Complex Formation between Purine and Indole Derivatives in Aqueous Solutions. Proton Magnetic Resonance Studies

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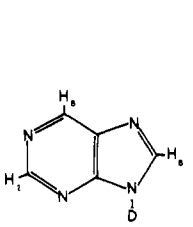
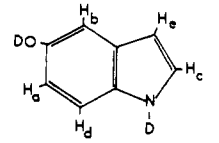
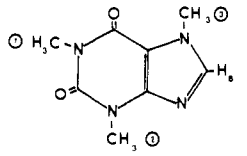
Abstract: Several mathematical models derived under simplifying assumptions are presented to account for proton magnetic resonance data of self-associating systems and of complex formation between compounds which self-associate through stacking interactions. These models are used to analyze the pmr data for self-association of purine in aqueous solutions and for interaction of purine with 5-hydroxyindole, tryptamine, and serotonin. The indole ring is able to substitute for purine molecules in purine stacks, and the magnetic anisotropy of the two rings are shown to be very similar. A model assuming intercalation of molecules in purine aggregates is also shown to account for the pmr data obtained for interactions of cytosine and caffeine with purine in aqueous solutions. The interaction of tryptophan with adenosine in acidic conditions is analyzed in terms of simultaneous formation of 1:1 and 2:1 complexes. The experimental pmr mixing curve obtained by the method of continuous variations fits quite well the curve calculated using the two equilibrium constants and the chemical shifts in the two complexes determined from the mathematical analysis.

The formation of intermolecular complexes between aromatic molecules in solution can be conveniently followed by proton magnetic resonance (pmr) spectroscopy. The large magnetic anisotropy of an aromatic molecule induces changes in the chemical shifts of a complexed neighboring molecule.[1] If the rate of exchange between complexed and free molecules is large on the pmr time scale, only one single resonance will be observed for each proton in a mixture of both molecules. Under these conditions, the measured chemical shift is the weighted average of chemical shifts for the free and complexed molecule. The concentration and temperature dependence of the magnitude and the direction (with respect to magnetic field) of these changes in chemical shifts for different protons of the same molecule, could, in principle, permit determination of the stoichiometry, association constants, thermodynamics parameters (enthalpy and entropy), and the stereochemistry of the complexes.[2]

In many cases, pmr data have been analyzed according to relationships derived in the case of 1:1 complex formation.[3] However, the formation of complexes with a stoichiometry different from 1:1 and self-association of the molecules engaged in the complexes can often play an important role and raise more complicated mathematical problems.[3] These problems were encountered in a study of stacking interactions between aromatic amines and nucleotides in aqueous solutions.[4] Some models of self-association and complex formation have already been proposed for hydrogen-bonded systems[5] and to analyze experimental data obtained from colligative methods.[6-8] We present here several simple models for analysis of pmr data of self-associating systems and of interactions between molecules which self-associate through stacking interactions. Experimental pmr results of interactions between purine and 5-hydroxyindole, tryptamine, or 5-hydroxytryptamine (serotonin) and other purine and pyrimidine derivatives, have been analyzed according to these different models.







I. Self-Association

(a) Dimer Formation. Molecule B is self-associating to give dimers according to the equation

$$B + B \rightleftharpoons B_2$$

The following relationship can be derived

$$\delta\sigma/B = 2K(\delta\sigma_{B_2} - \delta\sigma) \quad (1a)$$

$$\sqrt{\delta\sigma/B_0} = \sqrt{2K/\delta\sigma_{B_2}}(\delta\sigma_{B_2} - \delta\sigma) \quad (1b)$$

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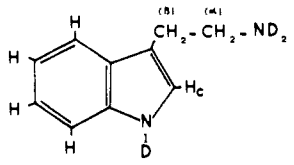
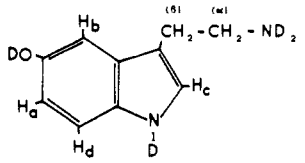


Tryptamine



Serotonin

where $\delta\sigma = \sigma - \sigma_B$ ($\delta\sigma_{B_2} = \sigma_{B_2} - \sigma_B$). B_0 and B represent the total concentration and the free concentration of molecule B, respectively. σ_B and σ_{B_2} are the chemical shifts of the investigated proton in the free B molecule and in the dimer. As there is no reason, *a priori*, to suppose that the corresponding protons of the two molecules are equivalent in the dimer, σ_{B_2} represents an average on all the possible environments.

A plot of $(\delta\sigma/B_0)^{1/2}$ against $\delta\sigma$ will give a straight line whose slope and x-axis intercept are $(2K/\delta\sigma_{B_2})^{1/2}$ and $(\delta\sigma_{B_2})$, respectively. Using eq 1a and 1b requires knowledge of σ_B which is obtained by extrapolation of the chemical shift to zero concentration. This of course raises some difficulties. However, we will see later that a small change of the extrapolated chemical shift does not lead to large variations of the association constants and of the chemical shifts of self-associated systems.

(b) Self-Association with Formation of *n*-Mers. Molecule B is assumed to associate in solution according to the following scheme.

$$B + B \rightleftharpoons B_2$$
$$B_2 + B \rightleftharpoons B_3$$
$$\cdots\cdots\cdots\cdots$$
$$B_{n-1} + B \rightleftharpoons B_n$$

We will make the following simplifying assumptions. (i) Successive association constants are identical. (ii) The effects of magnetic anisotropy of neighboring molecules are additive. (iii) Only the magnetic anisotropy of nearest neighbors is taken into account. This assumption appears quite reasonable since "ring current" effects at twice the intermolecular distance in the dimer (assumed to be around 4 Å) will be quite small. Then the change in chemical shift of a B molecule inside a *n*-mer (as compared to the free molecule) will be twice the change observed in the dimer. Only the two molecules at the end of every aggregate have the same chemical shifts as molecules in a dimer. The average chemical shift for a proton in a *n*-mer is

$$\sigma_{B_n} = [2(n-1)\sigma_{B_2} - (n-2)\sigma_B]/n \qquad (2)$$

The observed chemical shift (σ) will be given by the following relationship.

$$(B + 2KB^2 + \ldots + nK^{n-1}B^n + \ldots)\sigma =$$
$$\sigma_B B + 2KB^2\sigma_{B_2} + \ldots + nK^{n-1}B^n\sigma_{B_n} + \ldots \qquad (3)$$

Straightforward calculations lead to

$$\delta\sigma/B = 2K\delta\sigma_{B_2} \qquad (4a)$$

$$\sqrt{\delta\sigma/B_0} = \sqrt{K/2\delta\sigma_{B_2}}(2\delta\sigma_{B_2} - \delta\sigma) \qquad (4b)$$

A plot of $(\delta\sigma/B_0)^{1/2}$ against $\delta\sigma$ will thus give a straight line as already calculated when only dimers are formed but the slope will be half and the x-axis intercept twice the parameters obtained in the case of dimer formation.

II. Formation of Intermolecular Complexes

(a) Notations. The two interacting compounds will be named A and B. The concentration of compound B will be always much larger than that of A so that $B \simeq B_0$ if molecule B does not associate, with B and B_0 defined below.

The following symbols will be used: A and B, molar concentrations of free molecules; A_0 and B_0, total initial molar concentrations of compounds A and B; A_iB_j, concentration of complex containing i molecules of A and j molecules of B; σ, chemical shift of a proton of molecule A measured in the presence of compound B at a total concentration B_0; σ_0, chemical shift of the same proton in the free A molecule; $\sigma_{A_iB_j}$, average chemical shift of the same proton of molecule A engaged in complex A_iB_j.

(b) 1:1 Complex Formation. If only a 1:1 complex is formed between molecules which do not self-associate, the following equation can be derived under conditions where $B \simeq B_0$

$$\Delta\sigma/B_0 = -k(\Delta\sigma - \Delta\sigma_{AB}) \qquad (5a)$$

where $\Delta\sigma = \sigma - \sigma_0$, $\Delta\sigma_{AB} = \sigma_{AB} - \sigma_0$, and k is the association constant for complex formation ($A + B \rightleftharpoons AB$).

If different isomers of the 1:1 complex are formed, it can be easily shown that k represents the sum of all association constants and $\Delta\sigma_{AB}$ the weighted average of the different changes in chemical shifts.

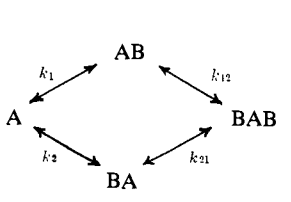
In the microscopic model described below (IIc), it will be assumed that two binding sites exist on molecule A with the same microscopic constant k_1. Equation 5a can then be rewritten as

$$\frac{\Delta\sigma}{B_0} = -2k_1\left(\Delta\sigma - \frac{\Delta\sigma_{AB} + \Delta\sigma_{BA}}{2}\right) \qquad (5b)$$

A plot of $\Delta\sigma/B_0$ *vs.* $\Delta\sigma$ should give a straight line. However, the linearity of such plots does not prove that only 1:1 complexes are formed since, under certain conditions, the simultaneous formation of 1:1 and 2:1 complexes will give a similar result as described below.

(c) Formation of 1:1 and 2:1 Complexes. A microscopic model for the simultaneous formation of 1:1 and 2:1 complexes can be represented by the Scheme I.[3] If it is assumed that the two binding sites

Scheme I



on molecule A are independent, then

$$k_{12} = k_2 \text{ and } k_{21} = k_1 \qquad (6)$$

$$\sigma_{BAB} = \sigma_{BA} + \sigma_{AB} - \sigma_0 \qquad (7)$$

Equation 8 can then be written with the assumption $B \simeq B_0$.

$$(1 + (k_1 + k_2)B_0 + k_1k_2B_0^2)\sigma = \sigma_0 + B_0(k_1\sigma_{AB} + k_2\sigma_{BA}) + k_1k_2B_0^2\sigma_{BAB} \quad (8)$$

If a linear relationship is observed between $(\Delta\sigma/B_0)$ and $\Delta\sigma$ apparent parameters K and $\Delta\tilde{\sigma}_{AB}$ can be introduced such that

$$\Delta\sigma/B_0 = -K(\Delta\sigma - \Delta\tilde{\sigma}_{AB}) \quad (9)$$

Identification of eq 8 and 9 for each value of B_0 leads to

$$K = k_1 = k_2,\ \Delta\tilde{\sigma}_{AB} = \sigma_{BAB} - \sigma_0$$

If it is assumed that molecule A has two independent binding sites with the same microscopic constant $k_1 = k_2$, then a plot of $\Delta\sigma/B_0$ against $\Delta\sigma$ will give a straight line at least in two cases.

(i) Formation of 1:1 complexes only: the slope yields $K = 2k_1$ and the x-axis intercept gives (see eq 5b)

$$\Delta\tilde{\sigma}_{AB} = (\Delta\sigma_{AB} + \Delta\sigma_{BA})/2$$

(ii) Formation of 1:1 and 2:1 complexes: the slope will give $K = k_1 = k_2$ and the x-axis intercept

$$\Delta\tilde{\sigma}_{AB} = \Delta\sigma_{AB} + \Delta\sigma_{BA} = \Delta\sigma_{BAB}$$

(compare also to the two cases for self-association described above in paragraphs Ia and Ib).

If it is not assumed that the two binding sites on molecule A are independent, the following equation can be written

$$[1 + K_1B_0 + K_1K_2B_0^2]\Delta\sigma = K_1B_0\Delta\tilde{\sigma}_{AB} + K_1K_2B_0^2\Delta\sigma_{BAB} \quad (10)$$

where $K_1 = k_1 + k_2$

$$K_2 = \frac{k_1k_{12}}{k_1 + k_2} = \frac{k_2k_{21}}{k_1 + k_2}$$

$$\Delta\tilde{\sigma}_{AB} = \frac{k_1\Delta\sigma_{AB} + k_2\Delta\sigma_{BA}}{k_1 + k_2}$$

Equation 10 can be rewritten as

$$\frac{K_1B_0\Delta\tilde{\sigma}_{AB} - (1 + K_1B_0)\Delta\sigma}{B_0^2} = K_1K_2\Delta\sigma - K_1K_2\Delta\sigma_{BAB} \quad (11)$$

If compound A does not self-associate and forms only 1:1 complexes when A is present in excess, it is possible to determine the value of K_1 when compound A is present in excess; an iterative procedure is used to find the value of $\Delta\tilde{\sigma}_{AB}$ which leads to a linear plot of the left-hand term of eq 11 against $\Delta\sigma$. Then the values of K_2 and $\Delta\sigma_{BAB}$ are determined. Such a case was met in the study of complex formation between adenosine and tryptophan in acidic solutions (see Experimental Section). It can be seen that in the case of two independent and equivalent sites the following relationships hold: $K_1 = 2k$, $K_2 = k/2$, $\Delta\sigma_{AB} = (\Delta\sigma_{AB} + \Delta\sigma_{BA})/2$, and $\Delta\sigma_{BAB} = \Delta\sigma_{AB} + \Delta\sigma_{BA}$.

(d) Compound B in Excess Self-Associates to Form n-Mers. The two binding sites in molecule A are assumed to have the same microscopic binding constant k_1. Compound B forms n-mers in solution according to the scheme described in paragraph Ib. Only nearest-neighbor effects are taken into account for the calculation of chemical shifts. We suppose that the populations of the different types of associates of the compound in excess are not affected by the presence of the other compound at much lower concentrations.

Two cases may be considered: (i) molecule A does not intercalate between aggregated B molecules and binds only to the ends of each aggregate; (ii) molecule A can bind not only to the ends of each aggregate but can also intercalate between B molecules.

Because of the low concentration of compound A, it will be further assumed that only one A molecule is able to bind to each aggregate of B molecules.

In these two cases, one can consider the equilibrium system as arising from the interaction between A molecules and B aggregates of concentration B', where

$$B' = \sum_{i=1}^{\infty} B_i = \frac{B}{1 - KB}$$

Then the first case (i) is equivalent to a model of 1:1 complexes between A molecules and B aggregates. The following equation can be derived.

$$\frac{\Delta\sigma}{B'} = -2k_1\left[\Delta\sigma - \frac{\Delta\sigma_{AB} + \Delta\sigma_{BA}}{2}\right] \quad (12a)$$

The other case is equivalent to a model in which 1:1 and 2:1 complexes are formed between A molecules (low concentration) and B aggregates. Then the following equation holds

$$\Delta\sigma/B' = -k_1[\Delta\sigma - (\Delta\sigma_{AB} + \Delta\sigma_{BA})] \quad (12b)$$

Strictly equivalent results can be obtained if we calculate the contribution of the different species present in the mixture (*cf.* Appendix). Equation 12a is equivalent to

$$\Delta\sigma/B = -(2k_1 - K)\Delta\sigma + k_1(\Delta\sigma_{BA} + \Delta\sigma_{AB}) \quad (13a)$$

and eq 12b to

$$\Delta\sigma/B = -(k_1 - K)\Delta\sigma + k_1(\Delta\sigma_{BA} + \Delta\sigma_{AB}) \quad (13b)$$

Using eq 4a, eq 13a and 13b can be modified to give

$$\frac{\Delta\sigma}{\delta\sigma} = \frac{K - 2k_1}{2K}\frac{\Delta\sigma}{\delta\sigma_{B_2}} + \frac{k_1}{2K}\frac{(\Delta\sigma_{AB} + \Delta\sigma_{BA})}{\delta\sigma_{B_2}} \quad (14a)$$

$$\frac{\Delta\sigma}{\delta\sigma} = \frac{K - k_1}{2K}\frac{\Delta\sigma}{\delta\sigma_{B_2}} + \frac{k_1}{2K}\frac{(\Delta\sigma_{AB} + \Delta\sigma_{BA})}{\delta\sigma_{B_2}} \quad (14b)$$

Thus if self-association of compound B can be described according to the model Ib, $\delta\sigma_{B_2}$, $\delta\sigma$, and K are known, and a plot of $\Delta\sigma/\delta\sigma$ against $\Delta\sigma/\delta\sigma_{B_2}$ will give a straight line from which k_1 and $(\Delta\sigma_{BA} + \Delta\sigma_{AB})$ can be obtained. The value of B can also be deduced from $\delta\sigma$ and a plot of $\Delta\sigma/B$ against $\Delta\sigma$ would give the same results.

When self-association of B molecules is limited to the formation of dimers, we have not been able to find any simple graphical representation.

The conclusions of the above models are summarized in Table I. It should be pointed out that in all the models proposed, the changes in chemical shifts for the different protons of the same molecule should be proportional to each other.

Results

As a model for the study of interactions between purine nucleotides and tryptophan derivatives in aque-

Table I. Summary of Graphical Representations for the Different Models of Self-Association and Complex Formation Described in the Text

Model	x	y	Slope = s	x-axis (x_0) intercept	K or k
			Self-association		
Ia, dimer formation	$\delta\sigma$	$\sqrt{\delta\sigma/B_0}$	$-\sqrt{2K/\delta\sigma_{B_2}}$	$\delta\sigma_{B_2}$	$K = x_0 s^2/2$
Ib, n-mer formation	$\delta\sigma$	$\sqrt{\delta\sigma/B_0}$	$-\sqrt{K/2\delta\sigma_{B_2}}$	$2\delta\sigma_{B_2}$	$K = x_0 s^2$
			Complex formation		
IIb, 1:1 complex	$\Delta\sigma$	$\Delta\sigma/B_0$	$-2k_1$	$(\Delta\sigma_{AB} + \Delta\sigma_{BA})/2$	$k_1 = -s/2$
IIc, 1:1 and 2:1 complexes (equivalent binding sites)	$\Delta\sigma$	$\Delta\sigma/B_0$	$-k_1$	$\Delta\sigma_{AB} + \Delta\sigma_{BA}$	$k_1 = -s$
IId, complex formation with a self-associating molecule	$\Delta\sigma$	$\Delta\sigma/B$	$K - k_1$	$\frac{k_1}{k_1 - K}(\Delta\sigma_{AB} + \Delta\sigma_{BA})$	$k_1 = (K - s)$
	$\Delta\sigma/\delta\sigma_{B_2}$	$\Delta\sigma/\delta\sigma$	$(K - k_1)/2K$	$\frac{k_1}{k_1 - K}\frac{\Delta\sigma_{AB} + \Delta\sigma_{BA}}{\delta\sigma_{B_2}}$	$k_1 = K(1 - 2s)$
IIe, complex formation with a self-associating molecule without sandwich complexes	$\Delta\sigma$	$\Delta\sigma/B$	$K - 2k_1$	$\frac{k_1}{2k_1 - K}(\Delta\sigma_{AB} + \Delta\sigma_{BA})$	$k_1 = (K - s)/2$
	$\Delta\sigma/\delta\sigma_{B_2}$	$\Delta\sigma/\delta\sigma$	$(K - 2k_1)/2K$	$\frac{k_1}{2k_1 - K}\frac{\Delta\sigma_{AB} + \Delta\sigma_{BA}}{\delta\sigma_{B_2}}$	$k_1 = K(1 - 2s)/2$

ous solutions, we chose to investigate the association of simpler compounds such as purine and 5-hydroxyindole. More complicated systems in which one or both of the aromatic rings are substituted and bear electric charges (purine + tryptamine or 5-hydroxytryptamine) have then been subjected to the same analysis. Although these systems do not behave as simply as the first one, the models described above allow some conclusions to be derived with respect to the mode of association and the effects of the charged substituents.

(1) Self-Association of Purine. It has been shown by osmotic and proton magnetic resonance studies that purine associates in aqueous solution and that vertical stacking is involved.[8,9] The pmr results have been analyzed using the different n-mer populations derived from osmotic studies with the assumption of identical constants for the successive steps of association. Two simplified models were presented.

In our case, according to the model leading to eq 4b a plot of $(\delta\sigma/B_0)^{1/2}$ against $\delta\sigma$ gives straight lines for the three protons of purine in the concentration range investigated (0.05–1.0 M) (Figure 1). From these straight lines, the microscopic association constant was calculated to be $K = 2.2 \pm 0.2\ M^{-1}$, a value quite close to the value deduced from osmotic measurements (2.1 M^{-1}).[8] When purine concentration increases, the changes in chemical shift for the different protons with respect to the monomer are proportional to each other. The chemical shifts of the protons of the different n-mers can then be calculated using the assumption made in eq 2. The changes in chemical shifts due to dimer formation are determined from extrapolation of the straight line of Figure 1 (Table II).

It must be emphasized that formation of purine dimers only would lead to a linear graph in the same representation (see Table I). The dimerization constant would then be half the above value (2.2 M^{-1}) and the changes in chemical shifts of the dimer compared to the monomer would be twice those given ($\delta\sigma_{B_2}$) in Table II. However, since purine has a plane of symmetry there is no reason *a priori* to suppose that self-association should be limited to dimers. Furthermore the extrapolated changes in chemical shifts are much too large when compared to those expected for dimers of purine. This is further supported by the results of complex formation between purine and 5-hydroxyindole (see below). Using the calculated value for K (2.2 M^{-1}), the concentration of free purine molecules can be calculated for each purine concentration.

(9) G. K. Helmkamp and N. S. Kondo, *Biochim. Biophys. Acta*, **145**, 27 (1967); G. K. Helmkamp and N. S. Kondo, *ibid.*, **157**, 242 (1968); J. Danner and G. K. Helmkamp, *ibid.*, **232**, 227 (1971).

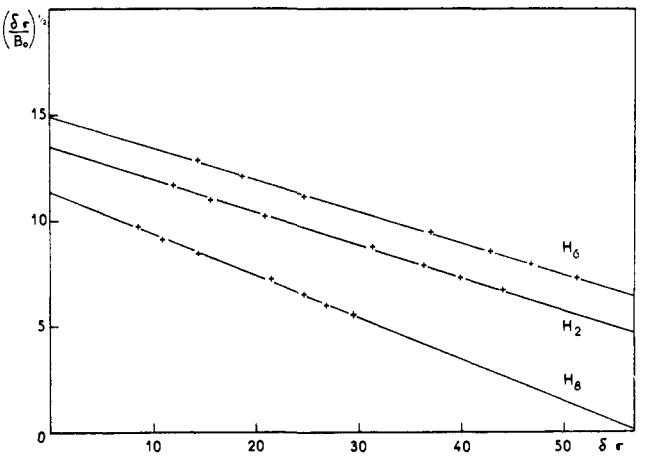


Figure 1. Self-association of purine in D_2O. The pmr results are analyzed according to eq 4b.

Table II. Change in Chemical Shifts of the Different n-Mers of Purine with Respect to the Monomers[a]

	$\delta\sigma_{B_2}$	$\delta\sigma_{B_3}$	$\delta\sigma_{B_4}$	$\delta\sigma_{B_\infty}$
H_6	49	65.5	73.5	98
H_2	43.5	58	65	87
H_8	29	39	43.5	58

[a] $\delta\sigma_{B_2}$ is calculated from extrapolations of linear plots according to eq 4b and other $\delta\sigma_{B_n}$ from eq 2.

(2) Self-Association of Indole Derivatives. Complex formation between nucleic acids and indole derivatives was previously investigated without taking into account the self-association of the indole derivatives.[4] However, the concentration dependence of the pmr spectra of tryptamine, 5-hydroxytryptamine (serotonin), and 5-hydroxyindole provides evidence for self-association (Table III). This self-association can also be

Table III. Self-Association of Indole Derivatives in Aqueous Solution (pH 7) and Uridine Effects on Self-Association

		H_a	H_b	H_c	H_d	H_e	$CH_{2\alpha}$	$CH_{2\beta}$
5-Hydroxyindole	$\Delta\delta_c$[a]	3.4	3.6	4.8	5.3	4.8		
Serotonin	$\Delta\delta_c$	6.3	7.5	10.8	8.6		10.6	11.4
	$\Delta\delta_u{}^1$[b]	4.5	4.8	3.1	4.9		0.3	1.7
	$\Delta\delta_u{}^2$[b]	2.5	2.2	0	2.4		−2.9[d]	−1.7[d]
	r[c]	0.32	0.35	0.28	0.29		0.30	0.30
Tryptamine	$\Delta\delta_c$[a]			4.9			6.2	5.6
	$\Delta\delta_u{}^1$[b]			3.5			0.5	2.2
	$\Delta\delta_u{}^2$[b]			1.6			−1.4[d]	0.5
	r[c]			0.39			0.30	0.30

[a] $\Delta\delta_c$ is the upfield shift observed when the concentration increases from 0.01 to 0.15 M. [b] $\Delta\delta_u{}^1$ and $\Delta\delta_u{}^2$ are the changes in chemical shifts of the indole derivative at low ($\Delta\delta_u{}^1$) and high ($\Delta\delta_u{}^2$) concentrations when uridine (0.15 M) is added to the solution. [c] r is the fractional destacking of indole rings at high concentration due to uridine and is calculated according to eq 15. [d] − indicates a shift toward lower fields.

Table IV. Changes in Chemical Shifts ($\Delta\sigma$) of the Different Protons of 5-Hydroxyindole (7×10^{-3} M) for Different Concentrations in Purine[a]

		H_a		H_b		H_c		H_d		H_e	
C	$\delta\sigma_{H_6}$	$\Delta\sigma$	r	$\Delta\sigma$	r	$\Delta\sigma$	r	$\Delta\sigma$	r	$\Delta\sigma$	r
0.960	51.5	56.4	1.09	67.3	1.31	42.1	0.82	66.2	1.28	56.1	1.09
0.746	46.9	51.7	1.10	62.5	1.33	38.5	0.82	61.9	1.32	51.5	1.10
0.582	42.8	47.0	1.10	57.0	1.33	34.7	0.81	55.8	1.30	46.6	1.09
0.420	37.0	40.9	1.11	49.5	1.34	30.1	0.81	49.6	1.34	39.6	1.07
0.200	24.8	27.9	1.12	33.6	1.35	19.9	0.80	32.4	1.31	26.7	1.08
0.130	18.8	21.0	1.12	25.4	1.35	14.7	0.78	24.6	1.31	20.2	1.08
0.089	14.4	15.5	1.08	19.0	1.32	11.0	0.77	18.3	1.27	14.5	1.08

[a] r is the ratio $\Delta\sigma/\delta\sigma_{H_6}$ where $\delta\sigma_{H_6}$ is the change in chemical shift of the H_6 proton of purine with respect to free purine.

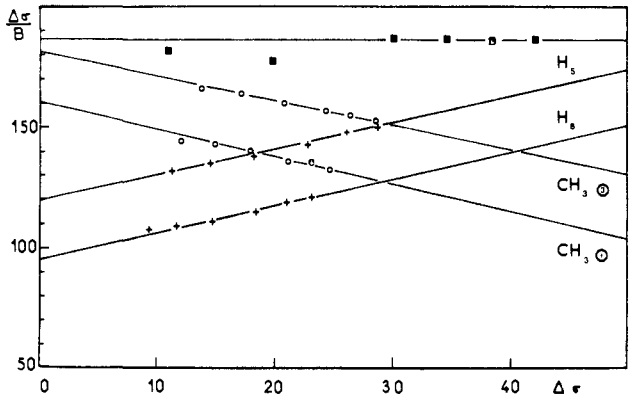


Figure 2. Complex formation between purine in excess and 5-hydroxyindole (proton H_c (■)), cytosine (protons H_5 and H_6 (+), $\Delta\sigma_{H_6} > \Delta\sigma_{H_5}$), and caffeine (protons CH_3 (1) and CH_3 (3) (○), $\Delta\sigma_{CH_3(3)} > \Delta\sigma_{CH_3(1)}$) each at a concentration of 8×10^{-3} M. The pmr data are plotted according to eq 13b.

demonstrated by the effect of uridine, a pyrimidine nucleoside whose ring-current effects are small, on the pmr spectra of indole derivatives. At low concentration of the latter (0.01 M), upfield shifts are observed upon addition of 0.2 M uridine ($\Delta\delta_u{}^1$ in Table III). This is due to complex formation between the two compounds resulting in small but measurable ring-current effects of the pyrimidine ring. At high concentration of the indole derivative (0.2 M), two opposite effects are expected: (i) downfield shifts due to destacking of the indole rings resulting from intercalation of uridine in indole aggregates, (ii) upfield shifts due to ring current effects of uridine on the indole protons. The net result depends on the particular proton investigated ($\Delta\delta_u{}^2$ in Table V). The association constant for the binding of uridine to the indole derivative is small (around 1 M^{-1}) so that whatever the concentration of the indole derivative between 0.01 and 0.2 M, the ratio of complexed to free indole molecules will have approximately the same value in the presence of 0.2 M uridine. Therefore, the value of $\Delta\delta_u{}^2$ can be calculated using the experimental values of $\Delta\delta_u{}^1$ (ring-current effects) and $\Delta\delta_c$ (change in chemical shifts of indole proton resonances when the concentration increases from 0.01 to 0.15 M)

$$\Delta\delta_u{}^2 = \Delta\delta_u{}^1 - r\Delta\delta_c \qquad (15)$$

where r represents fractional destacking of the indole rings. The values of r were calculated for each particular proton by using eq 15. The results presented in Table III for tryptamine and serotonin show that r is nearly constant for each proton in agreement with the above model.

Analysis of the pmr results for self-association of serotonin according to eq 4b does not lead to a linear representation indicating that the mode of association of this molecule is more complex than that of the simplified model described in section Ib.

(3) Complex Formation between Purine and Indole Derivatives. (a) Interaction between Purine and 5-Hydroxyindole. Purine in Excess. In Table IV, are reported the changes in chemical shifts of the different protons of 5-hydroxyindole (7×10^{-3} M) for different concentrations of purine. These changes have been compared to those measured for the H_6 proton of purine. The ratio $\Delta\sigma/\delta\sigma_{H_6}$ remains constant whatever the purine concentration and the proton of 5-hydroxyindole investigated. The H_6 proton of purine is chosen as reference since it gives the maximum variation in chemical shift. According to eq 14b, this would mean that $k_1 \simeq K = 2.2 \pm 0.2$ M^{-1}. Pmr results can be analyzed according to eq 13b where B represents the concentration of free purine molecules. As seen in Figure 2, a plot of $\Delta\sigma/B$ against $\Delta\sigma$ is a horizontal line which once again means that $k_1 \simeq K$. If purine association was limited to dimers, representation ac-

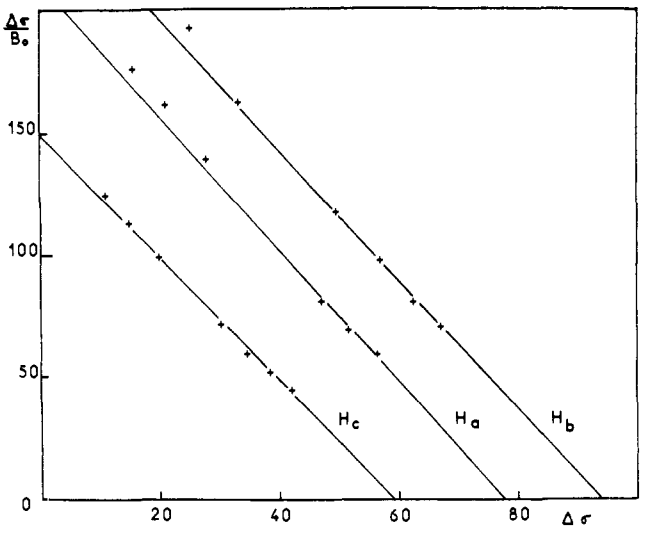


Figure 3. Complex formation between purine in excess and 5-hydroxyindole ($C = 7 \times 10^{-3}$ M). Analysis of pmr data according to eq 5a for three different protons of 5-hydroxyindole.

cording to eq 13 and 14 would not be expected to give the observed results. Thus, pmr results of interactions between purine and 5-hydroxyindole, when purine is in excess, are consistent with a model in which 5-hydroxyindole substitutes for purine in the aggregates, the association constants for purine–5-hydroxyindole and purine–purine complex formation being equal within experimental error. The changes in chemical shifts for the protons of 5-hydroxyindole bound to a purine molecule are given in Table V.

Table V. Changes in Chemical Shifts ($(\Delta\sigma_{AB} + \Delta\sigma_{BA})/2$) of the Different Protons of Various Indole Derivatives Bound to a Purine Molecule

	H_a	H_b	H_c	H_d	H_e	H_α	H_β
Hydroxyindole	54	65	39	64	53		
Tryptamine			47.5			36	16.5
Serotonin			50	70		38	16.5

Even in cases where the compound in excess (purine) strongly self-associates a plot of $\Delta\sigma/B_0$ against $\Delta\sigma$ may still give a straight line (Figure 3). As discussed in II (b and c), it might be concluded from this plot that either 1:1 complexes or a mixture of 1:1 and 2:1 complexes are formed with the characteristic parameters given in Table VI. It can be seen that assuming simultaneous formation of 1:1 and 2:1 complexes, the apparent association constant and chemical shifts determined in this way are similar to those determined when self-association of purine is taken into account (compare Table V and VI). However, the association constant is about 20% higher and the changes in chemical shifts are about 30% smaller when self-association of purine is not taken into account.

Table VI. Parameters Obtained from a Plot of $\Delta\sigma/B_0$ *vs.* $\Delta\sigma$ for the Various Protons of 5-Hydroxyindole in Presence of Various High Molarities of Purines[a]

	H_a	H_b	H_c	H_d	H_e
k_1, M^{-1}	2.7	2.6	2.5	2.6	2.7
$\Delta\sigma_{BAB}$, Hz	78	93	58	97	76

[a] Assuming simultaneous formation of 1:1 and 2:1 complexes with two independent and equivalent binding sites on 5-hydroxyindole and assuming also that purine does not undergo self-association.

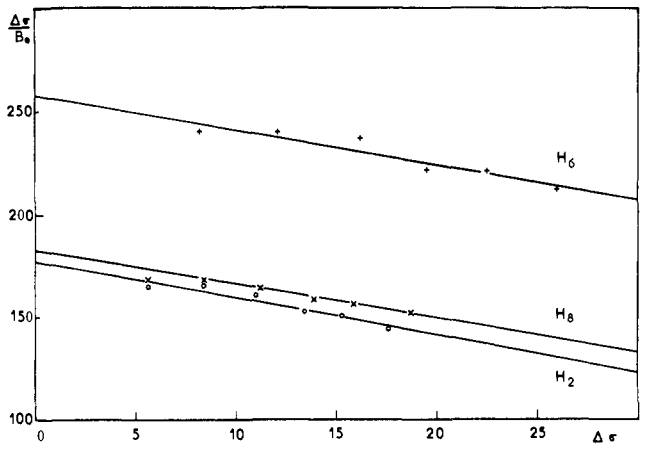


Figure 4. Complex formation between 5-hydroxyindole in excess and purine ($C = 7 \times 10^{-3}$ M). Analysis of pmr data according to eq 5a for the three protons of purine.

5-Hydroxyindole in Excess. The chemical shifts of 5-hydroxyindole protons are much less dependent upon concentration than the purine protons (compare Tables III and V). It can therefore be assumed that 5-hydroxyindole associates weakly in aqueous solutions as compared to purine. A straight line is obtained when $\Delta\sigma/B_0$ is plotted against $\Delta\sigma$ (Figure 4). The slope of the straight lines contained for the three protons of purine is -1.7 ± 0.1 M^{-1}. The x-axis intercepts are 152, 113, and 100 Hz for H_6, H_8, and H_2, respectively. Straight lines such as those shown in Figure 4 could be obtained at least in three cases (see Table I). If only 1:1 complexes were formed, x-axis intercepts would represent the difference in chemical shifts of purine protons in the complexed and free state. The values obtained above are much too high to represent such differences. If simultaneous formation of 1:1 and 1:2 complexes was taking place, the x-axis intercepts would represent the difference in chemical shifts between the free purine molecule and this molecule sandwiched between two 5-hydroxyindole molecules. Again, the experimental values are too high when compared to those obtained for purine intercalated between two purine molecules ($\Delta\sigma_{B_\infty}$, Table II). Moreover, the microscopic association constant would be 1.7 M^{-1}, too small a value when compared to that obtained when purine is in excess (2.2 M^{-1}). Finally, the model described in IId seems to account rather well for the experimental data with the assumption that self-association of 5-hydroxyindole is rather weak (in eq 13b B can then be replaced by B_0). This is in fact observed as stated at the beginning of this paragraph. The slope of the straight lines in Figure 4 will then be equal to $K - k_1$ where k_1 is the microscopic association constant determined when purine is in excess (2.2 M^{-1}). Thus, the association constant K for the self-association of 5-hydroxyindole is calculated to be 0.5 M^{-1}. This value is small enough to justify the assumption that $B \simeq B_0$ since the concentration of 5-hydroxyindole does not increases beyond 0.12 M. The variations of

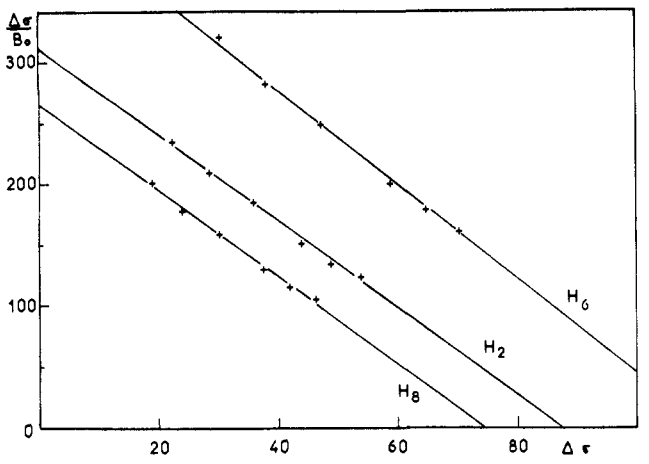


Figure 5. Complex formation between serotonin (5-hydroxytryptamine) in excess and purine ($C = 9.5 \times 10^{-3}$ M). Plot of data according to eq 5a for three protons of purine.

chemical shifts of the protons of purine in the complex with hydroxyindole are 54, 37.5, and 37.5 Hz for H_6, H_2, and H_8, respectively. These values are not very accurate because the error in the determination of the self-association constant of 5-hydroxyindole is of the same order of magnitude as this constant itself. However, these changes in chemical shifts are very similar to those observed for purine–purine interaction (see Table II).

(b) Purine–Serotonin and Purine–Tryptamine. Since it was shown that both compounds (purine and indole derivative) self-associate in solution, we have tried to use the equations derived in the models presented above.

Purine in Excess. It can be seen in Tables VII and VIII that the changes in chemical shifts of tryptamine and serotonin protons at different purine concentrations are proportional to those of purine itself. The H_6 proton is chosen as a reference since it gives the maximum variation in chemical shifts. In the two cases, a plot of $\Delta\sigma/B$ against $\Delta\sigma$ gives a straight line whose slope is nearly zero. According to eq 14, this means that $K \simeq k_1$. The microscopic association constant for complex formation between purine and tryptamine or serotonin is therefore quite similar to the self-association constant of purine (2.2 ± 0.2 M^{-1}). Comparing these results with those obtained with 5-hydroxyindole shows that the amine substituent does not interfere markedly with complex formation. This is further supported by the observation that the ratios $\Delta\sigma/\delta\sigma_{H_6}$ are quite similar for equivalent protons of the three indole derivatives (Tables V, VII, and VIII).

Tryptamine or Serotonin in Excess. Serotonin and tryptamine associate in aqueous solutions but this association does not follow the simple model described in paragraph Ib which was successful for purine. We have then plotted directly $\Delta\sigma/B_0$ against $\Delta\sigma$ (see paragraphs IIb and IIc). Straight lines are obtained as shown in Figure 5. The slopes are 4.0 ± 0.2 and 3.8 ± 0.2 M^{-1} for tryptamine and serotonin, respectively. The microscopic binding constant obtained when purine is in excess is 2.2 ± 0.2 M^{-1} (see above). Thus if only 1:1 complexes were formed when the indole derivative is the excess component, the association constant should be around 4.4 ± 0.4 M^{-1}. This value is higher than that derived above from the slopes. The extrapolated changes in chemical shifts appear to be larger than those expected for 1:1 complexes, especially in the case of serotonin. Due to self-association of the indole derivative, the plot of $\Delta\sigma/B_0$ against $\Delta\sigma$ gives association constants which are too small, and changes in chemical shifts which are too large.

(4) Complex Formation between Purine and Other Purine and Pyrimidine Derivatives. Complex formation between purine in excess and indole derivatives was analyzed above according to the model described in paragraph Id. In the three cases investigated, we found that the microscopic association constant for the binding of the indole derivative to purine was nearly identical to the self-association constant of purine. To test the proposed model, we looked for compounds which would give different association constants. We investigated the complexes formed between cytosine or caffeine and purine in excess. The effects of self-association of cytosine and caffeine were minimized by working at low concentrations of these products ($\sim 8 \times 10^{-3}$ M). The results presented in Figure 2 show that a plot of $\Delta\sigma/B$ against $\Delta\sigma$ (as defined in paragraph Id) gives straight lines in agreement with the model proposed. In the case of cytosine–purine complexes, the microscopic constant is smaller than the self-association constant of purine ($K - k_1 = 1.1$ M^{-1} and $k_1 =$

Table VII. Change in Chemical Shifts of Different Protons of Tryptamine (7×10^{-3} M) for Different Concentrations of Purine[a]

		—H_c—		—CH_2 (α)—		—CH_2 (β)—	
C	$\delta\sigma_{H_6}$	$\Delta\sigma$	$\Delta\sigma/\delta\sigma_{H_6}$	$\Delta\sigma$	$\Delta\sigma/\delta\sigma_{H_6}$	$\Delta\sigma$	$\Delta\sigma/\delta\sigma_{H_6}$
0.527	41.9	40.8	0.975	14.5	0.347	30.9	0.740
0.436	38.7	38.0	0.980	13.7	0.354	28.6	0.738
0.371	36.5	35.4	0.970	12.6	0.345	26.5	0.727
0.291	32.9	32.3	0.980	11.6	0.352	24.0	0.730
0.218	27.7	26.8	0.967	9.9	0.357	20.2	0.780
0.145	22.3	21.6	0.970	8.0	0.359	16.3	0.731
0.097	17.5	16.7	0.955	6.0	0.343	12.4	0.710

[a] See legend of Table IV.

Table VIII. Changes in Chemical Shifts of Different Protons of Serotonin (7×10^{-3} M) for Different Concentrations of Purine

		—H_c—		—H_d—		—CH_2 (α)—		—CH_2 (β)—	
C	$\delta\sigma_{H_6}$	$\Delta\sigma$	$\Delta\sigma/\delta\sigma_{H_6}$	$\Delta\sigma$	$\Delta\sigma/\delta\sigma_{H_6}$	$\Delta\sigma$	$\Delta\sigma/\delta\sigma_{H_6}$	$\Delta\sigma$	$\Delta\sigma/\delta\sigma_{H_6}$
0.501	40.6	40.6	1.0	58.0	1.43	13.1	0.323	30.7	0.755
0.432	37.9	38.7	1.02	54.7	1.44	12.6	0.333	29.2	0.770
0.360	34.9	35.4	1.015	50.7	1.455	11.3	0.324	26.9	0.770
0.260	29.6	30.5	1.03	43.5	1.47	10.2	0.345	22.7	0.767
0.187	24.7	25.5	1.03	35.6	1.44	7.9	0.320	19.0	0.769
0.133	20.4	21.1	1.035	29.5	1.44	7.2	0.352	15.8	0.775
0.0835	15.5	15.4	1.00	21.8	1.41	5.1	0.329	11.3	0.730

1.1 M^{-1}) while the reverse situation is observed in the case of caffeine–purine complexes ($K - k_1 = -1\ M^{-1}$ and $k_1 = 3.2\ M^{-1}$). The extrapolated chemical shifts for the complexes are quite comparable to those obtained for self-association of purine (compare Tables II and IX). The results obtained here are in good agreement with those already reported for similar complexes.[8-11]

Table IX. Changes in Chemical Shifts of the Different Protons of Cytosine and Caffeine Bound to a Purine Molecule

	H_5	H_6	H_8	$CH_3(1)$	$CH_3(2)$	$CH_3(3)$
Cytosine	54	39				
Caffeine			27	25	31	28

(5) Complex Formation between Adenosine and Tryptophan in 1 *N* DCl. In this case, the two components have substituted rings and possess electric charges. There is no concentration effect upon the pmr spectrum of adenosine in acidic solution (1 *N* DCl). The purine ring is protonated and this appears to prevent self-association of adenosine under these experimental conditions. In contrast, tryptophan molecules associate in acidic solutions as shown by the concentration dependence of their proton resonances. Analysis of pmr data for complex formation between adenosine and tryptophan in 1 *N* DCl according to eq 5b gives straight lines whatever the compound in excess. However, the association constant calculated from these plots is about twice higher when adenosine is the excess component (Table X). Also the extrapolated chemical shifts appear to be much too large when tryptophan is in excess. However, since tryptophan self-associates, it appeared reasonable to assume that both 1:1 and 2:1 complexes are formed when tryptophan is the excess component whereas only 1:1 complexes are formed when adenosine is in excess. The association constant for 2:1 complex formation may be determined according to eq 11 using the parameters $\Delta\bar{\sigma}_{AB}$ and K_1 calculated when adenosine is in excess (assuming only 1:1 complex formation under these conditions). The results are given in Table XI. It can be seen that the results are in quite good agreement with the simple model discussed in IIc assuming two independent binding sites for the adenosine molecule. In particular the change in chemical shift of the H_2 proton in the 2:1 complex is twice that in the 1:1 complex in agreement with this simple model. This is not verified for the H_8 proton which might imply that the two sites in the 2:1 complex are not rigorously magnetically equivalent. This may also explain, why the apparent constant K is not exactly the same for the proton H_8.

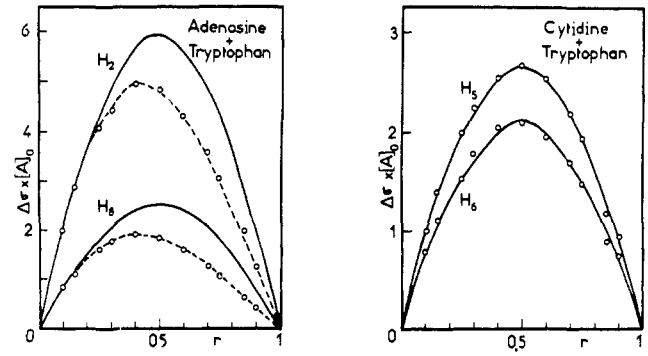


Figure 6. Mixing curves of complex formation between tryptophan and adenosine (left) or cytidine (right) in 1 *N* DCl (see ref 4). In each case the total concentration of both compounds is kept constant (0.28 *M*); r is $A/(A + B)$ where A is the concentration of the nucleoside and B the concentration of tryptophan. Full lines are theoretical curves calculated assuming the formation of 1:1 complexes only using the parameters (k_1 and $\Delta\sigma_{AB}$) obtained when adenosine is the excess component. The dotted line has been calculated assuming the simultaneous formation of 1:1 (AB) and 2:1 (BAB) complexes with the parameters given in Table XI (see text).

This is further supported by an analysis of mixing curves according to the method of continuous variations. If the sum of tryptophan B_0 and adenosine A_0 concentrations is kept constant and the ratio $r = A_0/B_0$ is varied, a plot of $A_0\Delta\sigma$ against r gives a curve similar to the classical Job plots in absorption[12] ($\Delta\sigma$ represents the change in chemical shifts of the adenosine protons when the concentration ratio is changed). The maximum of $A_0\Delta\sigma$ is observed for a value of r higher than

Table X. Association Constants and Changes in Chemical Shifts for the Interaction between Adenosine and Tryptophan in 1 *N* DCl Obtained from a Plot of $\Delta\sigma/B_0$ against $\Delta\sigma$ in the Presence of an Excess of Tryptophan or Adenosine

	a[a]		b[b]		
	$2k_1$	$\frac{1}{2}(\Delta\sigma_{AB} + \Delta\sigma_{BA})$	$2k_1$	$\frac{1}{2}(\Delta\sigma_{AB} + \Delta\sigma_{BA})$	Proton
Excess of tryptophan	5	105	10	52.5	H_2
	4.1	60	8.2	30	H_8
Excess of adenosine	10	26			H_α trypt

[a] Assuming that only 1:1 complexes are formed, eq 5b. [b] Assuming that 1:1 and 2:1 complexes are formed simultaneously, eq 9.

Table XI. Association Constants and Changes in Chemical Shifts for the Interaction of Tryptophan with Adenosine in 1 *N* DCl in Excess of Tryptophan Assuming Simultaneous Formation of 1:1 and 2:1 Complexes[a]

	K_1	K_2	$(\Delta\sigma_{AB} + \Delta\sigma_{BA})/2$	$\Delta\sigma_{BAB}$
H_2	10	2.3	55	111
H_8	10	2.3	19	54

[a] If the two binding sites on the adenosine molecule were independent and equivalent, one would expect $K_1 = 2k_1$, $K_2 = k_1/2$, $\Delta\sigma_{BAB} = \Delta\sigma_{AB} + \Delta\sigma_{BA}$ (see section Ic).

(10) M. E. Magar and R. F. Steiner, *Biochim. Biophys. Acta.*, **224**, 80 (1970); M. E. Magar, R. F. Steiner, and R. Kolinski, *Biophys. J.*, **11**, 387 (1971).

(11) W. B. Gratzer, *Eur. J. Biochem.*, **10**, 184 (1969); N. I. Nakano and S. J. Igarashi, *Biochemistry*, **9**, 577 (1970).

(12) P. Job, *Ann. Chim. (Paris)*, **9**, 113 (1928).

the 0.5 value that should be obtained if only 1:1 complexes were formed. This mixing curve can be calculated using the parameters for 1:1 and 2:1 complex formation determined above. It can be seen on Figure 6 that the agreement is quite good and this further supports the proposed model.

If adenosine is replaced by cytidine, a symmetrical curve is obtained with a maximum for $r = 0.5$ when $A_0\Delta\sigma$ is plotted against r. In the case of cytidine, the same association constant is obtained independent of whether tryptophan or cytidine is the excess component (Table XII). These results show that cytidine

Table XII. Parameters Obtained for the Interaction Cytidine–Tryptophan in 1 *N* DCl[a]

Proton obsd	$2k_1$	$\Delta\sigma$	
H_6	2.7	69	Tryptophan in
H_5	3.1	84	excess
Absorption measurements	3.0		Cytidine in excess

[a] The determination with cytidine in excess has been made from absorption measurements.[4]

and tryptophan form only 1:1 complexes in acidic solutions whatever the concentration range investigated justifying the assumption that self-association of tryptophan is not important in the behavior of mixtures. In fact, a theoretical mixing curve can be calculated using the extrapolated values of $K = k_1$ and $\Delta\sigma_{AB} = (\Delta\sigma_{AB} + \Delta\sigma_{BA})/2$ that fits quite well with the experimental curve (Figure 6).

Conclusion

Using simple models for self-association and for complex formation between self-associating molecules, we have been able to analyze quantitatively a few equilibria. Self-association of purine had been already investigated by various physical methods. The simple model that we have used here gives some details about a possible mode of association. We were then able to analyze the mechanism of interaction of purine with indole, purine, or pyrimidine derivatives. The main difficulty in this analysis arises from the need for extrapolation of chemical shifts of purine to zero concentration.

In cases where the simple models described in this paper do not apply, one has to rely upon computer fitting of the experimental curve. This has been already commented upon by different authors. In many cases a unique solution is not found and curve fitting is often limited by the accuracy of experimental determinations.

Analysis of these equilibria only in terms of 1:1 complex formation would lead to meaningless results, especially those concerned with chemical shifts in the complexes which are often much larger than those expected and cannot be used to propose a stereochemical model for these complexes.

Experimental Section

Purine and hydroxyindole were obtained from Aldrich and used without further purification. Tryptophan and nucleosides were purchased from California Corp. for Biochemical Research. Solutions were made in D_2O at pD 7.4. Proton magnetic resonance spectra were recorded with a Brüker HFX 90-MHz spectrometer equipped with a Fabritek 1072 computer. All chemical shifts were measured with respect to an external reference (HMS) and corrections for changes in bulk magnetic susceptibility were made when necessary.[4] All the concentrations are expressed in molarities and were determined by absorbance measurements. All pmr measurements were performed at a temperature of 28 ± 1°.

Appendix

Cases i and ii mentioned in paragraph IId above can be considered as shown. For case i

$$A_0\sigma = A\sigma_0 + k_1A\sum_{i=1}^{\infty}K^{i-1}B^i\sigma_{AB} + k_1A\sum_{i}K^{i-1}B^i\sigma_{BA}$$

and

$$A_0 = A(1 + 2k_1\sum_{i=1}^{\infty}K^{i-1}B^i)$$

Case i

Species in the solution	A	AB_i	B_iA
Concentration	A	$k_1K^{i-1}B^iA$	$k_1K^{i-1}B^iA$
Chemical shifts	σ_0	σ_{AB}	σ_{BA}

Straightforward calculations lead to

$$\frac{\Delta\sigma}{B} = -(2k_1 - K)\Delta\sigma + k_1(\Delta\sigma_{BA} + \Delta\sigma_{AB})$$

Case ii

Species in the solution	A	AB_i	B_iA	B_iAB_i	B_iAB_{i+j}	$B_{i+j}AB_i$
Concentrations	A	$k_1K^{i-1}B^iA$	$k_1K^{i-1}B^iA$	$k_1^2K^{2i-2}B^{2i}A$	$k_1^2K^{2i+j-2}B^{2i+j}A$	$k_1^2K^{2i+j-2}B^{2i+j}A$
Chemical shifts	σ_0	σ_{AB}	σ_{BA}	$\sigma_{AB} + \sigma_{BA} - \sigma_0$	$\sigma_{AB} + \sigma_{BA} - \sigma_0$	$\sigma_{AB} + \sigma_{BA} - \sigma_0$

For case ii

$$A_0\sigma = A\sigma_0 + k_1A\sum_{i}K^{i-1}B^i(\sigma_{BA} + \sigma_{AB}) + k_1^2A(\sigma_{AB} + \sigma_{BA} - \sigma_0) \times \sum_{i}(K^{2i-2}B^{2i} + 2\sum_{j}K^{2i+j-2}B^{2i+j})$$

and

$$A_0 = A\left[1 + \frac{2k_1B}{1 - KB} + \frac{k_1^2B^2}{1 - K^2B^2} + \frac{2k_1^2KB^3}{(1 - KB)(1 - K^2B^2)}\right]$$

Straightforward calculations lead to

$$\Delta\sigma/B = -(k_1 - K)\Delta\sigma + k_1(\Delta\sigma_{AB} + \Delta\sigma_{BA})$$