of 9-methyladenines is greater than that of the 7-methyladenines.

Since adenine is a bicyclic system there is no theoretical reason why the effects of C_8 substituents should be correlated by substituent constants derived for benzene systems. Attempts have been made to derive σ values for naphthalene (a)[45,191] and these may be extended to include the purine ring system.[191] For a reacting side chain at C_1 substituents at C_3 and C_4 are well-correlated by Hammett's σ values. Substituent constants C_5-C_7 may be estimated from non-bonding molecular orbital theory and are listed by Johnson[45] and Perrin.[191]

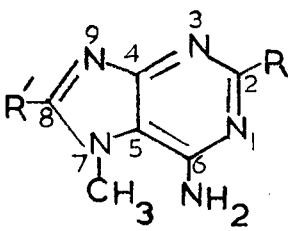
Let us consider 9-methyladenine to be a substituted naphthalene with the amino group equivalent to a reacting side chain at C_1 (b). Obviously a C_2 substituent in the adenine will be correlated using σ_m. For Hammett correlations of substituted five-membered heterocycles such as thiophen, the heteroatom is often regarded as replacing a -CH=CH- portion of a benzene ring. For the adenine to be considered a substituted naphthalene one of the endocyclic nitrogens in the imidazole ring may be considered as having replaced a -CH=CH- grouping in a benzene ring. If the methylated aza group, N_9, is regarded as having



(a)



(b)



(c)

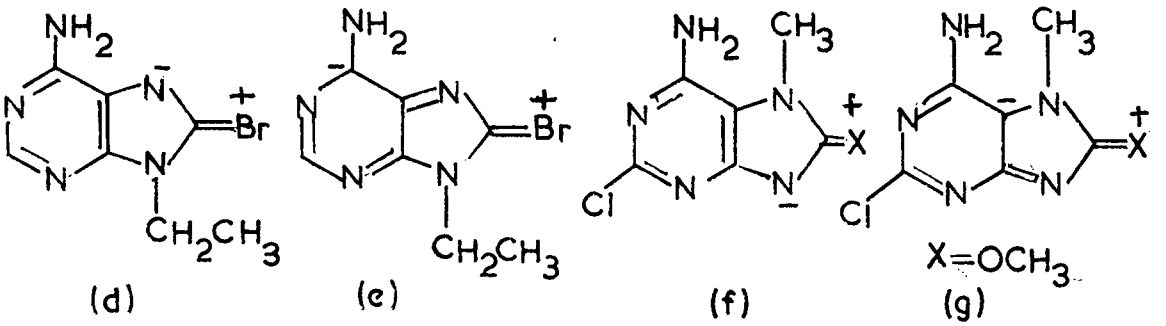
replaced such a grouping then the C_8 position in 9-methyladenine becomes equivalent to the C_7 position in naphthalene. The naphthalene σ_7 values for chloro and methoxy are respectively 0.21 and -0.07.[45]

Although the σ value of the chloro group is close to σ_p = 0.23 the value of the methoxy group is much too low. The methoxy group at C_8 in both adenines appears to have a large +R acid-weakening effect. The naphthalene σ_7^+ values for chloro and methoxy were calculated to be 0.16 and -0.28 respectively using Johnson's method.[45] These values are numerically close to the Hammett σ_p of 0.23 and -0.27 although one would not have expected that there would be an enhanced resonance interaction requiring σ^+ constants between two +R substituents such as amino and methoxy.

In 7-methyladenine (c) the C_8 position can also be regarded as equivalent to naphthalene's C_7 position rather than to C_6, as would be deduced if the methylated aza group N_7 is taken to be equivalent to a -CH=CH- grouping. At C_6 in naphthalene no resonance effects are felt,[45] but the C_8 methoxy group does exert a +R, acid-weakening effect in 2-chloro-8-methoxy-7-methyladenine. Thus the unmethylated aza group N_9 must be regarded as replacing the -CH=CH- grouping in this case.

Because the methoxy group is correlated by a σ^+ constant, it is possible that the methoxy group may interact with the pyrimidine ring rather than directly with the amino group itself. Evidence of resonance between N_7 and a +R substituent at C_8 in 9-alkyladenines has been obtained by Lord and Chen[208] from IR spectra of hydrogen-bonded complexes of 8-bromo-9-ethyladenine and 1-cyclohexyluracil in deutero-chloroform. The N_7 position can accept a hydrogen-bond because of the +R effect of bromine. If the -I effects of the bromo group alone were important, then N_7 would be electron-deficient and less likely to bind a proton. The resonance structure (d) below in which N_7 bears a negative

charge must therefore be important. This charge can be further delocalized into the pyrimidine ring such that hydrids in which C_4, C_2 or C_6 (e) bear a negative charge. Electronegative groups at C_2 might thus be expected to increase the +R effects of a C_8 substituent in a neutral 9-alkyladenine. Similar structures may be drawn for



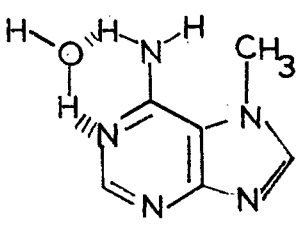
2-chloro-8-methoxy-7-methyladenine (f) and (g) where a negative charge resides at N_9, C_5, N_1 or N_3. Plotting the pK_{HA} values of the adenines against σ, using σ_m for C_2 substituents and σ_7^+ for C_8 substituents gave straight lines of r = 0.992, ρ = 2.05 for 7-methyladenines and r = 0.994, ρ = 3.26 for 9-methyladenines.

The lower ρ value for the acid ionization of 7- and 9-methyladenines may be explained by the charge transfer from the imidazo ring to the pyrimidine ring. The magnitude of a reaction constant is often interpreted as a measure of charge change at the reaction site.[45] In the aminoheterocycles the lone electron pair of the amino group is expected to be extensively delocalized into the aromatic ring so that the charge change at the amino group nitrogen from neutral to anionic species is expected to be large. In the adenines delocalization of the lone electron pair of the neutral amino group is not expected to be as large since the pyrimidine ring of the purine system will be less

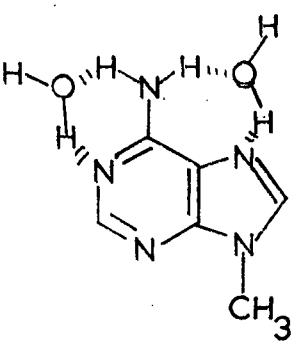
electron-deficient than in pyrimidine itself owing to electron-donation by the imidazole ring. Thus the charge change from neutral to anionic form is probably not as great, accounting for the lower ρ values.

(b) Effect of Aza Group Methylation on the Acidity of Adenines and Guanines: As can be seen in Table XXIX, 7-methyladenines are 1.5-2.1 pK units more acidic than the corresponding 9-methyladenines. The pK difference appears to increase as the derivatives become more weakly acidic but this change may not be significant in view of the lengthy extrapolations involved in evaluating the Bunnett-Olsen pK_{HA}'s. A possible explanation for the greater acidity of the 7-methyl system follows.

There is evidence suggesting that the amino group in unsubstituted adenine hydrogen-bonds to the N_7 position.[65] Such an interaction is possible in 9-methyladenine but not in 7-methyladenine because of the absence of a methyl group at N_7. Water-solvated structures similar to those suggested for the cytosines are shown below for 7- and 9-methyl-adenine (a), (b). A seven-membered hydrogen-bonded ring involving one of the amino group protons, N_7 of the imidazole ring, and a molecule of



(a)

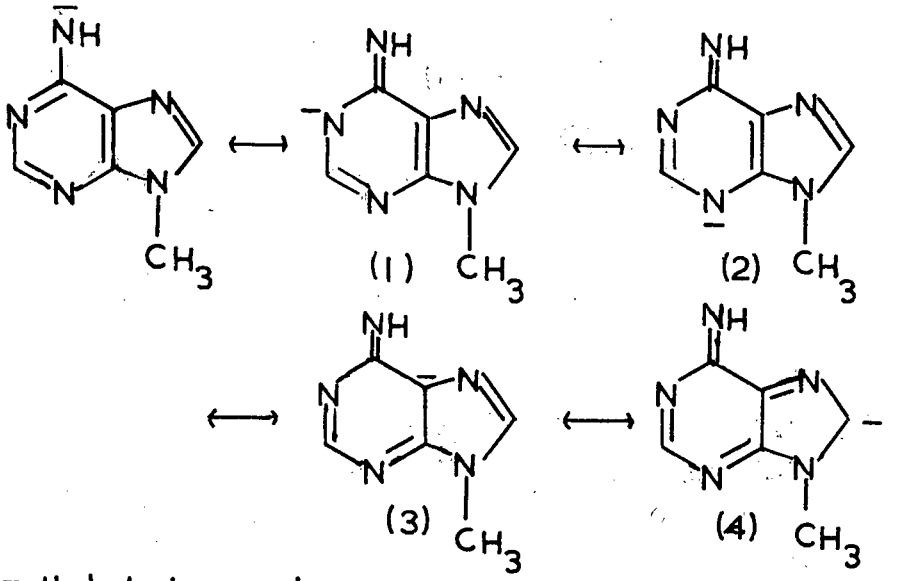


(b)

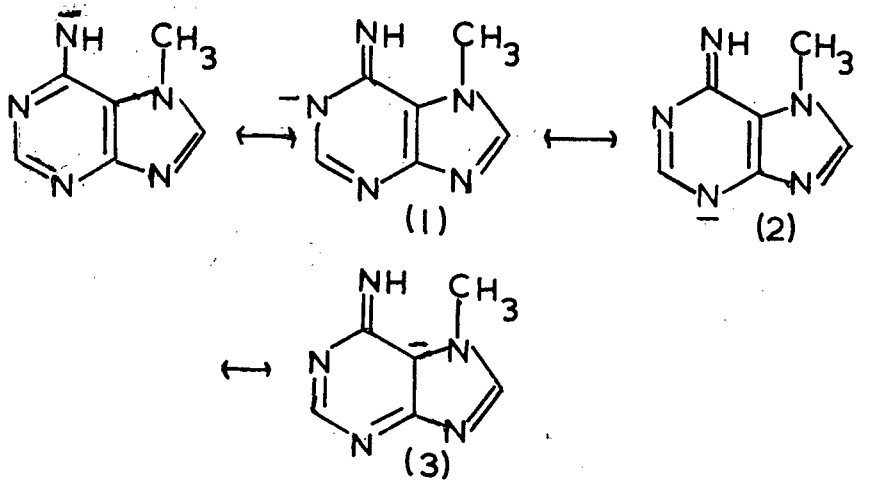
water is possible for 9-methyladenine but not for 7-methyladenine. Thus, the neutral molecule of 9-methyladenine is stabilized relative to its anion more than is 7-methyladenine and its acidity decreases.

The greater acidities of the 7-methyladenines is opposite to what one would expect from the resonance stabilization of the anions of 7- and 9-methyladenine. Similar structures 1, 2, and 3 may be drawn for both in which the negative charge is localized at N_1, N_3 and C_5. For 9-methyladenine a further structure (4) may be drawn in which the charge resides at C_8 in the imidazole ring. The C_8 position in purines is considered electron-deficient and readily undergoes nucleophilic substitution.[56] However, if one or more electron-donating groups are present in the pyrimidine ring electrophilic substitution such as bromination or nitration is possible, suggesting that structures like 4 are significant. One might expect that 9-methyladenine would be a stronger acid owing to greater stabilization of the anion by resonance. However, in 4 the negative charge resides on a carbon atom, which is less electronegative than a nitrogen atom. Structures such as 1 and 2 in which the aza groups bear the negative charge must be more important. Thus the stabilization of the neutral molecule of 9-methyladenine outweighs the additional stabilization gained by its anion via structure 4.

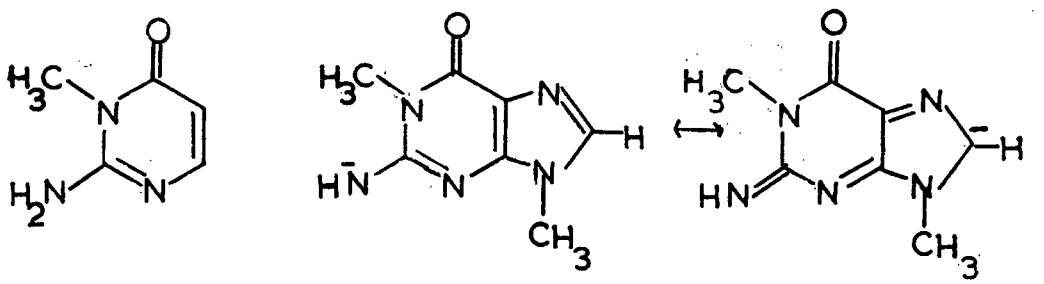
The two methylated guanines studied may be regarded as 3-methyl-isocytosine (36) (pK_{HA} 14.28), fused with an imidazole ring at C_5 and C_6. Since the amino group is well removed from the methylated imidazole aza group an increase in acidity of the 7-methyl derivative (pK_{HA} 14.91) over that of the 9-methyl derivative (pK_{HA} 14.46) is not expected, and



9 methyladenine anion



7- methyladenine anion



3-methylisocytosine

1,9-dimethylguanine anion

since a resonance structure analogous to structure 4, of the 9-methyladenine anion, may be drawn for the 1,9-dimethylguanine anion but not for the 1,7-dimethylguanine anion the former may be expected to be more acidic. Indeed, it is observed to be more acidic by 0.45 pK units. This modest difference is in accord with the assumption that structure 4 does not make a large contribution to the resonance stabilization of the adenine anions.

(c) The effect of the Imidazole Ring on Adenine and Guanine Acidities: Adenine may be considered to be 4-aminopyrimidine fused to an imidazole ring at C_5 and C_6. The presence of the imidazole ring increases the acidity of the amino group by 1.8 pK units when methylated at N_9 (pK_{HA} 9-methyladenine 16.74) and by 3.9 units when methylated at N_7 (pK_{HA} 7-methyladenine 14.67). (The greater acidity of 7-methyladenines was explained in Section 2(b) in terms of destabilization of the neutral forms in solution relative to the 9-methyladenines.) The acid-strengthening effect appears to be less, however, when there is an electronegative substituent meta to the amino group. Introduction of a chloro group at C_2 in 4-aminopyrimidine increases the acidity by 2.2 pK units (pK_{HA} 4-amino-2-chloropyrimidine 16.34), but a chloro group at C_2 in 7-methyladenine increases it by only 0.65 units (pK_{HA} 2-chloro-7-methyladenine 14.02) and in 9-methyladenine by 1.1 units (pK_{HA} 2-chloro-9-methyladenine 15.61).

These observations may be explained by two competing effects of the imidazole ring: stabilization of the anion by increased delocalization of the negative charge in the more extended purine system as compared to the pyrimidine system, which is acid-strengthening, and charge transfer by

the electron-rich imidazole ring into the electron-deficient pyrimidine ring[56] which is acid-weakening.

In the unsubstituted methyladenines the first effect predominates and the increased acidity, relative to 4-aminopyrimidine, may be explained by increased delocalization of charge in the anion. A chloro group at C_2 would be expected to increase charge-transfer from the imidazole into the pyrimidine ring, rendering it less electron-deficient. Furthermore, the anionic charge may be well accommodated by the pyrimidine ring because of the chloro group at C_2, so that the increased delocalization provided by the imidazole ring is less important. If both of these acid-weakening effects are important, a meta chloro group in the adenines will appear to be less acid-strengthening than in the aminopyrimidines.

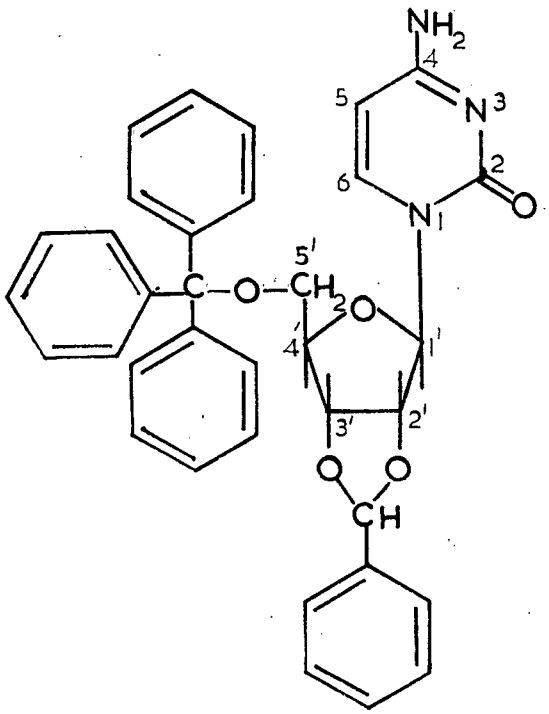
In contrast to the adenines, the imidazole ring actually appears to decrease the acidity of the methylguanines relative to 3-methylisocytosine (pK_{HA} 14.28). The acidity of 1,9-dimethylguanine (pK_{HA} 14.46) is decreased by 0.18 pK units, a value which is probably not significant considering the errors inherent in extrapolated pK_{HA} values. 1,7-Dimethylguanine (pK_{HA} 14.91), however, has its acidity decreased by 0.63 units relative to 3-methylisocytosine. That this difference is real is shown by the DMSO concentrations of the solutions in which the indicators are half-ionized. 3-Methylisocytosine is half-ionized at 27.9 mole % (section E (1)(b)), 1,9-dimethylguanine at 35.6 mole % and 1,7-dimethylguanine at 45.85 mole %. Possibly, the anionic charge is so well-accommodated by the carbonyl group that increased delocalization of the charge by the imidazole ring would have little importance. This

is analogous to the case of the nitroanilines where the anionic charge is well accommodated by a single nitro group and subsequent substitution of further nitro groups into the ring does not increase the aniline acidity as greatly as does a single nitro group.[15] Furthermore, the powerfully electronegative carbonyl group may increase charge-transfer into the pyrimidine ring to such an extent that the guanines actually become slightly weaker acids than 3-methyl-isocytosine.

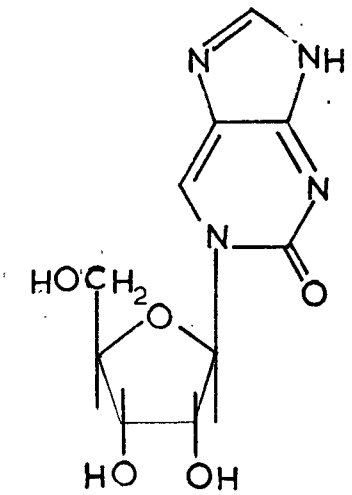
3. Effect of the Ribosyl Group on Nucleotide Base Acidity.

To test the validity of using the acidities of methylated nucleotide bases as a measure of nucleotide acidity in the DNA molecule, the pK_{HA} of a nucleoside was determined. In nucleosides the sugar hydroxyl protons at C_2' and C_3' ionize in strong base (pK_{HA} ca. 12[54]) and the C_5' hydroxyl proton might also be expected to ionize at high alkalinity. Fox[209] observed spectral shifts for cytidine, in the absorption due to the pyrimidine ring, upon ionization of the sugar hydroxyl protons which was interpreted as an interaction between the aglycon and the 2'-OH group. Small spectral shifts were also observed for 2',3'-O-isopropylideneuridine and were attributed to ionization of the 5'-OH group. To eliminate the possibility of these ionizations, the 2', 3' and 5'-OH groups were blocked with base-resistant groups. The nucleoside derivative used in this work is 2',3'-O-benzylidene-5'-O-tritylcytidine (I). The spectral changes observed as the basicity of the aqueous DMSO solution was varied could thus be interpreted as the ionization of the amino group.

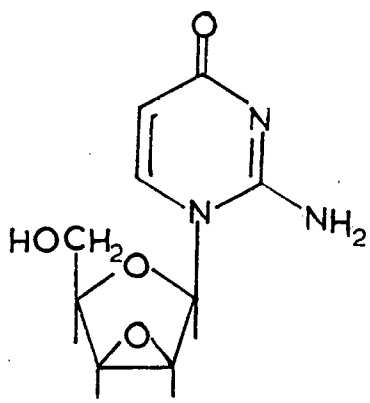
The spectral changes upon ionization of the cytidine were small



2',3'-O-benzylidene-5'-O-tritylcytidine (I)



1-β-D-ribofuranosyl-
2-oxypurine (II)



1-(2',3'-epoxy-β-D-lyxofuranosyl) isocytosine (III)

but a reasonably reliable pK_{HA} of 14.75 was calculated (indicator is half-ionized at 51.0 mole %), considerably lower than the pK of 1-methylcytosine (pK_{HA} of 16.69 and half-ionized at 65.0 mole %).

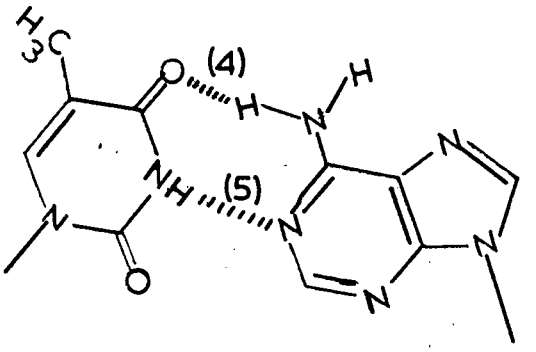
The stronger acidity of a nucleoside relative to the N-methylated nucleotide base has been observed by other workers.[67,69] Fox[67] determined the acidity of 1-methyl-5-nitrocytosine to be 10.55 and the acidity of 5-nitrocytidine to be 9.12, an increase in acidity of 1.4 pK units. The nucleoside 1-β-D-ribofuranosyl-2-oxypurine (II) is a stronger acid by 0.25 pK units than 1-methyl-2-oxypurine. The pK_{HA} of the nucleoside 1-(2',3'-epoxy-β-D-lyxofuranosyl)isocytosine (III) is 12.9,[69] 1.3 pK units stronger than the amino group acidity of 1-methylisocytosine, pK_{HA} 14.2. The cytidine studied in this work was 1.9 pK units stronger than 1-methylcytosine, comparable to the differences observed by Fox[67] and Hirata.[69] Fox[209] suggested that the increase in acidity was due to some interaction between the unionized 2'-OH group and the carbonyl group of the pyrimidine ring since spectral changes in the pyrimidine spectrum occurred upon the hydroxyl ionization. No such shifts were observed in 2'-deoxyribosyl derivatives where the 2'-OH group is replaced by a proton, nor in the oxyribosyl derivative of 2',3'-adenylic acid, even though hydroxyl ionization was detected potentiometrically. In these derivatives no interaction is possible between the 2'-OH group and the ring. However, the nucleoside examined in this work contained no 2'-OH proton and its acidity was still lower than that of 1-methylcytosine. Thus the increased acidity is not due to such hydrogen-bonding interaction. It is felt that the most likely explanation is that the ribosyl group exerts a -I effect on the

nucleotide base, by virtue of its many oxygen atoms, whereas the N_1 methyl group exerts a small +I effect.

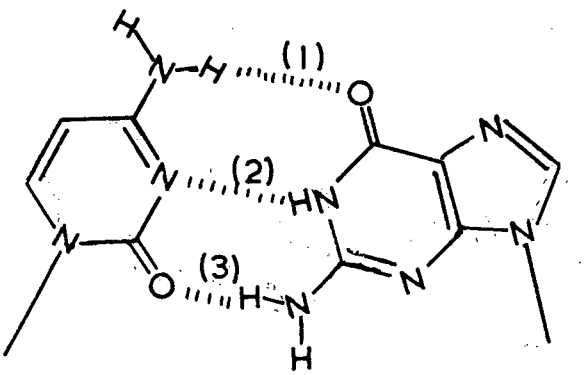
There is thus some uncertainty, roughly 1-2 pK units, in using the pK_{HA} of an appropriately methylated cytosine or isocytosine as the pK_{HA} of the corresponding nucleoside. A similar estimate cannot be made for the purine nucleosides since the spectral changes upon ionization of 2',3'-O-benzylidene-5'-O-tritylguanosine were too small to allow a meaningful estimate to be made of its pK_{HA}. It is assumed in the following discussion that the -I inductive effect of the ribosyl moiety is the same as that for 1-β-D-ribofuranosyl-2-oxypurine, i.e. 0.25 pK units acid-strengthening relative to the methylated aglycon, since the acidic site in adenosine and guanosine is farther removed from the sugar group than in cytidine.

The pK_{HA}'s of 1-methylcytosine, 9-methyladenine and 1,9-dimethylguanine may be used to estimate the pK_{HA} values of cytidine, adenosine and guanosine, respectively. The pK_{HA} of cytidine is taken to be 14.8, the pK_{HA} determined for the substituted cytidine. The pK_{HA} of guanosine is probably higher than the value 14.46 obtained for 1,9-dimethylguanine, since the methyl group at N_1 in the latter will increase its acidity. This increase is estimated to be 3.32 pK units, the difference between the acidity of 1-methylcytosine and 3-methylcytosine. Subtracting the pK unit correction for the ribosyl moiety, an estimate of 17.5 is made for the amino group ionization in guanosine. Applying the correction for the sugar ring to the pK_{HA} of 9-methyladenine gives an estimate of the pK_{HA} of adenosine of 16.5.

These pK_{HA} values may be used to estimate the strength of the



Adenine – thymine base pair (IV)



Cytosine–guanine base pair (V)

hydrogen-bonding interaction of the nucleotide bases in the nucleic acids DNA and RNA. The strength of hydrogen-bonding depends directly on acid strength[210] and the stronger the hydrogen bond the greater the association constants between the given base pairs.[208] Adenine bonds exclusively via two hydrogen bonds to thymine (5-methyl-uracil) in DNA and to uracil in RNA (IV). Cytosine always bonds to guanine via three hydrogen bonds in both nucleic acids.[167] (V)

Thus of the interactions (1), (3) and (4) the strength of the hydrogen bonds are (1) > (4) > (3), assuming that the strength depends solely on the acidity of the proton donor. The acidity of N_3 in thymidine is 9.8[54] and in uridine 9.3[54]; the acidity of N_1 in guanosine is 9.3.[54] Thus bonds (2) and (5) are probably of comparable strength. From these data it is then concluded that the association between guanine and cytosine (V) is stronger than the association between adenine and uracil (or thymine) (IV) not merely because the first pair possesses three hydrogen bonds whereas the second possesses two, but because the actual strength of one of the former pairs of hydrogen bonds is greater.

F. Comparison of Acidity and Basicity of Aminoheterocycles

Plotting the pK_{HA} values against the corresponding pK_{BH^+} values for a given series of homologous indicators helps to determine the relative effects of substituents on the acidity as compared to effects on basicity. For meta and para substituted diphenylamines, excluding those containing nitro groups, a straight line was obtained,[15c]

$$pK_{HA} = 21.4 + 1.30\ pK_{BH^+}$$

The coefficient in this equation indicates that the acidity of a given diphenylamine is more sensitive to substituent effects than is its basicity. For anilines and diphenylamines containing ortho and para nitro substituents, however, such a plot has a slope less than one,[9] owing to the anomalously large effect of a single nitro group on acidity.[15]

It was of interest to determine whether similar relations exist between the acidities and basicities of aminopyridines, pyrimidines and purines. Table XXX lists the basicities available in the literature for the indicators studied together with the B.O. pK_{HA} values determined herein. Examination of the table and Figure 16 reveals that there is little correlation between pK_{HA} and pK_{BH^+} for the aminopyridines, aminopyrimidines, cytosines or aminopurines. This is doubtless due to the fact that all aminoheterocycles protonate at the endocyclic nitrogen rather than at the exocyclic amino group.[54-57,71] Deprotonation, however, must occur at the exocyclic amino group in the indicators studied. Thus comparison of substituent effects on acidity and basicity is probably not meaningful since the location of substituents relative to the reaction site is different in each case. This is not so with anilines and diphenylamines.

Second protonation of the aminopyridines does occur at the exocyclic amino group as verified by NMR.[81] An attempt was therefore made to correlate the $pK_{BH_2^{++}}$ values of the aminopyridines with the pK_{HA} values. The $pK_{BH_2^{++}}$ values are those of Bellobono and Favini.[62] Since the diprotonated aminopyridine has three ionizable protons whereas the neutral molecule has two a statistical factor (log 1.5) was subtracted

TABLE XXX. Acidities and Basicities of Aminoheterocycles.

Indicator	pK_{HA}	pK_{BH^+}	ref.	$pK_{BH_2^{++}}$	ref.
2-aminopyridine	23.50	6.71	62	-7.65	62
2-amino-5-chloro-pyridine	21.74	4.71	199	--	
2-amino-3,5-dichloro-pyridine	20.87	2.80	198	--	
2-amino-3-nitro-pyridine	16.65	2.42	62	-12.4	62
2-amino-5-nitro-pyridine	15.69	2.80	62	-12.1	62
4-aminopyridine	22.26	9.15	62	-6.60	62
4-amino-3-nitropyridine	15.82	5.05	62	-11.9	62
2-aminopyrimidine	19.78	3.54	54		
2-amino-5-nitropyrimi-dine	14.60	0.35	73		
4-aminopyrimidine	18.53	5.71	54		
1,7-dimethylguanine	14.91	3.40	77		
1,9-dimethylguanine	14.46	3.28	77		
7-methyladenine	14.67	3.6	211		
9-methyladenine	16.74	3.25	211		
2,3-dihydro-1H-5-oxo-imidazo(1,2-c)pyrimi-dine hydrochloride	12.42	6.99	68		
1-methylcytosine	16.69	4.55	68		
3-methylcytosine	13.37	7.38	68		
1-methylisocytosine	14.22	4.0	69		
3-methylisocytosine	14.28	4.2	69		

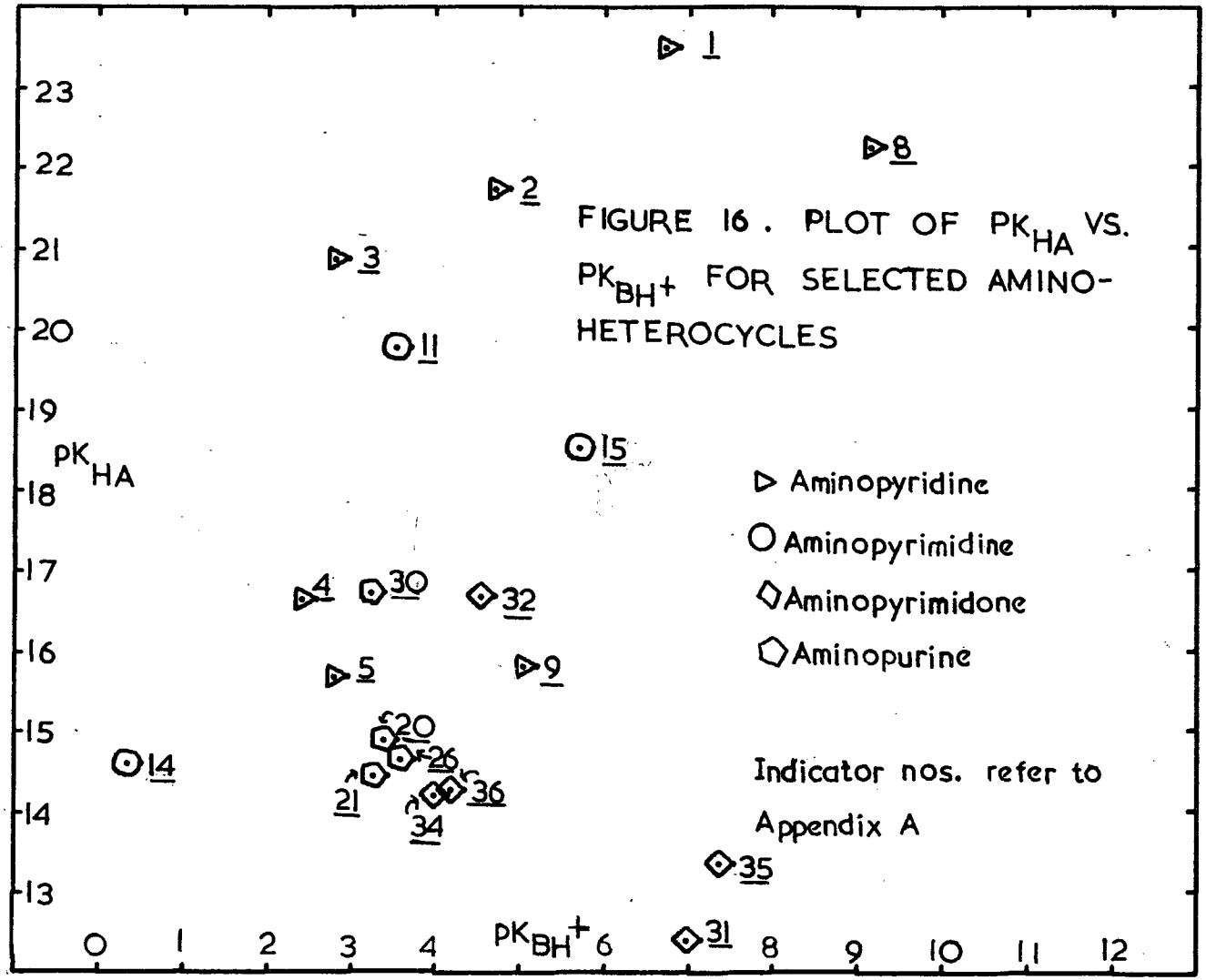


FIGURE 16. PLOT OF pK_{HA} VS. pK_{BH^+} FOR SELECTED AMINO-HETEROCYCLES

from all $pK_{BH_2}^{++}$ values before plotting them against pK_{HA}.

The correlation between the two ionization constants is poor, r = 0.957, m = 1.3. Possibly the nitro-substituted compounds form a different class, as with the anilines, since a single nitro group appeared to greatly enhance the acidity of aminopyridines, although not to the same extent as in the anilines. Plotting only the 2-aminopyridines gave a slightly better correlation, r = 0.986, m = 1.6, but since this plot only included three points it cannot be considered precise. The indication is, however, that the acidity of neutral aminopyridines is more sensitive to substituent effects than is the acidity of the corresponding dicationic acids.

SUGGESTIONS FOR FUTURE WORK

Arnett has found a linear relation between the pK_{HA} values of anilines and diphenylamines, standard state water, and their heats of deprotonation in pure DMSO.[1] It is possible that a similar relation exists for the compounds investigated in this work because of their structural similarity to anilines. Such a relation being established, Arnett's method would prove valuable in determining the pK_{HA} values of indicators which show little or no spectral change upon ionization, such as 1-methyl-5-bromocytosine and 3,7-dimethylisoguanine. Since the heat of deprotonation is measured in pure DMSO, the pK_{HA} values standard state water of very weak acids like 3-aminopyridine (whose pK_{HA} was estimated as 24 in this work), which are not detectably ionized in aqueous DMSO, could be estimated. The unsubstituted aminopyridines and pyrimidines are weak acids and the pK_{HA} values were determined for derivatives containing only electron-withdrawing substituents. In pure DMSO with potassium dimsyl as base the acidities of aminoheterocycles containing electron-donating substituents could also be determined, giving a more complete picture of the effect of substituents on amino group acidity.

Using the method of Ritchie and Uschold[36] or of Bordwell[37] the pK_{HA} values of the indicators could be redetermined in anhydrous DMSO with pure DMSO as the standard state. It would be valuable to investigate the variation of pK_{HA} with solvent particularly for the methylated cytosine and adenine derivatives whose order of acidity may depend on solvation by a molecule of water.

It has been suggested that an H_- function be established in the aprotic solvent hexamethylphosphoramide (HMPA) since hydroxide and t-butoxide ions are very strong bases in this solvent and it is often used as a reaction medium in organic synthesis.[15b] During the research for the work in this thesis this solvent was investigated. Aqueous HMPA/water/0.011 M TMAH appears to be a very strongly deprotonating system, stronger than DMSO/water/0.011 M TMAH. However, the HMPA commercially available in North America is contaminated by an impurity which absorbs at wavelengths less than 350 nm. This impurity cannot be removed by distillation or by treatment with metallic sodium or calcium hydride in vacuo. If supplies of HMPA free of this impurity are available or some method of its removal can be devised, establishment of an H_- function in HMPA should be straightforward.

APPENDIX A: Estimation of Ionization Ratios for the Indicators.

1. 2-Aminopyridine

Method: Flexser/Hammett/Katritzky (FHK)

Mole % DMSO	97.37	97.71	98.16	98.73	99.19
Log I*	-0.926	-0.727	-0.523	-0.148	0.285

Mole % DMSO at half-ionization: 98.9 (estimated graphically)

2. 2-Amino-5-chloropyridine

Method: FHK

Mole % DMSO	93.38	94.29	95.35	96.82	97.37	97.71	98.16
Log I	-0.571	-0.390	-0.112	0.302	0.518	0.693	0.940

Mole % DMSO at half-ionization: 95.8 (estimated graphically)

3. 2-Amino-3,5-dichloropyridine

Method: FHK

Mole % DMSO	74.37	77.65	79.14	81.50	83.89	86.18	87.39
Log I	-0.791	-0.500	-0.342	-0.161	0.205	0.540	0.762

Mole % DMSO at half-ionization: 81.65

4. 2-Amino-3-nitropyridine

The anionic spectrum consists of several transitions. These bands are smeared out between 77.7 and 86.6 mole % DMSO but are well-defined at 96.3 mole % with two peaks of equal e_{max} at 502 and 523 nm and a shoulder at 560 nm. The peak at 523 nm was used to calculate log I.

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* Log I values are given to three decimal places when the spectra were measured on the Cary 16 and to two decimal places when measured on the Cary 15. The mole % DMSO at half-ionization was calculated from the plots of log I vs. mole % DMSO unless otherwise stated. Where two alternative methods exist to estimate log I, the I values used in calculating the pK_{HA} are marked with an asterisk (*).

Method: FHK

Mole % DMSO	39.18	42.22	44.65	49.32	51.45	54.46	58.73
Log I	-0.940	-0.680	-0.443	-0.040	0.142	0.411	0.761

Mole % DMSO at half-ionization: 50.1

5. 2-Amino-5-nitropyridine

Method: FHK

Mole % DMSO	35.01	39.34	44.67	48.96	53.62
Log I	-0.692	-0.277	0.150	0.498	0.863

Mole % DMSO at half-ionization: 43.0

6. 2-Amino-3-chloro-5-nitropyridine

Method: FHK

Mole % DMSO	20.08	22.10	25.13	27.31	30.28	34.60
Log I	-0.759	-0.528	-0.205	0.027	0.340	0.777

Mole % DMSO at half-ionization: 27.05

7. 2-Amino-3,5-dinitropyridine

The anion was reasonably stable up to 50 mole % DMSO then decayed rapidly in solutions of higher DMSO content. At 96 mole % DMSO, the red color of the anion is visible immediately upon injection of the sample into the basic solution, but the color of the solution changes within seconds to a pale yellow. A peak at 398 nm (log e_{max} 4.37; sh 325 nm, log e 3.99) was observed, which decreased slowly with time. Neutralization with 1.0 M acetic acid did not restore the neutral molecule's spectrum, but gave an orange solution instead (λ_{max} 334 nm, 488 nm; sh 385 nm).

The anion decays much less rapidly in solutions of less than 50 mole % and neutralization of the basic solution gives the unionized species back unchanged. Readings of absorption for each basic solution were taken at two minute intervals for 10-15 min., then extrapolated to the time of injection of the sample. Since the anionic peak is broad and flat, error resulting from difficulties in estimating λ_{max}^{A-} will be small.

Method: FHK

Mole % DMSO	7.79	10.27	12.85	15.15	17.46	19.85	22.06
Log I	-0.848	-0.571	-0.279	0.000	0.274	0.571	0.871

Mole % DMSO at half-ionization: 15.0

8. 4-Aminopyridine

The tail edge of the neutral spectrum overlaps the absorption maximum of the fully ionized spectrum to a small extent. A_{HA} was considered to be negligible if log I was greater than 0.6 so that λ_{max} observed in solutions where log I is greater than 0.6 is probably λ_{max} of the anion. After estimating log I, using Katritzky's method (K) at 290 nm, λ_{max} was plotted against H_-^N for solutions where log I was greater than 0.6. Log I values obtained by both methods were nearly identical.

Mole % DMSO	93.38	94.29	95.35	96.30	96.82	97.37	97.71	98.16
*Log I (FHK)	-0.877	-0.657	-0.402	-0.028	0.055	0.195	0.423	0.695
Log I (K at 290 nm)	-0.874	-0.668	-0.416	-0.035	0.041	0.189	0.404	0.669

Mole % DMSO at half-ionization: 96.8 (estimated graphically using FHK I values).

9. 4-Amino-3-nitropyridine

Method: FHK

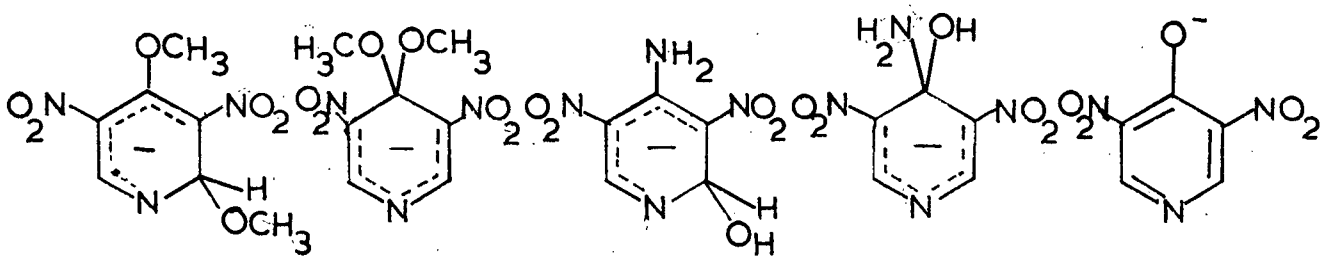
Mole % DMSO	30.10	34.52	42.22	44.65
Log I	-0.700	-0.177	0.580	0.832

Mole % DMSO at half-ionization: 36.6

10. 4-Amino-3,5-dinitropyridine

When a sample of indicator was injected into basic solution, the initial red color disappeared within seconds for all solutions from 10 to 55 mole % DMSO with a yellow color then appearing. The red species is most stable in solutions of about 50 mole % with a low, flat peak in the UV/vis spectrum at ca. 500 nm. The spectrum decreased continuously with concomitant formation of the yellow product (λ_{max} 412, 322 and 275 nm; log e_{max} 4.49, 3.88, and 3.76 respectively; sh 350 nm). Neutralization of the yellow or red solution with 1.0 M acetic acid recovered the unchanged neutral spectrum. This suggests that the spectral changes are due to formation of Meisenheimer adducts.

Meisenheimer adducts have been observed for 3,5-dinitro-4-methoxypyridine with methoxide ion in methanol/DMSO mixtures, the formation being favored by increasing DMSO content.[164] When the concentration of methoxide is greater than 0.01 M, I, illustrated below, is the kinetically favored product and is the one first observed, but II is thermodynamically more stable and eventually prevails.



I II III IV V

It is possible that the red color first observed for the amino-pyridine may be due to III, which rapidly disappears rearranging to IV. Alternatively, the red color may be due to the anionic form of the indicator, since 1,3-adducts such as I have lifespans of 1-2 sec., and the red color persists for a somewhat longer interval.

In 96 mole % DMSO the compound reacts irreversibly with base after initial formation of the red color. The species exhibited a maximum at 354 nm (log e_{max} 4.32) which decreased slowly with time. Neutralization about 5 min. after appearance of the pale yellow color did not restore the neutral spectrum but, rather, a species with λ_{max} 417 nm, sh 325 nm was formed which decayed rapidly with time. The yellow color may be due to a hydrolysis product V.[165]

11. 2-Aminopyrimidine

Method: FHK

Mole % DMSO	87.39	89.61	91.58	93.38	94.29	95.35	96.95
Log I	-0.994	-0.698	-0.410	-0.085	0.099	0.448	0.768

Mole % DMSO at half-ionization: 94.0 (estimated graphically)

12. 2-Amino-4-chloropyrimidine

Method: FHK

Mole % DMSO	68.31	71.90	74.37	76.18	79.14	81.24	83.89
Log I	-0.673	-0.409	-0.198	-0.069	0.189	0.381	0.635

Mole % DMSO at half-ionization: 76.5

13. 2-Amino-4,6-dichloropyrimidine

Method: FHK

Mole % DMSO	44.26	49.32	54.46	58.73	61.86
Log I	-0.771	-0.383	0.018	0.356	0.661

Mole % DMSO at half-ionization: 54.35

14. 2-Amino-5-nitropyrimidine

Method: FHK

Mole % DMSO	20.20	22.04	24.88	27.60	30.01	32.61
Log I	-0.712	-0.528	-0.262	0.000	0.207	0.490

Mole % DMSO at half-ionization: 27.5

15. 4-Aminopyrimidine

Method: FHK

Mole % DMSO	68.43	71.86	76.96	80.52	87.65
Log I	-0.739	-0.429	-0.102	0.291	0.966

Mole % DMSO at half-ionization: 77.1

16. 4-Amino-2-chloropyrimidine

Method: FHK

Mole % DMSO	44.32	49.47	51.41	54.50	58.45	62.78
Log I	-0.695	-0.335	-0.182	-0.011	0.330	0.710

Mole % DMSO at half-ionization: 54.2

17. 4-Amino-2,6-dichloropyrimidine

Method: FHK

Mole % DMSO

27.42	29.90	32.23	34.52	36.93	39.18	44.26	48.21

Log I

-0.873	-0.675	-0.487	-0.292	-0.086	0.083	0.482	0.834

Mole % DMSO at half-ionization: 38.0

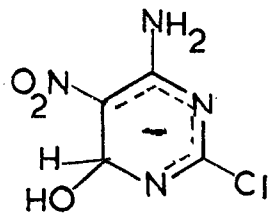
18. 4-Amino-2-chloro-5-nitropyrimidine

The anion of this indicator is not stable in basic DMSO/water solutions. The red color, which formed immediately upon injection of the indicator into the basic solution, was assumed to be that of the anion. (The spectrum showed a broad flat peak at about 430 nm.) In solutions with less than 55 mole % DMSO the anion persisted for 1-2 min., so that e_{A^-} could be estimated by extrapolation to the time of injection.

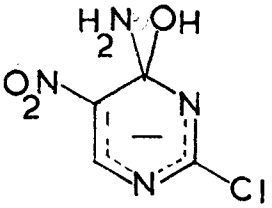
The rate of decay is low in solutions of less than 10 mole % DMSO, then increases up to 25 mole % and decreases slightly for solutions 40-55 mole %. Above 55 mole %, the rate increases rapdily, at 95 mole % the anion disappears almost immediately. As the anionic spectrum disappears another peak appears in the UV region, whose maximum shifts to shorter wavelengths and whose e_{max} increases with time. The same product appears to be formed in all solutions examined, from 5-96 mole % DMSO. At 96 mole % it has a maximum at 353 nm, log e_{max} 4.18, but there is a bathochromatic shift and an increase in e_{max} with increasing DMSO content. Neutralization with 1.0 M acetic acid gives a species whose λ_{max} is at 337 nm and 276 nm.

Meisenheimer complexes have been observed for 3,5-dinitropyridines[164] and for 2,4-dinitroanisoles.[165] It is possible that the red color is not due to the anion but to a kinetically favored species, I, which is rapidly converted to the more stable adduct II. Attack by the hydroxide ion on I would probably occur at C_4 or C_6 since base addition has not been observed at chlorine-bearing carbons (C_2 in this case). The irreversibly formed product may be III, an analog of the compound formed by reaction of 2,4-dinitroanisole with base.[165]

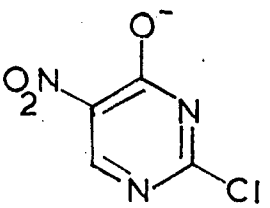
Taylor and Thompson, however, observed that 4-amino-2-chloro-5-nitropyrimidine is completely hydrolysed by 0.5 M sodium hydroxide at 100° to 5-nitrocytosine within 45 minutes.[128] That is, the chloro group is removed under these conditions and attack must occur at C_2. However, the product observed in this work is not 5-nitrocytosine because the spectrum of the latter in basic 96 mole % DMSO is different from that of the product obtained. In fact, 5-nitrocytosine itself appears to undergo an irreversible change in basic 96 mole % DMSO yielding a red species, λ_{max} 357 nm (log e_{max} 4.15); sh 420 nm (log e 3.74). Its spectrum in 0.1 M sodium hydroxide was reported as λ_{max} 219 nm, 253 nm, 353 nm (log e_{max} 3.98, 3.76, 4.16 respectively). Neutralization of the red solution produced by 5-nitrocytosine gave a species with λ_{max} 366 nm.



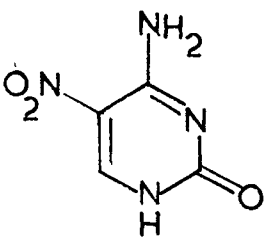
I



II



III



IV

Measurements were made in solutions less than 55 mole % at 430 nm where there is no contribution from the unionized spectrum nor from the irreversible reaction product. The anionic decay was assumed to be a pseudo-first order reaction and plots of ln A_{430} vs. time gave excellent straight lines ($r > 0.990$). The value of A_{430} in each solution, i.e. the extrapolated value at t_o, is the average of two runs. Log I was calculated using Katritzky's method.

Method: K at 430 nm

Mole % DMSO								
7.79	10.18	12.86	15.00	17.60	20.20	22.50	24.88	30.01
Log I								
-0.792	-0.559	-0.346	-0.211	-0.058	0.112	0.298	0.452	0.898

Mole % DMSO at half-ionization: 18.1

The low slope relative to the other pyrimidines is due to interference by product formation. Such divergent slopes were noted by O'Donnell under similar conditions.[10]

19. 2-Amino-s-triazine

Method: Katritzky at 315 nm

Mole % DMSO	34.62	38.96	44.65	49.32	54.46	58.68	62.84
Log I	-0.767	-0.506	-0.167	0.120	0.456	0.667	0.900

Mole % DMSO at half-ionization: 47.05.

20. 1,7-Dimethylguanine

Method: FHK

Mole % DMSO	29.84	34.73	38.80	44.07	48.82	53.61	59.01
Log I	-0.996	-0.699	-0.422	-0.092	0.202	0.517	0.843

Mole % DMSO at half-ionization: 45.9.

21. 1,9-Dimethylguanine

Method: FHK

Mole % DMSO	23.69	29.88	34.46	39.29	43.59	48.75
Log I	-0.908	-0.400	-0.045	0.252	0.545	0.879

Mole % DMSO at half-ionization: 35.85.

22. 3,7-Dimethylisoguanine

This is an example of medium effects being of the same order of magnitude as the spectral changes that accompany ionization, although both are small. The unionized and ionized spectra overlap greatly so there is no part of the ionized spectrum where there is not a sizeable contribution from the neutral compound. At 96 mole % DMSO (0.011 M TMAH), the ionized species has a maximum at 293 nm, e_{max} 10,500 and the unionized species 295 nm, e_{max} 9,500. The spectrum of the latter varies from this value to 289 nm, e_{max}

9,800, in 49.3 mole % DMSO to 285 nm, e_{max} 10,800, in water.

The indicator appears to ionize between 20 mole %-50 mole %. If a titration curve using e at 285 nm is plotted against H_-^N, poor results are obtained because Δe is never large enough to give meaningful results and does not vary regularly with solvent composition. The difference in areas is also too small to calculate log I.

23. 2,8-Dichloro-7-methyladenine

(i) DMSO/water/0.011 M TMAH: Two sets of measurements were made on 23 to test the reproducibility of the measurements. The data sets agree well with each other.

Set A

Mole % DMSO	10.17	12.94	15.09	18.25	20.09	24.78	29.53
Method: K at 300 nm							
Log I	-0.85	-0.61	-0.39	-0.05	0.09	0.47	0.86
*Method: Area							
Log I	-0.74	-0.47	-0.41	0.00	0.17	0.48	--

Set B

Mole % DMSO	10.29	12.52	15.18	17.53	20.13	25.01	29.67
Method: K at 300 nm							
Log I	-0.78	-0.58	-0.40	-0.14	0.06	0.53	0.82
*Method: Area							
Log I	-0.71	-0.46	-0.34	-0.04	0.17	0.66	--

Mole % DMSO at half-ionization: 18.4 (Set A and B plotted together using I values estimated by area method).

(ii) DMSO/water/0.1 M TMAH: Again two sets of measurements were made to test the reproducibility of the data. The extinction coefficients of the anion and the neutral molecule increased with increasing ionic strength. In the first set the spectrum of the unionized molecule was taken in DMSO/water solutions 0.1 M in tetramethylammonium bromide, in the second tetramethylammonium fluoride (TMAF) was used. The fluoride ion is closer in size to the hydroxide ion than is the bromide ion. Agreement between the two sets of data were good however.

Since one cannot assume that the acidity function obeyed by the adenine in 0.1 M base is parallel to an acidity function established in 0.011 M base,[12] titration curves were plotted against mole % DMSO rather than H_-^N.

Set A

Mole % DMSO								
0.22	1.21	2.94	5.61	8.22	9.83	12.48	14.53	17.53
Method: K at 300 nm								
Log I								
-0.885	-0.72	-0.54	-0.29	0.00	0.14	0.40	0.54	0.72
Method: Area								
Log I								
-0.67	-0.51	-0.44	-0.13	0.10	0.40	0.74	--	--

Set B

Mole % DMSO								
0.24	1.30	2.67	5.05	7.60	9.93	12.07	14.61	16.84
Method: K at 300 nm								
Log I								
-0.84	-0.68	-0.55	-0.32	-0.10	0.12	0.33	0.55	0.76

Method: Area

Log I

-0.73 -0.55 -0.48 -0.19 -0.03 0.22 0.48 0.79 --

24. 2-Chloro-7-methyladenine

Mole % DMSO	15.09	17.48	20.50	22.08	24.76	29.85	34.00
Method: K at 300 nm							
Log I	-0.84	-0.65	-0.40	-0.26	-0.07	0.41	0.68
*Method: Area							
Log I	-0.80	-0.61	-0.37	-0.22	-0.02	0.52	0.70

Mole % DMSO at half-ionization: 24.8 (Area method)

25. 2-Chloro-8-methoxy-7-methyladenine

Mole % DMSO	24.76	29.98	34.17	38.64	42.62	45.90
Method: K at 295 nm						
Log I	-0.76	-0.41	-0.12	0.23	0.53	0.84
*Method: Area						
Log I	-0.78	-0.47	-0.12	0.17	0.46	0.74

Mole % DMSO at half-ionization: 36.0 (Area method)

26. 7-Methyladenine

Mole % DMSO

29.85 33.95 38.36 42.65 49.14 53.89 58.77 60.77

Method: K at 300 nm

Log I

-1.00 -0.71 -0.40 -0.12 0.27 0.57 0.765 0.96

*Method: Area

Log I

-0.85 -0.57 -0.31 -0.04 0.40 0.64 -- --

Mole % DMSO at half-ionization: 43.2 (Area method).

27. 2,8-Dichloro-9-methyladenine

Mole % DMSO	29.96	34.18	38.64	44.46	49.00
Method: K at 295 nm					
Log I	-0.82	-0.53	-0.20	0.21	0.56
*Method: Area					
Log I	-0.83	-0.45	-0.12	0.31	--

Mole % DMSO at half-ionization: 40.3 (Area method).

28. 2-Chloro-9-methyladenine

Mole % DMSO	35.09	38.21	44.85	47.56	54.13	59.05
Method: K at 290 nm						
Log I	-0.95	-0.80	-0.27	-0.17	0.32	0.69
*Method: Area						
Log I	--	-0.77	-0.25	-0.16	0.36	--

Mole % DMSO at half-ionization: 49.1 (Area method).

29. 2-Chloro-8-methoxy-9-methyladenine

Mole % DMSO							
48.98	53.46	58.77	60.76	63.09	67.74	68.86	71.44
Method: K at 295 nm							
Log I							
-0.77	-0.51	-0.11	-0.03	0.19	0.56	0.69	0.83
*Method: Area							
Log I							
-0.82	-0.50	-0.12	--	0.18	0.55	0.65	0.83

Mole % DMSO at half-ionization: 60.3 (Area method)

30. 9-Methyladenine

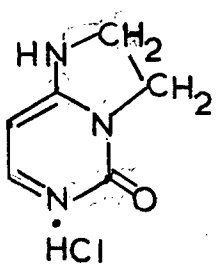
Because of its weak acidity, the upper arm of the titration curve is not long enough to allow a meaningful extrapolation to the

region of ionization, when areas are plotted against H_-^N. The anionic area increases with increasing DMSO content because of the lateral red shift (measurements are within a fixed frame from 260 nm to the end of long wavelength absorption and because of augmentation of e_{A^-}. It cannot be assumed that the anionic area is constant throughout the ionization region. Katritzky's method is more reliable since measurements are made at a broad, flat shoulder.

Mole % DMSO	58.13	63.09	67.74	68.87	71.44	77.49	81.42
*Method: K at 305 nm							
Log I	-0.84	-0.56	-0.29	-0.20	-0.05	0.45	0.66
Method: Area							
Log I	-0.78	-0.45	-0.21	-0.12	0.02	0.51	0.71

Mole % DMSO at half-ionization: 71.5 (K method).

31. 2,3-Dihydro-1H-5-oxoimidazo(1,2-c)pyrimidine Hydrochloride



This secondary amine, pK_{HA} 12.6,[68] was chosen as the anchor compound for the H_-^P acidity function. As it was isolated as the hydrochloride salt a slight excess of TMAH in the cell was necessary to fully remove the hydrochloride proton. In pure DMSO, the unionized spectrum is that of the salt, but the desired spectrum is that of the unprotonated species. To obtain its spectrum in

dilute DMSO solutions without any further deprotonation, a small volume (29 µl) of sodium sesquicarbonate buffer (pH 10.1)[166] was injected into the cell for neutral solutions less than 5 mole % DMSO. For solutions greater than 5 mole %, 120 µl of a saturated sodium bicarbonate buffer (pH 8.5)[166] was sufficient to maintain the indicator in unionized form.

Method: K at 325 nm

Mole % DMSO							
0.26	1.42	2.88	5.32	7.80	10.09	12.65	15.15
Log I							
-0.46	-0.33	-0.18	0.05	0.26	0.43	0.64	0.94

Mole % DMSO at half-ionization: 5.00.

32. 1-Methylcytosine

This indicator shows little spectral change upon ionization and the slope of log I vs. mole % DMSO, when log I is calculated by areas, differs widely from that obtained using log I values determined by Katritzky's method. Log I values found using the two methods diverge badly from each other in solutions greater than 62.8 mole % DMSO.

If e_{A^-} or Q is not extrapolated to the region of ionization and a value or e_{A^-} or Q determined for each solution therein, but an average, constant value of e_{A^-} or Q used throughout to calculate log I, then the slope of log I vs. mole % DMSO will be lowered. Since 32 is a weak acid, the fully ionized portion of the titration curve cannot be meaningfully extended. This may account for the deviation in values of log I. Because of the small change in area upon ionization, the Katritzky values are considered the

more reliable.

Mole % DMSO							
50.55	53.60	58.71	62.77	68.43	71.86	77.03	81.73

*Method: K at 305 nm

Log I							
-0.96	-0.80	-0.46	-0.14	0.22	0.46	0.87	--

Method: Area 265 nm ⟶

Log I							
-0.87	-0.765	-0.53	-0.28	0.06	0.20	0.43	0.65

Mole % DMSO at half-ionization: 65.0 (K method).

33. 1-Methyl-5-bromocytosine

This compound exhibits very little spectral change upon ionization and has no broad sections of the curve suitable for an accurate Katritzky calculation. Estimation of log I was made at 320 nm using Katritzky's method, but the measurements were taken on a steeply sloping portion of the curve and Δe is very small. Hence any error in Δe will greatly affect log I. Values of e_{A^-} in solutions greater than 77.7 mole % DMSO diverge from the flat upper arm of the titration curve defined by e_{A^-} in more dilute solutions and were omitted from the extrapolation. Log I values determined by area are probably more reliable but should, nevertheless, be regarded as approximate.

Mole % DMSO	29.72	29.90	34.52	36.93	39.65	44.26
Method: K at 320 nm						
Log I	-0.76	-0.67	--	-0.20	0.04	0.57
*Method: Area 265 nm ⟶						
Log I	-0.57	-0.50	-0.15	0.085	0.35	0.67

Mole % DMSO at half-ionization: 36.05 (Area method).

34. 1-Methylisocytosine

The anionic spectrum consists of a large absorption at wavelengths less than 260 nm and a low broad one at 285 nm (log e_{max} 3.25 at 63.4 mole % DMSO). In solutions greater than 68.2 mole %, the short wavelength transition shifted to the red more rapidly than that at 285 nm. In fact, at high mole % DMSO, the latter becomes a small shoulder on the former, λ290 nm, log e 3.13.

When performing the Katritzky calculations, all values of e_{A^-} in solutions greater than 68.2 mole % were omitted from the extrapolation. However, all spectral curves up to 96 mole % were used in the area calculation since this method is insensitive to the number of transitions comprising the spectral curve.

Mole % DMSO	17.69	20.04	22.37	25.02	27.31	29.67	35.23

*Method: K at 285 nm

Log I	-0.769	-0.560	-0.360	-0.135	0.081	0.273	0.725

Method: Area

Log I	--	-0.46	-0.28	0.03	0.07	0.29	--

Mole % DMSO at half-ionization: 26.4 (K method).

35. 3-Methylcytosine

The indicator's pK_{BH^+} is 7.0.[68] To maintain the compound in its neutral, unprotonated, state in dilute DMSO/water solutions, 31 µl of a sodium sesquicarbonate solution was injected into the cell before measuring e_{HA}.

Method: FHK

Mole % DMSO	7.83	10.07	12.49	15.07	20.00	22.01
Log I	-0.648	-0.471	-0.258	-0.051	0.436	0.602

Mole % DMSO at half-ionization: 15.4

36. 3-Methylisocytosine

Method: FHK

Mole % DMSO	17.46	19.85	25.09	30.10	34.52	38.96
Log I	-0.896	-0.699	-0.225	0.206	0.575	0.883

Mole % DMSO at half-ionization: 27.9

37. 2',3'-O-Benzylidene-5'-O-tritylcytidine

The absorption maxima of both the unionized and ionized forms were at wavelengths less than 260 nm so that only a portion of the total spectrum was visible. Since the change in area between ionized and unionized forms was small, Katritzky's method was used to calculate log I at 300 nm.

Method: K at 300 nm

Mole % DMSO	40.02	50.97	53.45	59.99	67.49
Log I	-0.605	0.02	0.21	0.465	0.83

Mole % DMSO at half-ionization: 50.8.

APPENDIX B. Spectral Data for the Indicators.

TABLE XXXI. Absorption Maxima and Molar Absorptivities of the Aminopyridines and Aminopyrimidines in Anhydrous DMSO.

Indicator	Observed Values		Literature Values		
	λ_{max} (nm)	log e_{max}	λ_{max} (nm)	log e_{max}	ref.
Aminopyridines					
2-amino	298.5	3.58	229	4.08	
			287.5	3.61[a]	62
2-amino-5-chloro	314	3.54	303.5	3.56[b]	207
2-amino-3,5-dichloro	321	3.72	240	4.04	198
			312	3.65[c]	
2-amino-3-nitro	398	3.86	396	3.86[d]	34
2-amino-5-nitro	sh300	3.57	360	4.18[d]	34
	362	4.22			
2-amino-3-chloro-5-nitro	sh305	3.68			
	359	4.20			
2-amino-3,5-dinitro	329	4.16	318.5	4.01[e]	212
	sh375	3.94			
4-amino	sh275	3.25	253	4.10[a]	62
			sh279	3.21	
4-amino-3-nitro	sh270	3.60	264	3.56[a]	62
	368	3.69	361.5	3.64	
4-amino-3,5-dinitro	sh295	3.39			
	383	3.85			
Aminopyrimidines					
2-amino	298	3.52	224	4.13[b]	54b
			292	3.50	
2-amino-4-chloro	300	3.57	229	4.14[b]	213
			296	3.62	
2-amino-4,6-dichloro	301	3.60	296	3.96[e]	214

TABLE XXXI (Continued)

Indicator	Observed Values		Literature Values		
	λ_{max} (nm)	log e_{max}	λ_{max} (nm)	log e_{max}	ref.
Aminopyrimidines (continued)					
2-amino-5-nitro	333	4.16			
4-amino	274	3.49	233	4.26[f]	54b
			268-9	3.72	
4-amino-2-chloro	277	3.60	232	3.93[b]	213
			272	3.70	
4-amino-2,6-dichloro	278	3.55			
4-amino-2-chloro-5-nitro	348	3.78			
2-amino-s-triazine	266	3.29			

[a] neutral form in water
[b] in water at pH 11.5
[c] in water at pH 7.0
[d] in 80.4% DMSO by weight
[e] in ethanol
[f] in water at pH 13.0

TABLE XXXII. Absorption Maxima and Molar Absorptivities of the Nucleotide Bases in Aqueous DMSO.

Substituent	Observed Values			Literature Values		
	λ_{max}	log e_{max}	Mole % DMSO	λ_{max}	log e_{max}	Ref.
Guanines						
1,7-dimethyl	285	3.84	49.6	250	3.75[a]	77
				283	3.87	
1,9-dimethyl	258	4.15	50.0	255	4.09[a]	77
	sh273	3.99		sh269	4.00	
3,7-dimethyliso-guanine	289	3.99	49.3			
Adenines						
2,8-dichloro-7-methyl	sh273	3.99	30.2			
	279	4.01				
	sh290	3.81				
2-chloro-7-methyl	sh274	4.00	43.6			
	278	4.01				
	sh294	3.79				
2-chloro-7-methyl-8-methoxy	275	4.07	50.2			
7-methyl	271	4.01	50.4	270	4.02[b]	54b
	sh285	3.78				
2,8-dichloro-9-methyl	270	4.22	50.2			
2-chloro-9-methyl	266	4.15	48.5			
2-chloro-8-methoxy-9-methyl	267.5	4.15	49.9			
9-methyl	262	4.18	54.9	262	4.08[b]	54b
2,3-dihydro-1H-5-oxo-imidazo(1,2-c)pyri-midine hydrochloride	297	4.01	0.0[c]	297	4.03[d]	68
	sh310	3.80				

TABLE XXXII (Cont'd.)

Substituent	Observed Values			Literature Values		
	λ_{max}	log e_{max}	Mole % DMSO	λ_{max}	log e_{max}	Ref.
Cytosines						
1-methyl	276	3.85	54.5	278	3.83[e]	74
1-methyl-5-bromo	291.5	3.84	49.3			
3-methyl	293	4.07	0.0[c]	294	4.08[f]	54b
Isocytosines						
1-methyl	258	3.67	50.1	260	3.74[g]	54b
3-methyl	288	3.88	49.6	225	3.86[h]	54b
				284	3.96	
2',3'-O-benzylidene-5'-O-tritylcytidine	[i]					

[a] in water at pH 6.0

[b] pH 11.0

[c] pH 10.0

[d] pH 9.45

[e] 100 mole % DMSO

[f] pH 12.0

[g] pH 13.0

[h] pH 9.8

[i] No absorption maximum at wavelengths greater than 260 nm.

TABLE XXXIII. Absorption Maxima and Molar Absorptivities of the Indicator Anions in Aqueous DMSO.

Substituent	Observed Values			Literature Values		
	λ_{max}	log e_{max}	Mole % DMSO	λ_{max}	log e_{max}	Ref.
Aminopyridines						
2-amino	288	3.48	100[a]			
	378	3.39				
2-amino-5-chloro	293	3.59	100[a]			
	395	3.31				
2-amino-3,5-dichloro	282.5	4.22	96.1			
	397	3.54				
2-amino-3-nitro	502	3.96	96.3	510	3.97[b]	34
	523	3.96				
2-amino-5-nitro	445	4.46	96.1	440	4.43[b]	34
2-amino-3-chloro-5-nitro	439	4.46	96.3			
2-amino-3,5-dinitro	489	4.02	49.3			
4-amino	291	4.18	100[a]			
4-amino-3-nitro	468	3.84	96.3			
Aminopyrimidines						
2-amino	373	3.30	100[a]			
2-amino-4-chloro	262	4.62	96.1			
	360	3.33				
2-amino-4,6-dichloro	351	3.38	96.3			
2-amino-5-nitro	412	4.38	96.2			
4-amino	267	4.19	96.1			
	324	3.27				
4-amino-2-chloro	262	4.19	96.1			
	319	3.35				
4-amino-2,6-dichloro	320	3.27	96.3			
4-amino-2-chloro 5-nitro	430	3.76	49.1			
2-amino-s-triazine	316	3.06	96.3			

TABLE XXXIII (Cont'd.)

Substituent	Observed Values			Literature Values		
	λ_{max}	log e_{max}	Mole % DMSO	λ_{max}	log e_{max}	Ref.
Guanines						
1,7-dimethyl	343	3.72	96.1			
1,9-dimethyl	275	4.13	96.2			
	311	3.80				
3,7-dimethylisoguanine	293	4.02	96.3			
Adenines						
2,8-dichloro-7-methyl	291.5	4.02	85.9[d]			
	290	4.03	71.1[e]			
2-chloro-7-methyl	289	4.07	77.5			
2-chloro-7-methyl-8-methoxy	278	4.13	96.2			
7-methyl	300	4.06	96.1			
2,8-dichloro-9-methyl	285	4.05	96.2			
	sh310	3.89				
2-chloro-9-methyl	282	4.17	96.7			
2-chloro-8-methoxy-9-methyl	274	4.16	96.2			
	sh305	3.64				
9-methyl	286	3.99	96.2			
	sh310	3.89				
2,3-dihydro-1H-5-oxo-imidazo(1,2-c)pyrimidine hydrochloride	323	3.89	96.3	309	3.85[f]	68
Cytosines						
1-methyl	288	3.82	96.3			
1-methyl-5-bromo	297	3.77	96.3			
3-methyl	307	4.00	96.1	294	4.04[g]	68

TABLE XXXIII (Cont'd.)

Substituent	Observed Values			Literature Values		
	λ_{max}	log e_{max}	Mole % DMSO	λ_{max}	log e_{max}	Ref.
Isocytosines						
1-methyl	285	3.25	63.4			
	sh290	3.13	96.1			
3-methyl	318	3.84	96.1			
2',3'-O-benzylidene-5'-O-tritylcytidine	sh275	3.89	96.1			

[a] 0.011 M potassium t-butoxide in anhydrous DMSO

[b] 80.4% DMSO by weight

[c] log e_{max} obtained by extrapolation to time of injection of sample

[d] 0.011 M TMAH

[e] 0.01 M TMAH

[f] 3 M sodium hydroxide

[g] λ_{max} and log e_{max} estimated from spectral curve in 3 M sodium hydroxide given in ref. 68.

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