

[CONTRIBUTION FROM THE DEPARTMENT OF RADIOLOGICAL SCIENCES, THE JOHNS HOPKINS UNIVERSITY, BALTIMORE, MARYLAND, AND THE DIVISION OF CHEMISTRY AND CHEMICAL ENGINEERING, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA¹]

Interaction and Association of Bases and Nucleosides in Aqueous Solutions. II. Association of 6-Methylpurine and 5-Bromouridine and Treatment of Multiple Equilibria²

BY PAUL O. P. TS'O³ AND SUNNEY I. CHAN

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The molal osmotic coefficients and molal activity coefficients of 6-methylpurine and 5-bromouridine in aqueous solution over the concentration range of 0.1 to 0.4–0.7 *m* have been determined by thermoelectric measurements of vapor pressure lowering at 25°. The data indicate that 6-methylpurine tends to associate more extensively than purine and 5-bromouridine more extensively than uridine. Analyses of the osmotic coefficients and activity coefficients based on a new treatment of multiple equilibria led to equilibrium constants for the associations and the apparent limiting sizes of the polymers.

Introduction

In a previous communication, the molal osmotic coefficients (ϕ) and molal activity coefficients (γ) of purine, uridine, and cytidine in the aqueous concentration range 0.1 to 1.0 *m* were reported.⁴ The data indicated that these solutes associate extensively in solution and that the association process does not proceed simply to the dimer stage, but continues to form higher polymers. At that time, we proposed that hydrophobic and vertical-stacking interactions (van der Waals interactions, π -electron interactions) are the predominant driving forces responsible for these associations. In the following paper of this series,⁵ the proton magnetic resonance spectra of purine and 6-methylpurine have also been examined to further elucidate the nature of these associations. From the concentration dependence of the n.m.r. spectrum, it was concluded that the mode of association is that of vertical stacking of rings in a partial overlapping fashion.⁵

In this paper, we report the osmotic coefficients and activity coefficients of 6-methylpurine and 5-bromouridine. It would be of interest to establish the effect of methyl and bromo substitution on the association tendencies of purine and uridine. The results indicate that these derivatives have a much stronger tendency to associate than do the unsubstituted compounds.

Considerable insight into the association processes was obtained from a mathematical analysis of the osmotic and activity coefficients in terms of various mathematical models for the multiple equilibria. A calculated fit to the experimental data on the basis of these assumed models is an obligatory though not necessarily a sufficient requirement to indicate the validity of these models. Based on these tested models, populations of the unassociated and associated species in these solutions have been calculated. The population distribution of these species so computed serves as a liaison between the thermodynamic osmotic results and the proton magnetic resonance results.⁵

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(3) The major part of this research was completed at the California Institute of Technology where P. O. P. T. was on leave of absence from The Johns Hopkins University.

(4) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Am. Chem. Soc.*, **85**, 1289 (1963).

(5) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. J. Helmkamp, *ibid.*, **86**, 4182 (1964).

Experimental

Materials.—6-Methylpurine was obtained from Cyclo Chemical Corp., Los Angeles, Calif. This compound was further purified by sublimation *in vacuo* (melting with decomposition 229–231°). The compound was used within one month's time and was stored at –10°. 5-Bromouridine was obtained from California Corp. for Biochemical Research, Los Angeles, as A grade material (nitrogen 8.66% and m.p. 217–219°). It was used without further purification.

Measurement of the Osmotic Concentration by Vapor Pressure Lowering.—Osmotic measurements were made using the Mechrolab Model 301 A osmometer, as reported previously.⁴

Definitions and Equations Relating the Osmotic and Activity Coefficients.—The osmotic concentration is defined as $\nu m\phi$, where ν is the number of species in solution per molecule of solute (one for purine and nucleoside), m is the molality of the solute, and ϕ is the molal osmotic coefficient.⁶

The relationship between the molal osmotic coefficient and the molal activity coefficient, γ , can be derived from the Gibbs-Duhem equation⁷ and is given by

$$\ln \gamma = (\phi - 1) + \int_0^m (\phi - 1) d \ln m \quad (1)$$

In this work an empirical sixth-degree polynomial, eq. 2, calculated from the experimental data by the method of least squares,⁸ was used to express ϕ as a function of m . By direct integration of this empirical polynomial, γ was obtained as a function of m .

$$\phi = 1 + a_1 m + a_2 m^2 + a_3 m^3 + a_4 m^4 + a_5 m^5 + a_6 m^6 \quad (2)$$

$$\ln \gamma = 2a_1 + \frac{3}{2}a_2 m^2 + \frac{4}{3}a_3 m^3 + \frac{5}{4}a_4 m^4 + \frac{6}{5}a_5 m^5 + \frac{7}{6}a_6 m^6 \quad (3)$$

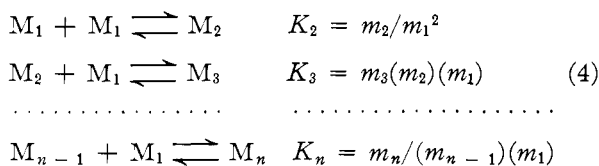
Interpretation and Analysis of the Osmotic Data.—The osmotic properties of these solutions were analyzed in terms of a multi-stage association process. Certain general relationships pertinent to these analyses will first be presented. In particular, an extremely useful relation between the molal activity coefficient and the fraction of monomers in solution will be derived. This will be followed by a discussion of specific multiple equilibria based upon different assumptions concerning the association constants for each successive association step.

The association process is assumed to occur by way of the following steps

(6) The convention and symbols are essentially the same as those employed by R. A. Robinson and R. H. Stokes, in "Electrolyte Solutions," Academic Press, Inc., New York, N. Y., 1959.

(7) G. N. Lewis, M. Randall, K. S. Pitzer, and L. Brewer, "Thermodynamics," 2nd Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1961, p. 322.

(8) The actual calculations were carried out on the IBM 7090 computer at the computer center of the California Institute of Technology. We would like to acknowledge F. Lawler for his assistance in setting up the computer program.



Here, we have denoted the concentration of monomer (M_1), dimer (M_2), trimer (M_3) species in solution by m_1, m_2, m_3 , etc. Because of the above association, the true molality of the solution, \bar{m} , is then

$$\begin{aligned}
 \bar{m} &= m_1 + K_2(m_1^2) + K_2K_3(m_1)^3 + \dots + K_2K_3\dots K_n(m_1)^n \\
 &= \sum_{i=1}^n \left(\prod_{j=1}^{i-1} K_j \right) (m_1)^i \quad K_1 = 1
 \end{aligned}
 \tag{5}$$

The stoichiometric molality, m , is by definition

$$\begin{aligned}
 m &= m_1 + 2K_2(m_1)^2 + 3K_2K_3(m_1)^3 + \dots + nK_2K_3\dots K_n(m_1)^n \\
 &= \sum_{i=1}^n i \left(\prod_{j=1}^{i-1} K_j \right) (m_1)^i
 \end{aligned}
 \tag{6}$$

It is readily shown upon differentiation that

$$m/m_1 = d\bar{m}/dm_1 \tag{7}$$

In dilute solution,

$$\phi \cong \frac{\bar{m}}{m} \quad \text{or} \quad \bar{m} = \phi m \tag{8}$$

Differentiating eq. 8, we get

$$d\bar{m} = \phi dm + m d\phi \tag{9}$$

which when combined with eq. 7 yields

$$d \ln \frac{m_1}{m} = (\phi - 1) d \ln m + d\phi \tag{10}$$

Integrating eq. 10, and noting that both m_1/m and ϕ approach unity at infinite dilution, we find that

$$\ln \frac{m_1}{m} = (\phi - 1) + \int_0^m (\phi - 1) d \ln m \tag{11}$$

A comparison of eq. 1 and 11 yields the interesting result that

$$m_1/m = \gamma \tag{12}$$

Equation 11 has previously been derived by Kreuzer⁹ in another connotation. However, eq. 12, which asserts that the molal activity coefficient is equal to the mole fraction of monomers in solution, while an obvious result, has not been fully exploited before.

It is important to note that eq. 11 and 12 are completely general and are applicable over the same concentration range where eq. 8 is valid. In theory, no assumptions need to be made regarding the various association constants and the limiting size of the polymer formed. In practice, however, it would be impossible to consider the general case where all the association constants are different and therefore certain simplifying assumptions must be made about them before useful information can be extracted from the osmotic data. Fortunately, these simplifying assumptions, as we shall show, are extremely reasonable for the systems under study.

The usefulness of eq. 11 or 12 will now be shown. In this connection, several models for the multiple equilibria pertinent to our interpretation of the osmotic data for purine, 6-methylpurine, and 5-bromouridine will be discussed. The various models differ in their assumptions regarding the association constants for the successive association steps and the size of the highest polymer.

(1) We will first assume that the association constants are the same for each successive step, and to be as general as possible, we

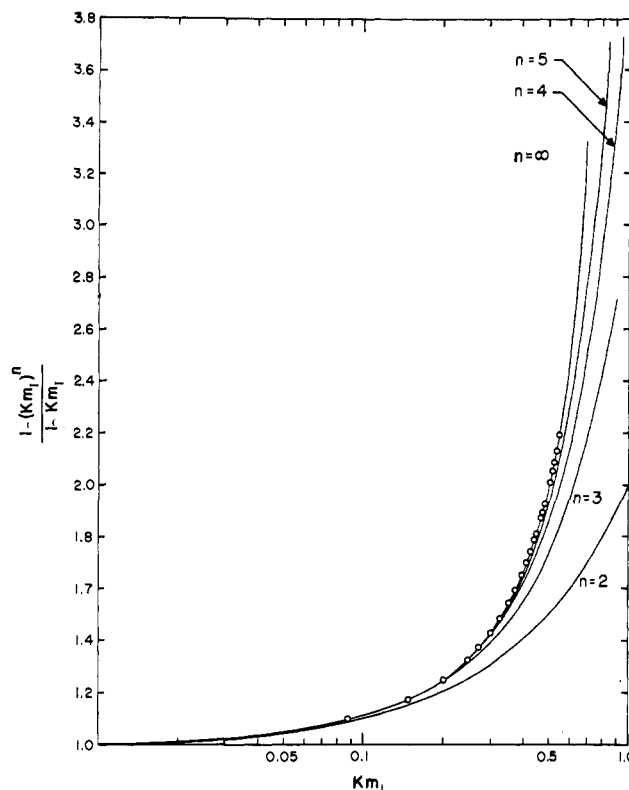


Fig. 1.—Graphic determination of n and K by eq. 13

$$\phi/\gamma = [1 - (Km_1)^n]/(1 - Km_1)$$

solid lines, theory; O, purine data.

will arbitrarily set the size of the limiting polymer at n units. That is, $K_2 = K_3 \dots K_n = K$, and $K_{n+1} = 0$. From eq. 5 and 12, then

$$\phi/\gamma = [1 - (Km_1)^n]/(1 - Km_1) \tag{13}$$

A graphical representation of eq. 13 is given in Fig. 1. The family of curves corresponds to plots of ϕ/γ vs. $\log Km_1$ for different values of n .

To extract K and n from the osmotic data, we resort to a graphical method. ϕ/γ is known as a function of m from the osmotic coefficients and the activity coefficients computed from the osmotic data. For each solute concentration m , m_1 is given by eq. 12. Thus, the experimental ϕ/γ 's can also be plotted against $\log m_1$. In this manner, n can be determined by matching the experimental curve to fit a member of the curves on the "master plot" and K determined from the translation along the abscissa required to yield the fit. A treatment of the data previously reported for purine is illustrated in Fig. 1. Application of the aforementioned technique yields $n \rightarrow \infty$ and $K = 2.1$ for the association in purine, in excellent agreement with the conclusions of Ts'o and co-workers.⁴

Equation 13 can easily be shown to lead to more familiar results in the case of two particular multiple equilibria. Thus, when $n = \infty$, that is, there is no restriction on the size of the polymer

$$\phi/\gamma = 1/(1 - Km_1) \tag{14}$$

and from eq. 7,

$$1/\gamma = 1/(1 - Km_1)^2 \tag{15}$$

Combining eq. 14 and 15, we find that

$$\phi^2 = \gamma \tag{16}$$

Substitution of eq. 16 into eq. 14 leads to

$$K = (1 - \phi)/(\phi^2 m) \tag{17}$$

as developed previously.^{4,10}

(9) J. Kreuzer, *Z. physik. chim.*, **B53**, 213 (1943).

(10) J. A. Scheffman, *Compt. rend. trav. Lab Carlsberg, Ser. Chim.*, **29**, 223 (1956).

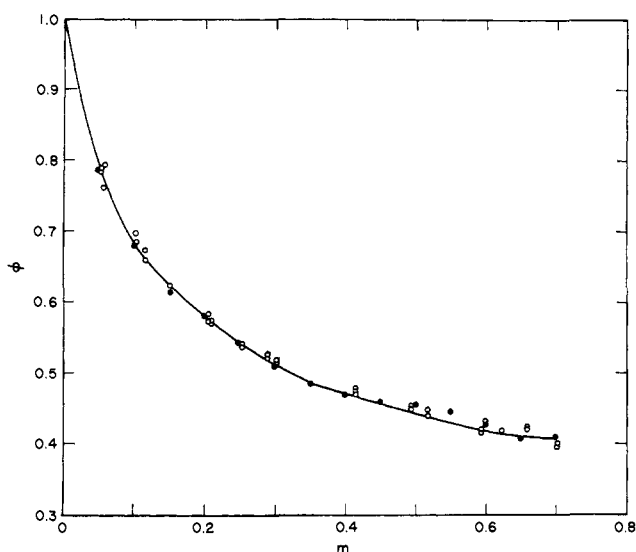


Fig. 2.—The osmotic coefficients (ϕ) of 6-methylpurine vs. molal concentrations at 25°: experimental (O); fitted (●).

When only dimers are formed, that is, $K_2 = K$, and $K_3 = K_4 \dots K_n = 0$

$$\phi/\gamma = 1 + Km_1 \quad (18)$$

$$1/\gamma = 1 + 2Km_1 \quad (19)$$

Combining eq. 18 and 19, we find that

$$\gamma = 2\phi - 1 \quad (20)$$

and

$$K = (1 - \phi)/[(2\phi - 1)^2 m] \quad (21)$$

as shown before.⁴

(2) We now turn our attention to a slightly more general situation. Here we shall relax one of the restrictions invoked in the above discussion and assume that the equilibrium constant for dimer formation is different from the association constants for the remaining successive associations steps. That is $K_2 \neq K_3 = K_4 \dots = K_n = K$. Again, we shall for general discussion first set the size of the limiting polymer at n units, so that $K_{n+1} = 0$. From eq. 5 then,

$$\phi/\gamma = 1 + K_2 m_1 \{1 - (Km_1)^{n-1}\} / (1 - Km_1) \quad (22)$$

and from eq. 6 and 7

$$\frac{1}{\gamma} = 1 + \frac{K_2 m_1}{(1 - Km_1)^2} \{2 - Km_1 - (n+1)(Km_1)^{n-1} + n(Km_1)^n\} \quad (23)$$

For low concentrations, where we may keep only terms linear with respect to m_1

$$\begin{aligned} 1/\gamma &\longrightarrow 1 + 2K_2 m_1 \\ \phi/\gamma &\longrightarrow 1 + K_2 m_1 \end{aligned} \quad (24)$$

so that K_2 may be obtained from

$$K_2 = \lim_{m \rightarrow 0} (1 - \phi) / [(2\phi - 1)^2 m] \quad (25)$$

Once K_2 is determined, the remaining two unknowns, namely K and n , may be obtained graphically by comparing a plot of $(\phi - \gamma) / \gamma^2 K_2 m$ vs. $\log m$ with the master plot given in Fig. 1, since from eq. 22

$$\frac{(\phi - \gamma) / (\gamma^2 K_2 m)}{(1 - Km_1)^{n-1}} = \frac{1 - (Km_1)^{n-1}}{(1 - Km_1)} \quad (26)$$

Note that if Fig. 1 is used, the value of n that fits the experimental data must be increased by one before it corresponds to the size of the limiting polymer.

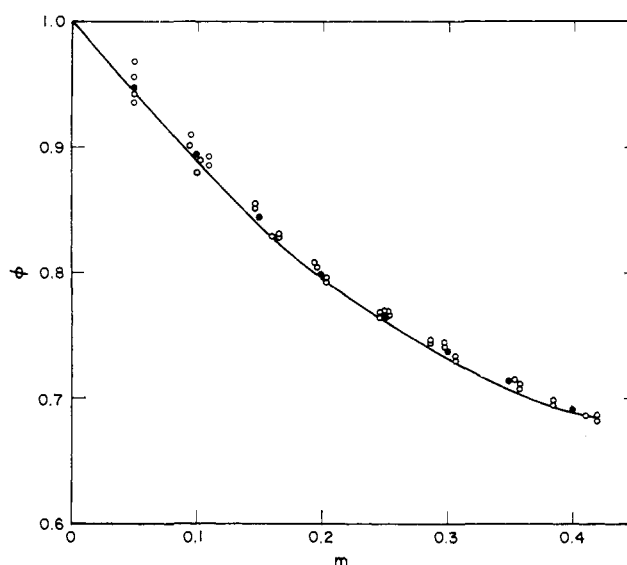


Fig. 3.—The osmotic coefficients (ϕ) of 5-bromouridine vs. molal concentrations at 25°: experimental (O); fitted (●).

The situation where $n \rightarrow \infty$ has been treated by Davies and Thomas.¹¹ Their method of extracting K_2 is identical to our eq. 25. Here K need not be determined graphically.

We note from eq. 22 and 23 that for $Km_1 < 1$

$$\frac{\phi - \gamma}{\gamma} = K_2 m_1 / (1 - Km_1) \quad (27)$$

$$\frac{1 - \gamma}{\gamma} = K_2 m_1 \left\{ \frac{2 - Km_1}{(1 - Km_1)^2} \right\} \quad (28)$$

Thus, dividing eq. 28 into eq. 27, we obtain,

$$K = (2\phi - \gamma - 1) / [m(\phi\gamma - \gamma)] \quad (29)$$

This result is simpler than the following equation previously derived by Davies, *et al.*

$$K = \frac{1}{m\phi} \pm \left(2 - \frac{1}{\phi} \right) \left[\frac{K_2}{m(1 - \phi)} \right]^{1/2} \quad (30)$$

However, K may be inferred from eq. 30 from the osmotic coefficients directly without direct reference to the activity coefficients.

Results

Osmotic Coefficients.—The molal osmotic coefficients of 6-methylpurine over the concentration range 0.1 to 0.7 m and 5-bromouridine over the concentration range 0.1 to 0.4 m at 25° are reported in Table I and Fig. 2 and 3. The experimental data for 6-methylpurine were fitted by a sixth-degree polynomial and 5-bromouridine by a fourth-degree polynomial as described above. A comparison of the fitted values with the experimental results is given in Table I and Fig. 3 and 4. The fit is seen to be satisfactory. The numerical coefficients of the two polynomials are listed in Table II.

Activity Coefficients.—Molal activity coefficients for 6-methylpurine and 5-bromouridine over the concentration range investigated were calculated from eq. 3. These are reported in Table III. The extensive lowering of the activity coefficients of these two compounds suggests the association or polymerization of these solutes in aqueous solution. At comparable concentrations, the activity coefficients of 6-methylpurine are lower than those of purine, and those of 5-bromouridine

(11) M. Davies and D. K. Thomas, *J. Phys. Chem.*, **60**, 763 (1956).

TABLE I
EXPERIMENTAL AND FITTED VALUE^a OF MOLAL OSMOTIC COEFFICIENTS AT 25°

Experimental		Fitted	
Molal concentration	ϕ	Molal concentration	ϕ
A. 6-Methylpurine			
0.0517	0.785	0.05	0.786
0.0573	0.777		
0.116	0.665	0.1	0.682
0.103	0.692		
0.151	0.623	0.15	0.624
0.209	0.571	0.20	0.582
0.205	0.577		
0.252	0.539	0.25	0.544
0.288	0.523	0.30	0.510
0.302	0.515		
		0.35	0.484
0.415	0.472	0.40	0.469
0.415	0.474		
		0.45	0.461
0.516	0.443	0.50	0.456
0.495	0.451		
		0.55	0.446
0.595	0.428	0.60	0.427
0.599	0.429		
0.624	0.419	0.65	0.407
0.660	0.423		
0.702	0.400	0.70	0.410
B. 5-Bromouridine			
0.0502	0.945	0.05	0.948
0.0504	0.951		
0.0950	0.906	0.10	0.894
0.103	0.885		
0.110	0.889		
0.148	0.854	0.15	0.844
0.161	0.828		
0.166	0.829		
0.194	0.810	0.20	0.801
0.196	0.804		
0.204	0.795		
0.247	0.766	0.25	0.766
0.251	0.769		
0.251	0.769		
0.286	0.746	0.30	0.738
0.298	0.744		
0.306	0.733		
0.354	0.715	0.35	0.715
0.357	0.711		
0.384	0.698	0.40	0.693
0.409	0.687		
0.421	0.686		

^a See eq. 2 and Table II for the coefficients of the polynomials.

TABLE II
COEFFICIENTS OF THE FITTED POLYNOMIALS (EQ. 2) IN RELATING MOLAL OSMOTIC COEFFICIENT, ϕ , TO THE MOLAL CONCENTRATION

	a_1	a_2	a_3
6-Methylpurine	-6.0253	43.056	-183.94
5-Bromouridine	-0.9971	-1.4636	+9.6714
	a_4	a_5	a_6
6-Methylpurine	422.91	-482.56	214.07
5-Bromouridine	-11.348

lower than those of uridine, indicating that methyl and bromo substitution of the parent molecules have enhanced the association.

Analysis of the Osmotic Data.—We now present details of our analysis of the osmotic data of 6-methyl-

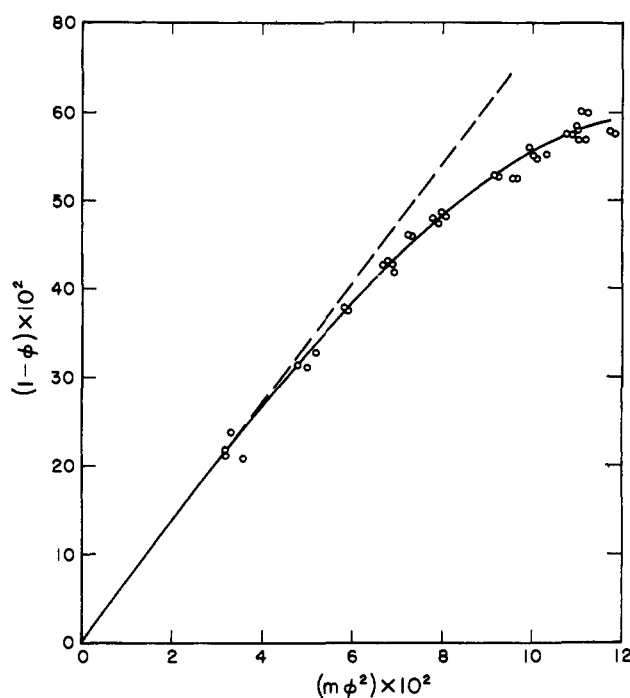


Fig. 4.—Plot of $(1 - \phi)$ vs. $m\phi^2$ for 6-methylpurine (eq. 17).

purine and 5-bromouridine in terms of the multiple equilibria described above.

For 6-methylpurine, the data were first treated by plotting $(1 - \phi)$ vs. $m\phi^2$. Such a plot should yield a straight line if each step in the association process is equally favorable and if there is no restriction on the size of the polymer. According to eq. 17, the association constant K can then be determined from the

TABLE III
MOLAL ACTIVITY COEFFICIENTS^a AT 25° COMPUTED FROM THE FITTED OSMOTIC COEFFICIENTS^b

Molal concentration	6-Methylpurine	5-Bromouridine
0.05	0.626	0.902
0.10	0.469	0.811
0.15	0.385	0.732
0.20	0.329	0.666
0.25	0.287	0.613
0.30	0.255	0.569
0.35	0.230	0.533
0.40	0.211	0.502
0.45	0.196	...
0.50	0.185	...
0.55	0.173	...
0.60	0.162	...
0.65	0.152	...
0.70	0.146	...

^a See eq. 3. ^b See Table I.

slope of this linear plot. However, as seen in Fig. 4, the curve is not linear over the entire concentration range studied. Instead, it starts to bend toward the abscissa above 0.3 m . This suggests that there is a progressive dropoff in the association constants for the successive association steps; or, alternately, if the successive steps are equally favorable, there is a rapid cutoff beyond a limiting polymer of n units. For the second alternative, mathematical analyses have already been developed (eq. 13) to determine the values of n and K . Thus, ϕ/γ was plotted vs. $\log m_1$ and an attempt was made to match the resulting plot with a member of the curves on the master plot given in Fig. 1.

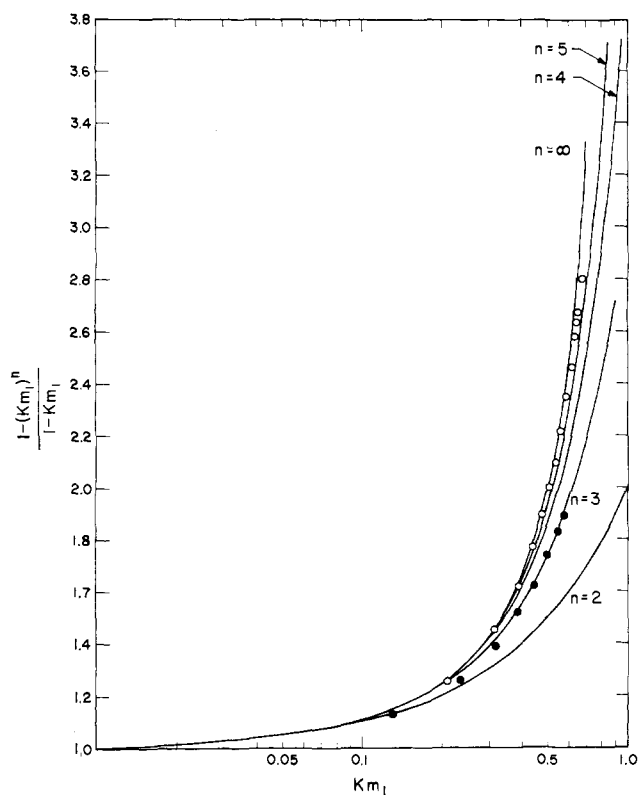


Fig. 5.—Graphic determination of n and K by eq. 13

$$\phi/\gamma = [1 - (Km_1)^n] / (1 - Km_1)$$

solid lines, theory; (O), 6-methylpurine; (●), 5-bromouridine.

A match of the experimental data with one of the theoretical curves is given in Fig. 5. The translation of the abscissa required to provide the match corresponds to a K of $6.7 M^{-1}$. Since the slope for the linear portion of the curve in Fig. 5 is also 6.7, the data, therefore, favor the interpretation suggested by the second alternative mentioned above. That is, for 6-methylpurine, $K_2 = K_3 \dots = K_n = K$ and $K_{n+1} = 0$. The size of the limiting polymer was determined from Fig. 5 to be ≥ 5 .

A plot of $(1 - \phi)$ vs. $m\phi^2$ for 5-bromouridine is given in Fig. 6. Here a smooth S-shape curve was obtained. At low concentrations, the curve is seen to bend away from the abscissa whereas at the high concentration end it bends towards the abscissa. This suggests that the association constant for dimer formation may be smaller than the association constants the next few succeeding steps. Also, there is the possibility that the association may terminate after a finite number of steps. Equations 25, 26, and 29 may, therefore, be pertinent. A plot of $(1 - \phi) / [(2\phi - 1)^2 m]$ vs. m was extrapolated to infinite dilution in accordance with eq. 25 to yield K_2 , the association constant for dimer formation. The extrapolation was linear and a value of $1 M^{-1}$ was obtained within 10% accuracy. A plot of $(\phi - \gamma) / (\gamma^2 K_2 m)$ vs. $\log m_1$ was then compared with the master plot in Fig. 1 and a match of the experimental curve with one of the theoretical curves was sought. The best fit is depicted in Fig. 5, from which it was determined that 5-bromouridine associates in aqueous solution to form polymers up to the tetramer with an association constant K of 2.9 for the second and the third steps.

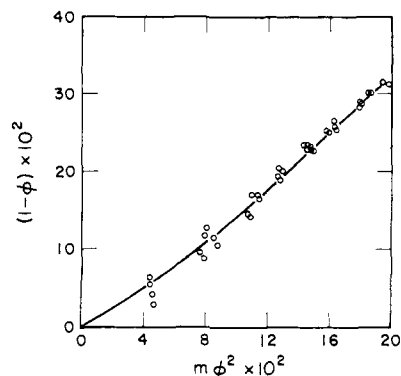


Fig. 6.—Plot of $(1 - \phi)$ vs. $m\phi^2$ for 5-bromouridine (eq. 17).

A summary of the results pertinent to the association of 6-methylpurine and 5-bromouridine in aqueous solutions at 25° and as determined from the above analyses of the osmotic data is given in Table IV. For sake of comparison, the corresponding results for purine and uridine previously communicated are also included.

TABLE IV
SUMMARY OF THE ANALYSES OF THE OSMOTIC DATA BASED
ON TREATMENTS OF MULTIPLE EQUILIBRIA

	K (Molal ⁻¹)	ΔF° ($-RT \ln K$, cal.)	n ($K_n = 0$)
Purine ^a	2.1	-440	$5 > n > \infty$
6-Methylpurine ^b	6.7	-1120	$5 > n > \infty$
Uridine ^c	0.61	+290	...
Cytidine ^d	0.87	+80	...
5-Bromouridine ^e	$K_1 = 1.0$ $\bar{K} = 2.9$	0 -630	$n = 4$
Urea ^f	0.041	+1190	...

^a Equation 17, ref. 4. ^b Equation 13. ^c Equation 17, ref. 4. ^d Equation 17, ref. 4. ^e Equations 25 and 26. ^f Equation 17, ref. 10.

Populations of the Associated Species.—Populations of the associated species in aqueous solutions of purine, 6-methylpurine, and 5-bromouridine are tabulated in Table V. These have been computed using the information summarized in Table IV. In the case of 6-methylpurine, where $n \geq 5$, the value of 6 was chosen for the population computations. The results reported in Table V serve the following dual purpose. First they can be used to provide a check on the accuracy of the models. The column labeled "remainder" in Table V contains, for each concentration of solute, the difference between the stoichiometric molality and the sum of all the associated species computed from the model. Theoretically, these remainders should be zero if the system is a perfect replica of the model assumed. As seen in Table V, these remainders are essentially random in nature and are usually around 1%. Secondly, these computed populations can serve as stepping stones toward the interpretation of other physical properties of these solutions. For instance, these computed populations have been employed in the following paper to analyze the concentration dependence of the n.m.r. spectra of purine and 6-methylpurine in aqueous solution.

Discussion

The results of the present investigation are significant in the light of the following experimental findings with regard to interactions in polynucleotides. Recent measurements of the melting temperatures, T_m , of various polynucleotides indicate that methyl substitu-

TABLE V
THE COMPUTED DISTRIBUTION OF THE POPULATION OF THE
ASSOCIATED SPECIES IN AQUEOUS SOLUTIONS^{a, b}

Molal	F(1)	F(2)	F(3)	F(4)	F(5)	$1 - \sum_{n=1}^5 F(n)$	
1. Purine							
0.05	0.833	0.146	0.019	0.002	0.000	0.000	
0.10	0.720	0.218	0.049	0.010	0.002	0.000	
0.15	0.638	0.256	0.077	0.021	0.005	0.003	
0.20	0.575	0.278	0.101	0.032	0.010	0.004	
0.25	0.525	0.290	0.119	0.044	0.015	0.007	
0.30	0.484	0.294	0.135	0.055	0.021	0.011	
0.35	0.449	0.296	0.147	0.064	0.027	0.017	
0.40	0.419	0.296	0.156	0.073	0.032	0.024	
0.45	0.394	0.294	0.164	0.081	0.038	0.029	
0.50	0.372	0.290	0.170	0.088	0.043	0.036	
0.55	0.352	0.286	0.175	0.095	0.048	0.044	
0.60	0.335	0.282	0.179	0.100	0.053	0.051	
0.65	0.319	0.278	0.181	0.105	0.057	0.060	
0.70	0.305	0.274	0.184	0.110	0.062	0.066	
0.75	0.292	0.268	0.185	0.114	0.065	0.076	
0.80	0.280	0.264	0.186	0.117	0.069	0.084	
0.85	0.269	0.260	0.187	0.120	0.072	0.092	
0.90	0.260	0.254	0.188	0.122	0.072	0.101	
0.95	0.250	0.250	0.188	0.125	0.078	0.109	
1.00	0.242	0.246	0.188	0.127	0.081	0.117	
Molal	F(1)	F(2)	F(3)	F(4)	F(5)	F(6)	Remainder
2. 6-Methylpurine							
0.05	0.626	0.263	0.0826	0.0231	0.0061	0.0015	-0.002
0.10	0.469	0.292	0.139	0.0582	0.0229	0.0086	0.008
0.15	0.385	0.298	0.173	0.0892	0.0431	0.0200	-0.008
0.20	0.329	0.276	0.192	0.113	0.0621	0.0329	-0.019
0.25	0.287	0.261	0.199	0.128	0.0766	0.0442	-0.010
0.30	0.255	0.248	0.201	0.137	0.0880	0.0541	0.003
0.35	0.230	0.239	0.201	0.144	0.0973	0.0630	0.017
0.45	0.211	0.232	0.202	0.153	0.1079	0.0732	0.014
0.45	0.196	0.232	0.205	0.162	0.120	0.085	0.001
0.50	0.185	0.229	0.213	0.176	0.136	0.101	-0.04
0.55	0.173	0.221	0.211	0.179	0.143	0.109	-0.03
0.60	0.162	0.211	0.206	0.179	0.146	0.114	-0.018
0.65	0.152	0.201	0.200	0.176	0.146	0.116	0.009
0.70	0.146	0.200	0.205	0.187	0.160	0.132	-0.031
3. 5-Bromouridine							
0.05	0.902	0.0814	0.0160	0.003			-0.002
0.10	0.811	0.132	0.0464	0.0146			-0.003
0.15	0.732	0.161	0.0768	0.0326			-0.002
0.20	0.666	0.177	0.103	0.0529			0.001
0.25	0.613	0.188	0.125	0.0742			0.000
0.30	0.569	0.194	0.144	0.0952			-0.003
0.35	0.533	0.199	0.161	0.116			-0.009
0.40	0.502	0.202	0.176	0.137			-0.016

^a See Table IV for all conditions. ^b $F(i) = M_i / \sum_{n=1}^n mn_n$.

tion on polyU has greatly increased the T_m of its complex with polyA as well as the T_m of the helical homopolymer.¹² Similarly, the melting temperatures of bromodeoxyribopolynucleotides¹³⁻¹⁵ as well as those for

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bromoribopolynucleotides¹⁶ also indicate that the helices of the bromo-substituted polymers are more stable toward thermal uncoiling than those of the unsubstituted polymers. It is interesting and perhaps pertinent then that the enhancement in the stability of these chemically modified polynucleotides toward thermal uncoiling is also reflected in the increased tendencies of purine and uridine toward self-association with methyl and bromo substitution.

The increase in the self-association tendencies of 6-methylpurine and 5-bromouridine with respect to the parent molecules does not appear to be readily accounted for in terms of increase in hydrogen bonding. In view of the recent n.m.r. results,⁵ an interpretation in terms of changes in the hydrophobic interaction seems more reasonable. Because of the hetero atoms (nitrogens) in the aromatic rings of these bases and nucleosides, there is considerable charge separation and it is certainly likely that changes in the van der Waals' interactions between the π -systems of the aromatic rings can account for the enhanced associations observed.

It is the principal objective of this research to compare and correlate the interactions between simple bases and nucleosides with the corresponding interactions in polynucleotides. While it is premature to suggest that the enhanced stability toward thermal uncoiling of the aforementioned methyl and bromo polynucleotides does not arise partly from changes in hydrogen-bonding interactions, it appears that changes in the hydrophobic interactions are certainly not negligible and need also to be considered.

The present investigation of the association of solutes in terms of the thermodynamic osmotic properties of the solutions has also been fruitful in another respect. Analyses of the osmotic and activity coefficients in terms of various models for the association process have led to a rather detailed and quantitative description of the process. We do not wish to imply that the model invoked replicates the actual association processes at hand. Nevertheless, the type of treatments presented here does yield numerical results which can be used to correlate the osmotic properties with other physical properties of these solutions. Thus, the n.m.r. results on the purine and 6-methylpurine solutions were analyzed in terms of the population distribution of polymeric species calculated on the basis of these models.⁵

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(16) Unpublished results from this laboratory.