Desrosiers, R. C., Mulder, C., & Fleckenstein, B. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3839–3843.
Drahovsky, D., & Morris, N. R. (1971a) *J. Mol. Biol. 57*, 475–489.
Drahovsky, D., & Morris, N. R. (1971b) *J. Mol. Biol. 61*, 343–356.
Drahovsky, D., & Morris, N. R. (1972) *Biochim. Biophys. Acta 277*, 245–250.
Fuchs, R., & Daune, M. (1971) *FEBS Lett. 14*, 206–208.
Fuchs, R., & Daune, M. (1972) *Biochemistry 11*, 2659–2666.
Fuchs, R. P. P. (1978) *Anal. Biochem. 91*, 663–673.
Fuchs, R. P. P., Lefèvre, J.-F., Pouyet, J., & Daune, M. P. (1976) *Biochemistry 15*, 3347–3351.
Grunberger, D., & Weinstein, I. B. (1979) in *Chemical Carcinogens* and *DNA* (Grover, P., Ed.) Vol. 2, pp 59–94, CRC Press, Boca Raton, FL.
Holliday, R., & Pugh, J. E. (1975) *Science (Washington, D.C.) 187*, 226–232.
Kalousek, F., & Morris, N. R. (1969) *J. Biol. Chem. 244*, 1157–1193.
Kay, F. R. M., Simmons, N. S., & Dounce, A. L. (1952) *J. Am. Chem. Soc. 74*, 1724–1726.
Kriek, E. (1972) *Cancer Res. 32*, 2042–2048.
Kriek, E. (1974) *Biochim. Biophys. Acta 355*, 177–203.
Kriek, E., Miller, J. A., Juhl, U., & Miller, E. C. (1967) *Biochemistry 6*, 177–182.
Lapeyre, J. N., & Becker, F. F. (1979) *Biochem. Biophys. Res. Commun. 87*, 698–705.
Lotlikar, P. D., Scribner, J. D., Miller, J. A., & Miller, E. C. (1966) *Life Sci. 5*, 1263–1269.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1975) *J. Mol. Biol. 193*, 265–275.
McGhee, J. D., & Ginder, G. D. (1979) *Nature (London) 280*, 419–420.
Miller, J. A. (1970) *Cancer Res. 30*, 559–576.
Millete, R. L., & Fink, L. M. (1975) *Biochemistry 14*, 1426–1432.
Pogo, A. O., Allfrey, V. G., & Mirsky, A. (1966) *Proc. Natl. Acad. Sci. U.S.A. 56*, 550–557.
Riggs, A. D. (1975) *Cytogenet. Cell Genet. 14*, 9–25.
Salas, C. E., & Sellinger, O. Z. (1978) *J. Neurochem. 31*, 85–91.
Salas, C. E., Pfohl-Leszkowicz, A., Lang, M. C., & Dirheimer, G. (1979) *Nature (London) 278*, 71–72.
Scarano, E. (1971) *Adv. Cytopharmacol. 1*, 13–24.
Simon, D., Grünert, F., Von Acken, U., Döring, H. P., & Kröger, H. (1978) *Nucleic Acids Res. 2*, 2153–2167.
Sneider, T. W., Teague, N. N., & Rogachevsky, L. M. (1975) *Nucleic Acids Res. 2*, 1685–1700.
Vanyushin, B. F., Mazin, A. L., Vasilyev, V. K., & Belozersky, A. N. (1973) *Biochim. Biophys. Acta 299*, 397–403.
Waalwijk, C., & Flavell, R. A. (1978) *Nucleic Acids Res. 5*, 4631–4641.
Westra, J. G., Kriek, E., & Hittenhausen, H. (1976) *Chem.-Biol. Interact. 15*, 149–164.

Affinities of Nucleic Acid Bases for Solvent Water†

P. M. Cullis and R. Wolfenden*

ABSTRACT: Equilibria of transfer of pyridine and benzene derivatives, from the vapor phase to dilute aqueous solution, are enhanced by the introduction of exocyclic amino and hydroxyl substituents. Much larger increases are associated with the introduction of imino and keto substituents. Purine derivatives exhibit comparable behavior. These observations are discussed in relation to group transfer potentials, the observed affinities of nucleic acid bases for the active sites of proteins, environmental influences on the occurrence of rare tautomers that lead to errors in base pairing, and hypotheses concerning the origins of the genetic code.

The polar character of amino acids serves as an important determinant of their tendencies to be found at the surface of globular proteins (Kauzmann, 1959; Perutz, 1965), and these tendencies are closely correlated with the absolute affinities of amino acid side chains for solvent water (Wolfenden et al., 1981). Similarly, it seems evident that base pairing and stacking interactions in nucleic acids must usually occur in competition with solvent interactions with the participating groups (Ts'o, 1970). Furthermore, the strengths and specificities of binding of purine and pyrimidine derivatives by biological receptors, including the active sites of enzymes, presumably reflect the requirement that solvent water be stripped away (at least in part) from both the ligand and the receptor. It would therefore be of interest to have information concerning the affinities of nucleic acid bases for watery surroundings. This information would also be useful for determining the influence of changing solvation on the observed equilibria of metabolic transformation of purine and pyrimidine derivatives in aqueous solution and for testing certain hypotheses concerning factors that may have influenced the early evolution of the genetic code.

Affinities of organic compounds for solvent water can be evaluated, in an absolute sense, by measuring equilibrium constants for their transfer from dilute aqueous solution to the vapor phase. The hydrophilic character of complex molecules, determined in this way, can generally be predicted with reasonable accuracy from their constituent groups (Butler, 1937; Hine & Mookerjee, 1975). In the present study, purine and pyrimidine bases were found to be insufficiently volatile for detection in the vapor phase at room temperature, and pyridine derivatives were therefore examined as models. Derivatives of the nucleic acid bases themselves were examined in solvent–solvent distribution experiments in order to investigate the validity of these models.

† From the Department of Biochemistry, University of North Carolina, Chapel Hill, North Carolina 27514. *Received October 2, 1980.* Supported by grants from the National Science Foundation (PCM-7823017) and the National Institutes of Health (GM-18325). P.M.C. was supported by a fellowship from the Jane Coffin Childs Foundation.

0006-2960/81/0420-3024$01.25/0

In neutral aqueous solution, equilibria of nucleic acid bases have been found to favor keto over enol tautomers, and amino over imino tautomers, by factors in the neighborhood of 10^4–10^5 in derivatives of cytosine (Kenner et al., 1955), uracil (Katritzky & Warning, 1962), and adenine (Wolfenden, 1969a). During the biosynthesis of nucleic acids and proteins, errors in base pairing may arise as a result of the occurrence of rare tautomers (Watson & Crick, 1953; Topal & Fresco, 1976). If free energies of solvation of two tautomers differ, then the position of their tautomeric equilibrium is expected to be sensitive to changes in the nature of the solvent environment. In pyridine derivatives, there is evidence that effects of this kind can be of considerable magnitude (Beak & Fry, 1973; Beak et al., 1976b). Accordingly, we have attempted to compare the strengths of interaction of different tautomers with solvent water, by determining the hydrophilic character of appropriately substituted derivatives.

Materials and Methods

1-Methyl-2-iminopyridine was prepared by the method of Tschitschibabin et al. (1921) and recrystallized from ethanol as the hydrogen iodide salt. Other pyridine derivatives were obtained from Aldrich Chemical Co. Of these, 2-pyridone and 2-aminopyridine were purified by repeated sublimation, while 1-methyl-1-pyridone and 2-methoxypyridine were purified by repeated fractional distillation under reduced pressure. Methylated purine and pyrimidine derivatives, obtained from Vega Biochemical Co., were used without further purification.

Distribution coefficients between solutions and the vapor phase were determined by measuring the ultraviolet absorbance of solutes in the gas space over solutions of known concentration. These measurements were made in cylindrical cuvettes, of 10- and 100-cm path length, maintained at 25 °C with water-heated brass jackets. After introduction of a small volume of aqueous solute, the supernatant gas space was scanned at intervals until a stable spectrum was observed. With the notable exception of 2-pyridone, spectra in the vapor phase were closely similar to spectra in hexane, so that is seemed reasonable to use extinction coefficients determined in this solvent to determine concentrations in the vapor phase. Errors introduced by this approximation are likely to be minor in comparison with the large differences in observed distribution coefficients between the various compounds included in this study.

Two compounds included in this study, 2-pyridone and 1-methyl-2-pyridone, proved insufficiently volatile to be detectable over aqueous solutions at concentrations in excess of 1 M. Their water-to-vapor distribution coefficients were estimated by combining the vapor pressures of the pure compounds (determined as described above for aqueous solutes), their solubilities in an organic solvent (hexane or 1-octanol), and their coefficients of distribution between this organic solvent and water. Distribution coefficients between the organic solvent and water were determined by ultraviolet absorbance, after the two phases had been shaken together at 25 °C for 30 min.

For derivatives of the nucleic acid bases, all of which were sparingly soluble, distribution coefficients were estimated from the ratio of solubilities of the pure crystalline material in water and in 2-butanol. Solubilities were measured by shaking an excess of the solid with an appropriate volume of solvent in a sealed ampule. Samples were allowed to equilibrate during a minimum of 48 h at 25 °C before the amount of material in the supernatant was determined by ultraviolet absorbance, and measurements were checked at later intervals to confirm that saturation had been achieved. Further measurements were made after additional solid had been added, to ensure that the observed equilibrium was not dependent on the amount of solid added (as might have been the case if soluble contaminants had been present).

Where solubilities and sensitivities of detection permitted, distribution coefficients were determined, and shown to be invariant, over at least a 5-fold range of solute concentrations. Exceptions are noted below.

Table I: Water Affinities of Model Pyridine Compounds

		log $(C_w/C)^b$		
	pK_a[a]	vapor[c]	hexane[c]	octanol[d]
	3.28	2.96	0.49	−1.36
	5.17	3.44[e]	0.65	−0.64
	6.7	5.96	1.82	−0.54
	13.02		2.4[g]	
	0.32	7.35[f]	2.96	0.24
	0.75	≥10[f]	5.52[h]	0.58

[a] pK_a values taken from the *CRC Handbook of Biochemistry*; 1-methyl-2-iminopyridine (Cook et al., 1972). [b] The results of this study were determined by ultraviolet absorbance using the following spectroscopic data: 2-methoxypyridine, UV (decane) λ_{max} 275 nm (ϵ 4000) (Beak et al., 1976b) and UV (water, pH 7) λ_{max} 269 nm (ϵ 3230) (Beak et al., 1976a); pyridine, UV (cyclohexane) λ_{max} 251 nm (ϵ 3100) (*CRC Handbook of Chemistry and Physics*) and UV (water) λ_{max} 256 nm (ϵ 3150) (this study); 2-aminopyridine, UV (hexane) λ_{max} 288 nm (ϵ 4500) (this study) and UV (water) λ_{max} 289 nm (ϵ 3700) (this study); 1-methyl-2-iminopyridine, UV (0.1 M HCl) λ_{max} 299 nm (ϵ 5900) (this study); 1-methyl-2-pyridone, UV (decane) λ_{max} 310 nm (ϵ 3000) (Beak et al., 1976b) and UV (water, pH 5) λ_{max} 297 nm (ϵ 5700) (Beak et al., 1976a); 2-pyridone, UV (water, pH 6) λ_{max} 392 nm (ϵ 5900) (Beak et al., 1976a). [c] Results of this study. [d] Hansch & Leo (1979). [e] Hine & Mookerjee (1975). [f] Derived from the vapor pressure over the pure compound, its solubility in 1-octanol, and its distribution coefficient for transfer from 1-octanol to water. [g] The distribution was determined from 2 M KOH to maintain the pyridine derivative substantially in the un-ionized state; the amount of solute transferred to the hexane phase was determined by evaporating an appropriate volume of the hexane phase with a stream of nitrogen, dissolving the residue in 0.1 M HCl, and determining the concentration by UV absorbance using the above extinction coefficient. [h] The distribution was measured directly at a concentration in the aqueous phase of 0.09 M and corrected for the extent of solute association in the organic phase, assuming an association constant of 5.7×10^6 M^{-1} (Beak et al., 1976b).

Results

Of the pyridine derivatives examined, 2-pyridone was extreme in its affinity for water (Table I). Its distribution between water and hexane was found to be concentration dependent, in accord with earlier observations by Beak et al. (1976b) that this compound is strongly associated in nonpolar solvents. The value shown in Table I has been corrected for the amount of dimer present in hexane, estimated from an association constant of $\sim 5.7 \times 10^6$ M^{-1} measured earlier in decane and cyclohexane (Beak et al., 1976b) and confirmed by us in hexane. Its distribution between water and the vapor phase (Table I) is estimated as an upper limit, calculated from a combination of the vapor pressure of the crystalline material, its solubility in 1-octanol, and its distribution coefficient be-

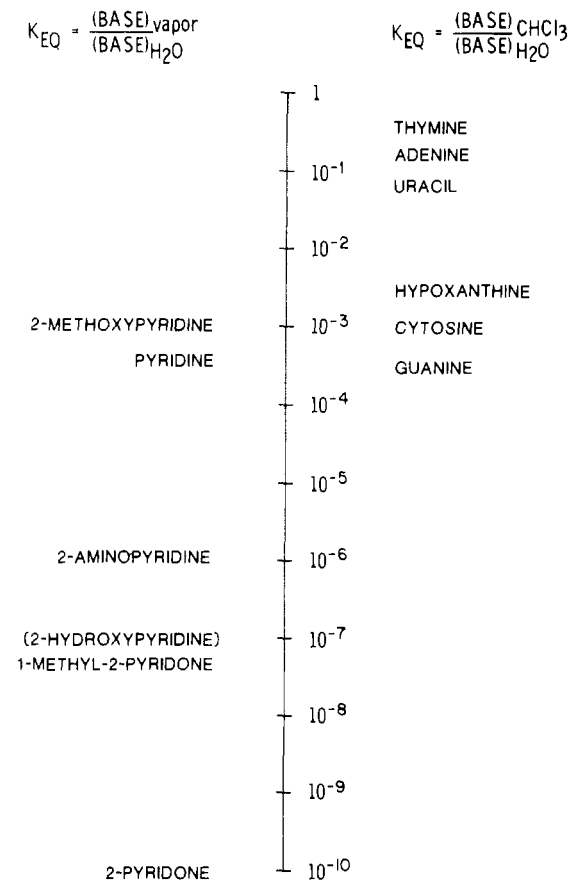


FIGURE 1: Relative affinities of pyridines and methylated nucleic acid bases for solvent water.

tween 1-octanol and water. The vapor pressure of the pure crystalline material at 25 °C was found to be so low that, even with a cuvette of 1-m path length, the ultraviolet absorbance of the vapor phase was near the lower limits of detection.

Substituted pyridine showed distribution coefficients, for transfer from dilute aqueous solution to the vapor phase, with a range of values that differed by a factor $>10^7$ (Table I). For their transfer from water to hexane, distribution coefficients were considerably more favorable and spanned a range of values that differed by a factor of $\sim 10^5$ (Table I). As shown in the final column of Table I, distribution coefficients determined earlier, for transfer of these pyridine derivatives from water to 1-octanol, are more favorable still, with a range of reported values that varies by a factor of <100. Distribution coefficients for some correspondingly substituted benzenes are shown in Table II.

Derivatives of the nucleic acid bases were found to be insufficiently volatile for detection in the vapor phase over their aqueous solutions and were therefore examined in solvent–solvent distribution experiments. Table III shows distribution coefficients of methyl-substituted purines and pyrimidines between water and chloroform and between water and 2-butanol. In both sets of measurements the range of values was relatively modest compared with the range of values in Table I, but a reasonably clear distinction could be made between the bases in terms of their distributions between water and chloroform.

For purposes of discussion, the water-to-vapor distributions of substituted pyridines, along with the relative water-to-chloroform distributions of the nucleic acid bases, are displayed on a logarithmic scale in Figure 1.

Discussion

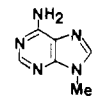
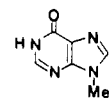
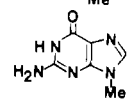
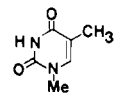
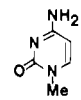
Influence of Structure on Hydrophilic Character. Despite

Table II: Water Affinities of Related Substituted Benzenes

	log (C_w/C)		
	vapor[a]	hexane[b]	octanol[b]
	0.66	−2.22	−2.14
	0.77	−2.10	−2.08
	4.15[c]	0.065	−0.9
	4.79	0.82	−1.5

[a] Hine & Mookerjee (1975). [b] Hansch & Leo (1979). [c] Result of this study; the concentration in the vapor phase was determined by UV absorbance assuming the extinction coefficient in the vapor phase to be the same as in hexane, λ_{max} 287 nm (ϵ 1800).

Table III: Water Affinities of Nucleic Acid Bases

	log (C_w/C)[a]	
	chloroform[b]	2-butanol[c]
	0.78	0.79
	2.52[c]	1.77
	3.52[c]	1.35
	0.45	0.82
	1.21	1.37
	3.00	1.79

[a] The distribution coefficients were estimated from the ratio of the absorbance in the aqueous phase to that in the organic phase (assuming the extinction coefficients in the two phases to be the same; see text). The following λ_{max} values were used: 9-methyladenine, 260 nm; 9-methylhypoxanthine, 250 nm; 9-methylguanine, 251 nm; 1-methylthymine, 271 nm; 1-methyluracil, 265 nm; 1-methylcytosine, 272 nm. [b] Measured directly by solvent–solvent distribution. [c] The ratio of the solubility in water over that in the organic solvent.

apparently similar opportunities for hydrogen-bonding interactions with solvent water, 2-aminopyridine is several orders of magnitude less hydrophilic than 2-pyridone, regardless of whether hexane or the vapor phase is used as a reference. It thus appears that an amino group adjacent to the nitrogen atom in a heterocyclic base is very much less hydrophilic than a keto group at an equivalent position. In keeping with this generalization, 9-methyladenine is found to be much less hydrophilic than 9-methylhypoxanthine (Table III).

On comparison of 2-aminopyridine with pyridine itself, the introduction of an amino group in place of hydrogen is seen to result in an increase in hydrophilic character (Table I). Similarly, 9-methylguanine is somewhat more hydrophilic than

9-methylhypoxanthine (Table III). In the present study, and in earlier reports (Tinker & Brown, 1948; Garel et al., 1973), little difference was observed between guanine and hypoxanthine in their distributions between water and alcohols. A likely explanation lies in the levelling effect of the reference phase. When the nonpolar solvent is an alcohol, hydrogen-bonding interactions are possible in both phases and differences in distribution coefficient are reduced accordingly. Table I shows, for example, that differences between 2-aminopyridine and pyridine, substantial in terms of their water-to-vapor and water-to-hexane distributions, almost vanish when 1-octanol is used as the reference phase.

1-Methyl-2-iminopyridine and 1-methyl-2-pyridone are similar in hydrophilic character and both are more hydrophilic than 2-aminopyridine. These findings suggest that the imino function, like the keto function, is strongly hydrophilic, since the methyl substituent is itself somewhat hydrophobic as compared with substituent hydrogen. Beak et al. (1976) have examined keto–enol equilibria of 2-pyridone in the vapor phase and find that the enol tautomer that is rare in water becomes relatively abundant at equilibrium in the vapor phase. In the pyridine series, the absence of an appropriate model obviates the possibility of comparing substituent hydroxyl and amino functions directly, with respect to their hydrophilic character. Water-to-vapor distribution coefficients observed for phenol and aniline (Table II) suggest that these substituents are similar in their affinities for solvent water.

These findings indicate that in heterocyclic bases, amino and hydroxyl functions adjacent to ring nitrogen atoms confer moderately hydrophilic tendencies on heterocyclic bases, whereas imino and keto functions adjacent to ring NH groups exert a more profoundly hydrophilic influence. The unusual hydrophilic character of the –CO–NH– function has been noted previously in models for the peptide bond (Wolfenden, 1978).

An exception to these tendencies is noted if 1-methylcytosine is compared with 1-methyluracil (Table III). There were no indications of self-association of either of these bases, nor did UV spectra in chloroform differ significantly from spectra in water, rendering it unlikely that the anomaly was due to changes in tautomeric equilibria in passing from one phase to the other. In previous investigations, hydrophilic character has seldom been found to depart very much from expectations based on the additivity of effects of substituent groups. The few recorded exceptions have involved compounds that can establish intramolecular hydrogen bonds in the absence of water, such as ethylene glycol (Hine & Mookerjee, 1975), or that have arrangements of atoms such that hydrogen-bonding interactions with water at one site tend to reinforce interactions at another site by inductive effects, as in water itself (Frank & Wen, 1957), *p*-nitrophenol (Hine & Mookerjee, 1975), and imidazole (Wolfenden et al., 1981). Uracil and cytosine, in which many potential hydrogen-bonding groups are tightly clustered together, may be especially susceptible to effects of this kind. Anomalies may also arise from specific attractive or repulsive interactions between water molecules within the highly structured solvation shell that probably surrounds these highly polar molecules.

Solvent Effects on Group Transfer Potential and Enzyme–Substrate Interaction. The free energy change associated with hydrolysis of adenosine to inosine, expressed in terms of nonionized reactants and products, is –2.2 kcal/mol at 25 °C (Wolfenden, 1967), with water activity taken as unity in dilute aqueous solution. If the observed difference in free energy of solvation between 2-pyridone and 2-aminopyridine (from water-to-vapor distribution coefficients) applies also to inosine and adenosine, then the favorable free energy change associated with adenosine hydrolysis, mentioned above, is somewhat exceeded by the increase in negative free energy of solvation (–3.3 kcal/mol or more) when the reactants are converted to the products. Thus, the reaction would be endergonic in the vapor phase and can be considered to be "driven" by changing solvation.

A knowledge of absolute affinities for water may be useful in attempting to rationalize the relative affinities that various ligands exhibit for the active site of the same enzyme. The substrate adenosine exhibits an apparent dissociation constant (3.1×10^{-5} M) from the active site of adenosine deaminase that is considerably lower than the dissociation constant of its complex with the product inosine (1.6×10^{-4} M) (Wolfenden, 1969b). A specific attactive force, involving the 6-amino group of adenosine, might be postulated to explain this difference in affinities, but it has been shown that purine ribonucleoside, with hydrogen as a 6 substituent, is bound even more tightly ($K_i = 9.3 \times 10^{-6}$ M) (Wolfenden et al., 1969). This order of binding affinities seems understandable in terms of the relative difficulty of removing the model compounds 2-aminopyridine, 2-pyridone, and unsubstituted pyridine from solvent water (Table I).

Similarly, 2-aminopyridine and 2-pyridone are structurally related to each other as aminopterin is related to folic acid. The sense and approximate magnitude of difference between the free energies of solvation of 2-aminopyridine and 2-pyridone roughly matches the very large difference in apparent free energies of dissociation of aminopterin and folic acid from the active site of dihydrofolate reductase (Werkheiser, 1961). This would seem understandable if binding of both compounds were associated with removal of part of the pteridine ring from solvent water into an environment of low dielectric constant. The water-leaving character of 2-aminopyridine greatly exceeds that of 2-pyridone but it itself exceeded by the water-leaving character of unsubstituted pyridine (Table I). If binding affinities of dihydrofolate reductase are determined by the relative ease with which its ligands can be removed from water, then it would seem worthwhile to examine the possibility that an analogue of folic acid, in which hydrogen replaced oxygen as a substituent at the 4 position, might inhibit the enzyme effectively.

Influence of Solvent Water on Tautomeric Equilibria. As mentioned earlir, Beak & Fry (1973) have shown that 2-pyridone favors the hydroxy tautomer at equilibrium in the vapor phase by a factor of ~2.5, whereas the keto tautomer is favored overwhelmingly in solution. Comparison of pK_a values of the methylated tautomers (Figure 1) suggests that in water, the keto tautomer is ~1000-fold more abundant than the hydroxy tautomer at equilibrium. Combining these values with the present limiting value for the water affinity of the keto form of 2-pyridone, one may estimate a distribution coefficient in the neighborhood of 10^{-7} for transfer of the hydroxy tautomer from water to the vapor phase. This value is comparable in magnitude to that for 2-aminopyridine, just as the value for phenol is comparable with that for aniline (Table II).

Values observed for the pK_a of 2-aminopyridine and 1-methyl-2-iminopyridine (Cook et al., 1972) suggest that the amino tautomer predominates in aqueous solutions of 2-aminopyridine by a factor in the neighborhood of 10^6. In terms of its distribution from water to hexane, 1-methyl-2-iminopyridine is more hydrophilic than 2-aminopyridine (Table I). Since the imino tautomer of 2-aminopyridine can be expected

to be even more hydrophilic than 1-methyl-2-iminopyridine, these results suggest that in the vapor phase, equilibrium favors the amino tautomer even more than it does in water.

In keeping with these models, the apparent tautomeric equilibria of the nucleic acid bases in water strongly favor forms with exocyclic keto and amino groups (see introduction). The findings of Beak & Fry (1973) suggest, by analogy with the behavior of 2-pyridone, that hydroxy forms of nucleic acid bases may occur much more abundantly in an environment of low dielectric constant. The present results suggest that the dominance of amino tautomers in water, as in 2-aminopyridine, is likely to be reinforced by transfer to nonpolar surroundings. The few studies that have been done on the nucleic acid bases in the vapor phase have shown that, at elevated temperatures, the major tautomers present are the same in the vapor phase as in aqueous solution (Shugar, 1979). In the present investigation also, spectra of the bases in chloroform at room temperature were found to resemble those in water, both in band shape and λ_{max}. In hexane, Beak et al. (1976b) have observed that the spectrum of 2-pyridone does not resemble its spectrum in the vapor phase, due to extensive dimerization. In the present experiments, self-association of purine and pyrimidine derivatives did not appear to occur to a significant extent in chloroform, as judged from the absence of any dependence of their distribution coefficients on solute concentration. In general, interactions of this kind have been found to be weak: 1-cyclohexyluracil, for example, was found to exhibit an association constant in chloroform of 6.1 M^{-1} at 25 °C (Kyogoku et al., 1967).

One is left to conclude that nucleic acid bases may undergo substantial shifts in the positions of their tautomeric equilibria when they are removed from water to a medium of low dielectric constant but that the tautomers that are favored in water continue to be favored in its absence. Transitions that would depend on the occurrence of rare enol tautomers appear more likely to occur in nonpolar environments. Transitons that would require rare imino tautomers seem even less likely in the absence of water than in its presence.

Water Affinities and the Genetic Code. Following the early recognition that similar code words pertain to amino acid with apparently similar properties (Nirenberg et al., 1963), considerable attention has been devoted to the possible origin of this relationship [for discussions, see Crick (1968), Woese (1969), and Jungck (1978)]. Nagyvary & Fendler (1974) have proposed one model for the early evolution of the genetic code that would have involved cocompartmentation of amino acids and nucleotides with similar physical properties. Weber & Lacey (1978) have proposed a specific relationship between the water affinity of amino acids and the water affinity of the second base that appears in each of the corresponding anticodons in tRNA. This hypothesis can be tested against the present results, in conjunction with the absolute affinities of amino acid side chains for solvent water at pH 7 (Wolfenden et al., 1979, 1981). We find that uracil, the most common anti-code letter at the second position among the more hydrophilic amino acids, is itself quite hydrophilic. Adenine, which serves as the second anti-code letter for many hydrophobic amino acids, is less hydrophilic than uracil. However, cytosine and guanine, with greater affinities for water than that of uracil, serve as second anti-code letters for amino acids that are distributed throughout the scale of hydrophilic character. These results cannot rule out the possibility that a relationship, of the kind proposed by Weber and Lacey, may have existed at some point during the early evolution of the genetic code when a simpler set of components may have been present. At the present time, there appears to be no strict relationship between the water affinities of amino acid side chains and the water affinities of bases at the second positions of their codons or anticodons.

References

Beak, P., & Fry, F. S., Jr. (1973) *J. Am. Chem. Soc. 95*, 1700–1702.

Beak, P., Fry, F. S., Jr., Lee, J., & Steele, F. (1976a) *J. Am. Chem. Soc. 98*, 171–179.

Beak, P., Covington, J. B., & Smith, S. G. (1976b) *J. Am. Chem. Soc. 98*, 8284–8286.

Butler, J. A. V. (1937) *Trans. Faraday Soc. 33*, 229–236.

Cook, M. J., Katritzky, A. R., Linda, P., & Tack, R. D. (1972) *J. Chem. Soc., Perkin Trans. 2*, 1295–1302.

Crick, F. H. C. (1968) *J. Mol. Biol. 38*, 367–379.

Frank, H. S., & Wen, W. Y. (1957) *Discuss. Faraday Soc. 24*, 133–139.

Garel, J. P., Filliol, D., & Mandel, P. (1973) *J. Chromatogr. 78*, 381–390.

Hansch, C., & Leo, A. J. (1979) *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York.

Hine, J., & Mookerjee, P. K. (1975) *J. Org. Chem. 40*, 292–298.

Jungck, J. R. (1978) *J. Mol. Evol. 11*, 211–224.

Katrizky, A. R., & Waring, A. J. (1962) *J. Chem. Soc.*, 1540–1552.

Kauzmann, W. (1959) *Adv. Protein Chem. 16*, 1–63.

Kenner, G. W., Reese, C. B., & Todd, A. R. (1955) *J. Chem. Soc.*, 855–864.

Kyogoku, Y., Lord, R. C., & Rich, A. (1967) *J. Am. Chem. Soc. 89*, 496–504.

Nagyvary, J., & Fendler, J. H. (1974) *Origins Life 5*, 357–362.

Nirenberg, M. W., Jones, O. W., Leder, P., Clark, F. C., Sly, S., & Petska, S. (1963) *Cold Spring Harbor Symp. Quant. Biol. 28*, 549–557.

Perutz, M. (1965) *J. Mol. Biol. 13*, 646–670.

Shugar, D. (1979) in *Antimetabolites in Biochemistry, Biology and Medicine* (Skoda, J., & Langen, P., Eds.) pp 139–150, Pergamon Press, New York.

Tinker, J. F., & Brown, G. B. (1948) *J. Biol. Chem. 173*, 585–588.

Topal, M. D., & Fresco, J. R. (1976) *Nature (London) 263*, 285–289.

Tschitschibabin, A. E., Konowalowa, R. A., & Konowalowa, A. A. (1921) *Ber. Dtsch. Chem. Ges. 54B*, 814–820.

Ts'o, P. O. P. (1970) *Biol. Macromol. 4*, 49–190.

Watson, J. D., & Crick, F. H. C. (1953) *Nature (London) 171*, 964.

Weber, A. L., & Lacey, J. C., Jr. (1978) *J. Mol. Evol. 11*, 199–210.

Werkheiser, W. C. (1961) *J. Biol. Chem. 236*, 888–893.

Woese, C. (1969) *J. Mol. Biol. 43*, 235–240.

Wolfenden, R. (1967) *J. Biol. Chem. 242*, 4711–4714.

Wolfenden, R. (1969a) *J. Mol. Biol. 40*, 307–310.

Wolfenden, R. (1969b) *Biochemistry 8*, 2409–2411.

Wolfenden, R. (1978) *Biochemistry 17*, 201–204.

Wolfenden, R., Kaufman, J., & Macon, J. B. (1969) *Biochemistry 8*, 2412–2415.

Wolfenden, R., Cullis, P. M., & Southgate, C. C. B. (1979) *Science (Washington, D.C.) 206*, 575–577.

Wolfenden, R., Andersson, L., Cullis, P. M., & Southgate, C. C. B. (1981) *Biochemistry 20*, 849–856.