Studies on Purine-Pyrimidine Hydrogen Bonded Base Pairing by Means of Absorption and Emission Spectroscopy

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Procedures are described for measurement of association constants between potentially complementary purine and pyrimidine bases by hydrogen bonding in non-aqueous medium by means of absorption and emission spectroscopy. The methods require that one of the two constituents be fluorescent and exhibit a long wavelength absorption band not, or only partially, overlapped by the second constituent. The foregoing has been applied to measurements of the association constants, and other parameters, of hydrogen-bonded complexes of 2-aminopurine with 1-substituted uracil, thymine and barbital in chloroform solution, both in the ground and excited states. It was established that the 1:1 hydrogen-bonded base pairs 2-aminopurine : 1-cyclohexyluracil and 2-aminopurine: 1-octylthymine maintain the ground-state equilibrium during the life time of the excited state, whereas for the corresponding pair 2-aminopurine: 1-methylbarbital this equilibrium is perturbed on excitation. The influence of N-alkylation of the 2-aminopurine residue on base-pairing with the pyrimidines has also been examined. The results are compared with those obtained by other methods. The specific advantages of the procedures employed, and their possible applications to studies on the behaviour of fluorescent residues in polynucleotides, are discussed.

Base-pairing of potentially complementary purine and pyrimidine derivatives in non-aqueous media has been extensively investigated by means of infrared [1–4] and proton magnetic resonance [5, 6] spectroscopy. Both of these procedures possess the advantage that it is frequently possible to identify one or more of the positions involved in base pairing.

No attention appears hitherto to have been devoted to the possibility of studying such base pairing by means of emission spectroscopy, possibly because of the fact that the naturally occurring base residues in nucleic acids normally exhibit only weak fluorescence in fluid media at room temperature. There are, however, a number of base analogues, such as the mutagen 2-aminopurine, which are fluorescent at room temperature, and the absorption bands of which are displaced sufficiently to the red of the absorption bands of the normal bases as to permit of their specific excitation and an examination of the resultant luminescence spectra in the presence of a potentially complementary base.

Hence, under conditions where base pairing is known to occur, it should be possible to estimate association constants and relevant thermodynamic parameters from modifications in emission spectra. Such a procedure would possess the advantage that complex formation could be followed at concentrations 1 to 3 orders of magnitude lower than those normally required for IR and PMR studies. Furthermore, data on the influence of association by hydrogen bonding on emission properties of fluorescent bases might assist in the interpretation of the emission properties of the latter when incorporated in polynucleotide chains.

The present communication describes the application of the foregoing procedure to the study of association between 2-aminopurine derivatives and N_1-substituted uracil, thymine and barbital.

Materials and Methods

2-aminopurine was a product of Waldhof (Mannheim, GFR), while 2-amino-9-ethylpurine and 2,6-diamino-9-ethylpurine were obtained from Cyclo Chem. Corp. (Los Angeles, U.S.A.). N^2,N^2-dimethylaminopurine was prepared according to Kempter *et al.* [7], 1-cyclohexyluracil according to Kuśmierek and Shugar [8], 1-methylbarbital according to

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Abbreviations: 2-AP, 2-aminopurine; 9-ethyl-2-AP, 2-amino-9-ethylpurine; 9-ethyl-2,6-diAP, 2,6-diamino-9-ethylpurine; 7-octyl-2-AP, 2-amino-7-octylpurine; 1-CHU, 1-cyclohexyluracil; 1-OT, 1-octylthymine; 1-MeB, 1-methylbarbital.

Fischer and Dilthey[9], and 1,3-dimethylbarbital by the procedure of Dox[10].

The preparation of 2-amino-7-octylpurine was based on the treatment of 2-AP with octyl bromide in dimethylformamide. This gave a mixture of the 7-octyl and 9-octyl derivatives, which were separated by thin-layer chromatography.

We are grateful to Dr. J. T. Kuśmierek and Mr. R. Jaskulski for preparation of the following compounds by the general procedure of Baker and Jackson[11]: 1-octylthymine, m.p. 137 – 138 °C; 1-octyl-6-methyluracil, m.p. 134 °C; and 3-octyl-6-methyluracil, m.p. 190 – 192 °C. These three compounds are isomers and theoretical elementary analysis should give C, 65.51%; H, 9.31%, N, 11.76%. Experiment gave, in the order cited above: C, 65.99%, 65.66%, 65.68%; H, 9.31%, 9.42%, 9.23%; N, 11.76%, 11.75%, 11.35%.

All compounds (except 2-amino-7-octylpurine) were recrystallized and all were checked for chromatographic homogeneity in several solvent systems.

Spectral measurements were carried out in commercial spectral grade chloroform, which was further distilled over P_2O_5 to remove traces of water and ethanol (checked by infrared absorption) and collecting the fraction which passed at 61.2 °C. The chloroform was stabilized[12] by addition of 0.04% (v/v) pentene-2.

Ultraviolet absorption spectra were run on a Zeiss (Jena, GDR) VSU-2 manual spectrophotometer and on a Perkin-Elmer Model 450 recording instrument. Difference spectra were obtained with the use of tandem cuvettes, the matching of which was checked. Emission spectra were recorded on an Aminco-Bowman spectrofluorimeter, and were not corrected for photomultiplier sensitivity. For both absorption and emission spectra, the cuvettes were located in thermostated carriages through which circulated an aqueous glycol mixture from a Hoeppler ultrathermostat; temperatures were controlled and measured to an accuracy of ±0.5 °C. All cuvettes used were fitted with machined teflon stoppers; as an additional check on posible loss of solvent by evaporation, optical densities were checked prior to and following each experiment.

Fluorescence lifetimes were measured to an accuracy of ±0.1 nsec on a phase fluorimeter constructed at the Physics Department of the Mikołaj Kopernik University in Toruń. We are indebted to Dr. R. Bauer for making this instrument available to us.

Results

Interactions of 2-AP and 1-CHU and of 2-AP and 1-OT

In chloroform solution the principal long-wavelength absorption maximum of 2-AP, located at 304 nm, is well to the red of those for 1-CHU (λ_{max} 268 nm) and 1-OT (λ_{max} 273 nm), while 1-MeB

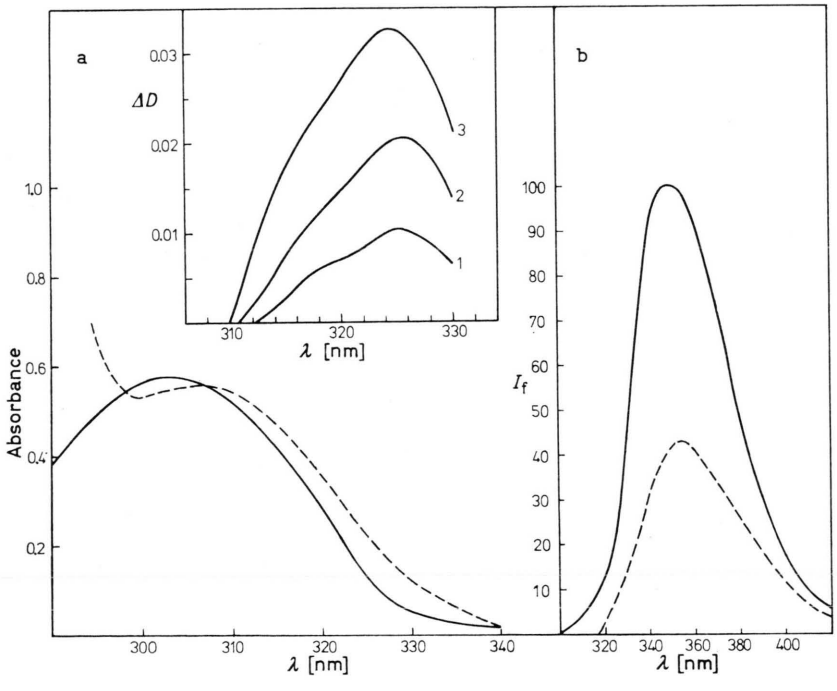


Fig. 1. Modifications in (a) absorption spectrum, and (b) emission spectrum, of 10^{-4} M 2-aminopurine in chloroform solution on addition of 1-cyclohexyluracil to a concentration of 10^{-3} M: ———, prior to addition of 1-CHU; – – –, following addition of 1-CHU. *Insert* to 1(a): Difference absorption spectrum, resulting from addition to 10^{-4} M solution of 2-AP, of 1-CHU to final concentrations of (1) 1.2×10^{-4} M, (2) 3.2×10^{-4} M, (3) 5.9×10^{-4} M.

exhibits no absorption whatever to the red of 270 nm. It is thus relatively simple to specifically excite 2-AP (as well as its N-alkylated analogues, see below) in the presence of the foregoing 1-substituted pyrimidines. The fact that these do not exhibit fluorescence under the conditions of experiment is an additional advantage.

Both the absorption and emission spectra of 2-AP are modified on addition of 1-CHU. At a 2-AP concentration of $\sim 10^{-4}$ M, the addition of 1-CHU to a concentration of 10^{-3} M leads to a red shift in the absorption maximum of 2-AP by about 4 nm (Fig. 1 a) with accompanying modifications in extinction, which may be followed by difference spectrophotometry over the wavelength range 315 – 330 nm. There is also pronounced quenching of the 2-AP emission spectrum, the maximum of which is simultaneously shifted to the red by about 5 nm (Fig. 1 b). The isosbestic point for the absorption spectra is at 308 nm, and excitation was at this wavelength for following changes in emission intensity as a function of the added 1-CHU concentration.

Replacement of 1-CHU by comparable concentrations of 1,3-dimethyluracil was without observable effect on either the absorption or emission spectrum of 2-AP, thus excluding classical Stern-Volmer fluorescence quenching under these conditions.

Addition of 1-OT in place of 1-CHU led to similar changes in the absorption and emission spectra of 2-AP. The precise shift in the absorption maximum of 2-AP was not determined in this case because of overlapping by the 1-OT spectrum, but the isosbestic point was 309 nm as compared to 308 nm for 1-CHU. With a 10^{-3} M concentration of 1-OT, the emission band of 2-AP was red shifted by about 6 nm, and the intensity appreciably reduced as with 1-CHU.

Interactions of N-alkyl derivatives of 2-AP with 1-CHU

Replacement of 2-AP by its N_7- or N_9-alkyl derivatives abolished the modifications in absorption spectrum of both of these on addition of 1-CHU at concentrations up to 10^{-3} M, and considerably decreased the change in emission intensity, the maximum for which was shifted to the red by only about 2 nm for both derivatives. These results point to a lower degree of interaction as a result of N-alkylation. With N^2,N^2-dimethylpurine there was likewise no change in absorption spectrum on addition of 1-CHU; but fluorescence quenching was more pronounced than with the N_7- and N_9-alkyl derivatives, although still less so than with free 2-AP.

The existence of hydrogen-bonded complexes between 9-ethyl-2-AP and 1-CHU in the ground state had, in fact, earlier been demonstrated by Kyogoku *et al.*[1] by means of infrared absorption spectroscopy, the association constant being 45 M^{-1}. Evidence for similar ground state association between 7-octyl-2-AP and 1-CHU is provided by the infrared spectra in Fig. 2, although the association constant in this case was not calculated.

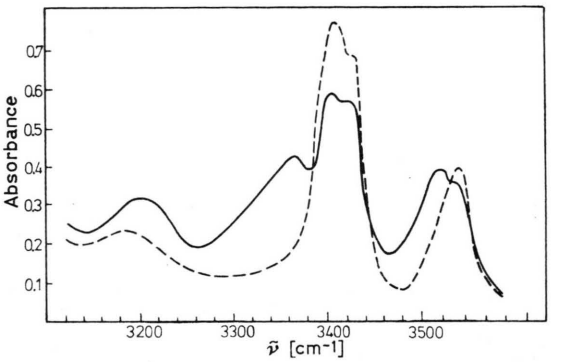


Fig. 2. Infrared absorption spectrum in chloroform solution of a mixture of 4.6×10^{-2} M 2-amino-7-octylpurine and 4.6×10^{-2} M 1-cyclohexyluracil: ———, observed spectrum; — — —, calculated spectrum on assumption of no interaction between the two components.

Interaction of 2-AP derivatives and 1-MeB

This was readily placed in evidence by the marked changes in absorption and emission spectra of 2-AP on addition of 1-MeB. With a 10^{-4} concentration of 2-AP, addition of 10^{-4} to 10^{-3} M 1-MeB provoked a red shift of the 2-AP absorption band by about 2 nm, with a small decrease in absorbance (Fig. 3 a). The emission maximum was also shifted to the red by 6 nm, with a simultaneous marked *increase* in intensity (Fig. 3 b). Replacement of 1-MeB by comparable concentrations of 1,3-dimethylbarbital completely abolished the effects on both the absorption and emission spectra of 2-AP, discounting Stern-Volmer quenching under these conditions, and consistent with formation of a hydrogen-bonded complex between 2-AP and 1-MeB. It is of interest that 1-cyclohexyl-5,6-dihydrouracil, with as saturated pyrimidine ring like 1-MeB, also *increased* the emission intensity of 2-AP.

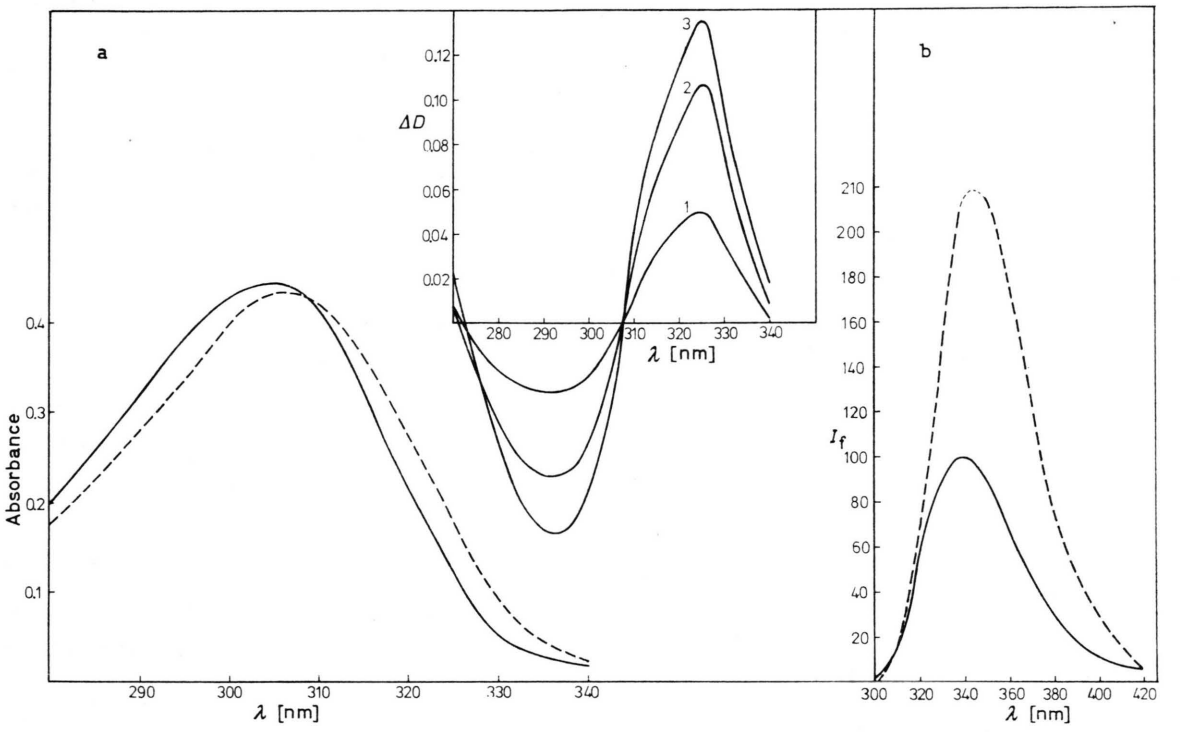


Fig. 3. Modifications in (a) absorption spectrum, and (b) emission spectrum, of a 10^{-4} M chloroform solution of 2-aminopurine on addition of 1-methylbarbital to a final concentration of 10^{-3} M: ———, prior to addition of 1-MeB; — — —, following addition of 1-MeB. *Insert* to 1(a): difference absorption spectrum, resulting from addition to 10^{-4} M solution of 2-AP, of 1-MeB to final concentrations of (1) 3.5×10^{-4} M, (2) 10^{-3} M, (3) 1.7×10^{-3} M.

When 1-MeB ($10^{-4} - 10^{-3}$ M) was added to a chloroform solution of 9-ethyl-2-AP, there was no detectable modification of either the absorption or emission spectra. This situation was unchanged when 9-ethyl-2-AP was replaced by 9-ethyl-2,6-diAP. Only when the 1-MeB concentration was raised to $10^{-2} - 10^{-1}$ M did these systems exhibit a small red shift in the absorption bands with a decrease in extinction, and a red shift of the emission bands with a small *increase* in intensity.

Complexing of 2-AP with 3-alkyluracil

Since the natural nucleosides are N_1-glycosides, it is most logical to study base-pair complexing with the corresponding N_1-substituted pyrimidines, such as 1-CHU or 1-OT, which can base pair *via* two or three hydrogen bonds. It appeared of interest, however, to examine the behaviour of an N_3-substituted pyrimidine, which can hydrogen bond to a purine *via* only two bonds, the $C_2=O$ and N_1H. Since 3-methyluracil is insufficiently soluble in chloroform, the available 6-methyl-3-octyluracil was employed. This compound, at a concentration of 10^{-3} M, was without effect on the absorption spectrum of 2-AP, but did provoke a 2 nm red shift in the emission spectrum, together with a small decrease in emission intensity, testifying to weaker complexing than with 1-CHU. The role of the 6-methyl substituent was of no consequence in complex formation, since a control experiment demonstrated that 6-methyl-1-octyluracil reacted with 2-AP quantitatively like 1-CHU. With the N_7- and N_9-alkyl derivatives of 2-AP, 6-methyl-3-octyluracil produced no observable effects either on the absorption or emission spectra, indicating that, if complex formation occurred, it was too feeble to measure by these methods.

Calculation of Association Constants

The observed modifications in absorption and emission of the foregoing systems may be related to the association constants of the two constituents in each case in either the ground or excited states.

Here the fluorescent component A exhibits an absorption band to the red of the non-fluorescent component B. If we neglect autoassociation of either of the two components (see below), and assume that we are dealing exclusively with 1:1 complexes, then the following derived relationships may be applied:

a. For modifications in absorption [13],

$$\frac{1}{\Delta D} = \frac{1}{\Delta D_{max}} + \frac{1}{K_g \Delta D_{max}} \cdot \frac{1}{[B]} \qquad (1)$$

where ΔD is the change in optical density of 2-AP (or A) in the presence of a concentration [B] of 1-CHU, 1-OT or 1-MeB;
ΔD_{max} is the maximal change in absorbance;
K_g is the association constant in the ground state $-[AB]/([A][B])$.

b. For modifications in emission [14],

$$\frac{I/I_0 - 1}{[B]} = a - b \cdot \frac{I}{I_0} \qquad (2)$$

where I_0 and I are the emission intensities of A prior to, and following, addition of a concentration [B] of 1-CHU, 1-OT or 1-MeB. For an unperturbed equilibrium in the ground state,

$$a = \frac{k_f' \tau' \varepsilon_{AB}}{k_f \tau \varepsilon_A} K_g \quad \text{and} \quad b = \frac{\varepsilon_{AB}}{\varepsilon_A} K_g$$

where k_f', k_f represent the rate constants for radiative processes, τ' and τ the measured lifetimes, and ε_{AB} and ε_A the molar extinction coefficients, of the complex AB and free A, respectively. Experimentally, excitation for determination of association constants was in all instances at the isosbestic point of the absorption spectra, so that $\varepsilon_{AB}/\varepsilon_A = 1$.

If, on the other hand, a new equilibrium is established in the excited state, then

$$a = (k_f'/k_f) K_e \quad \text{and} \quad b = (\tau/\tau') K_e .$$

The association constant in the excited state is

$$K_e = [AB]^*/([A]^*[B]) .$$

Under the experimental conditions here employed, autoassociation of 2-AP may be discounted from the observed linear dependence of emission on concentration up to 10^{-4} M, as well as the absence of any observable shift in location of the emission band. From published data obtained by means of infrared spectroscopy at much higher concentrations [1-3, 12], autoassociation of $10^{-4} - 10^{-3}$ M concentrations of 1-CHU, 1-OT and 1-MeB can be shown to be less than $2-3\%$, and may therefore be neglected. Finally the foregoing systems fulfil the relationships (1) and (2) (see *e.g.* Fig. 4) consistent with the formation of 1:1 associates [13, 14].

From the temperature dependence of the association constants K_g and K_e, application of the van't Hoff equation provided the values for the corresponding enthalpies and entropies of the as-

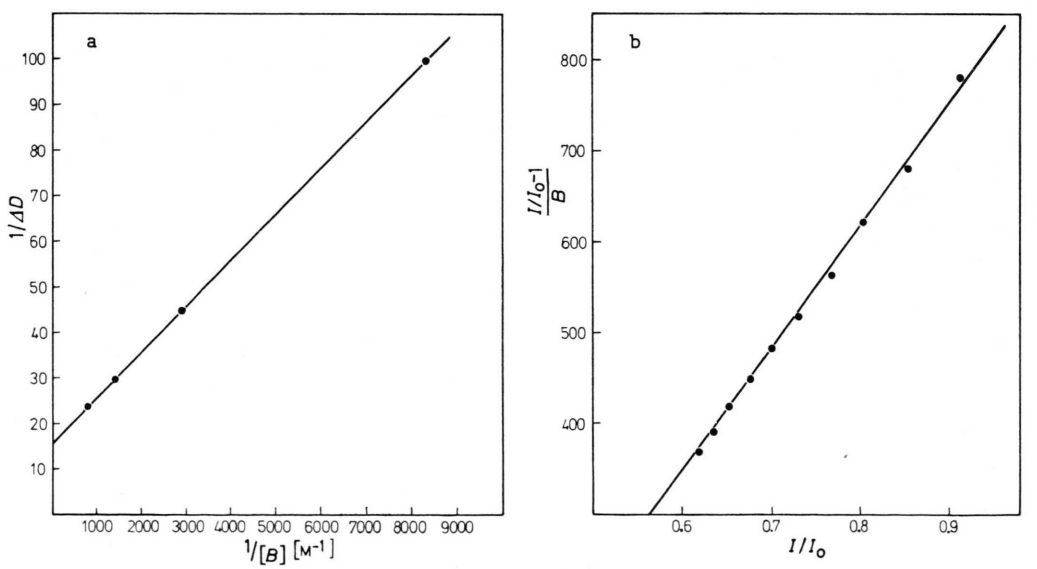


Fig. 4. Interaction of 2-AP at a concentration of 10^{-4} M with various concentrations, [B], of 1-CHU, according to (a) Eqn (1) for changes in absorbance at 326 nm; (b) according to Eqn (2) for changes in fluorescence emission at 348 nm.

Table I. Association constants at 25 °C in chloroform solution, and corresponding parameters for hydrogen-bonded complex formation between 2-aminopurine derivatives and 1-substituted uracil, thymine and barbital.

Complex	K_g [M^{-1}]	a [M^{-1}]	b [M^{-1}]	K_e [M^{-1}]	ΔH^0 [kcal M^{-1}]	ΔS^0 [cal deg^{-1} M^{-1}]
2-AP : 1-CHU [a]	1280±150	700±30	1380±160	8700+500 [c]	7.5±0.5 [e]	11±2 [e]
2-AP : 1-OT [a]	1700±300	740±60	1600±100	—	—	—
2-AP : 1-MeB [a]	725± 60	400±80	1300±200	4700±700 [d] 3900±330 [e]	8.6±2 [f] 11.0±2 [e]	16±5 [f] 20±7 [f]
9-ethyl-2-AP : 1-*MeB* [b]	40± 8	—	—	—	10.0±2 [f]	25±6.5 [f]
9-ethyl-2,6-diAP : 1-MeB [b]	200± 20	—	—	—	9.0±2 [f]	20±6 [f]

[a] 10^{-5}—10^{-4} M 2-AP and 10^{-4}—10^{-3} M 1-CHU, 1-OT or 1-MeB.
[b] 10^{-5}—10^{-4} M 9-ethyl-2-AP or 9-ethyl-2,6-diAP and 10^{-2}—10^{-1} M 1-MeB.
[c] Calculated from the Förster cycle.
[d] Calculated from Eqn (2).
[e] Calculated from temperature-dependent changes in emission spectrum.
[f] Calculated from temperature-dependent changes in absorption spectrum.

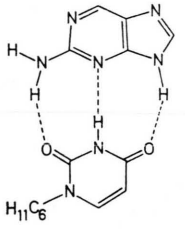
sociation reactions. The measured values of K_g, K_e, a and b, and the calculated magnitudes of ΔH and ΔS are listed in Table I. The excited state lifetimes of free 2-AP and its complexes with 1-CHU and 1-MeB are shown in Table II.

Table II. Excited state lifetimes ($\tau \pm 0.1$ nsec) of 2-AP and its hydrogen-bonded complexes with 1-CHU and 1-MeB in chloroform at 25 °C.

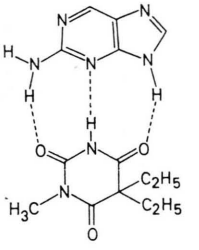
2-AP or complex	τ [nsec]
2-AP	1
2-AP : 1-CHU	0.7
2-AP : 1-MeB	3.4

Discussion

From the foregoing results, taking into account particularly the effects of N-alkylation, it is clear that 1-CHU, 1-OT and 1-MeB each associates with 2-AP in chloroform solution *via* more than one hydrogen bond. The possible schemes of hydrogen bonding in such complexes are shown in Schemes 1 and 2. Furthermore, from a comparison of the results obtained by means of the modifications in absorption and emission spectra for the systems



Scheme 1. Base-paired structure of 2-aminopurine with 1-cyclohexyluracil involving formation of three hydrogen bonds.



Scheme 2. Base pairing of 2-aminopurine with 1-methylbarbital *via* three hydrogen bonds.

2-AP : 1-CHU and 2-AP : 1-OT (Table I), it is clear that the effects observed in the fluorescence spectra reflect the association equilibria in the ground state. In agreement with this conclusion is the ratio of the association constants with 2-AP of 1-CHU and 1-OT (Table I), ~0.8, a value comparable with that reported for the association of these two uracil derivatives with 9-ethyladenine (0.77) by Kyogoku *et al.* [1], using infrared absorption techniques. The somewhat higher association constant for 2-AP : 1-OT, as compared to 2-AP : 1-CHU, is also in agreement with the higher thermal stability of a thymine-containing helical polynucleotide as compared to one containing uracil residues [15].

The association constant for the complex 2-AP: 1-CHU, calculated from the emission data according to Eqn (2), corresponds to the equilibrium constant in the ground state ($K_g = 1280$ M^{-1}, $b = 1380$ M^{-1}, see Table I). On the other hand, the association constant calculated from the Förster cycle is 8700 M^{-1}. These values indicate that the ground state equilibrium for this complex is maintained during the lifetime of the excited state. The fact that the lifetime of the excited state of the complex is approxi-

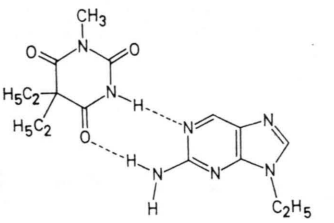
mately the same, or even shorter, as that for free 2-AP (Table II) also points to the equilibrium being unchanged during the lifetime of the excited state.

For the 2-AP:1-MeB complex the observed emission emanates from a state involving perturbation of the ground state equilibrium, as may be seen from a comparison of the parameters a, b and K_g (Table I), and in accord with the increase in lifetime (to 3.4 nsec) of the excited 2-AP:1-MeB complex as compared to the 1 nsec lifetime for excited 2-AP itself (Table II). The values of K_e for the 2-AP:1-MeB pair, calculated by both methods, *i. e.* Eqn (1) and the Förster cycle, are similar (Table I).

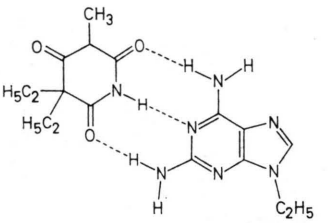
The appreciably weaker associations observed with the N_7- and N_9-alkyl derivatives of 2-AP point to the importance of the additional imidazole hydrogen in the complexing properties of 2-AP itself. The alkyl derivatives are capable of complexing with 1-CHU *via* only two hydrogen bonds, as compared to three hydrogen bonds with 2-AP. Similarly N^2,N^2-dimethylaminopurine is capable of complexing only *via* two hydrogen bonds; the more pronounced effects observed in the emission spectra of complexes of N^2,N^2-dimethylaminopurine, as compared to 9-ethyl-2-AP, may be interpreted as due to stronger complexing *via* N_1 and N_9, as compared to N^2 and N_1 or N^2 and N_3.

Similar decreases in association prevail for the pairs 9-ethyl-2-AP:1-MeB and 9-ethyl-2,6-diAP:1-MeB as compared to 2-AP:1-MeB. However, the data obtained for these are only approximate; with the concentrations of 1-MeB used in these instances ($10^{-2}-10^{-1}$ M), autoassociation would be in excess of 10%. Furthermore concentration quenching may be expected; and, in fact, although such concentrations of 1,3-dimethylbarbital (which cannot complex) did not shift the λ_{max} for absorption or emision, or modify the extinction coefficient, of the 9-ethyl derivatives, they led to about 15% quenching of emission.

The association constants for complexes involving alkylated derivatives of 2-AP are lower than those for complexes with free 2-AP (Table I). It should be noted that 9-Et-2-AP may associate with 1-MeB at most by two hydrogen bonds (Scheme 3). By contrast, 9-Et-2,6-diAP may complex with 1-MeB *via* three hydrogen bonds (Scheme 4), and the association constant in this instance is comparable to the value for the system 9-Et-2,6-diAP:1-CHU



Scheme 3. Base pairing of 2-amino-9-ethylpurine with 1-methylbarbital *via* two hydrogen bonds.



Scheme 4. Base pairing of 2,6-diamino-9-ethylpurine with 1-methylbarbital *via* three hydrogen bonds.

(Table III [1]), which may also associate *via* three bonds. Comparison of the values of the association constants of the systems 9-Et-2,6-diAP:1-MeB and 9-Et-2,6-diAP:1-CHU with the value for 2-AP:1-MeB (Table III) also supports the suggestion (see above) that complexes involving the N_9 position are stronger than those involving the amino groups.

Table III. Ground state association constants, K_g [M^{-1}] in chloroform solution at 25 °C of various purine and pyrimidine base pairs.

	9-etA	2-AP	9-et-2-AP	9-et-2,6-diAP
1-CHU	100	1300 [a]	45	170
5,6-DH-1-CHU	30	—	10	100
1-mephobarb.	200	—	—	—
1-MeB	—	725 [a]	40 [a]	200 [a]

[a] Values obtained in the present paper; other values are taken from Kyogoku *et al.* [1, 3].

For purine-pyrimidine hydrogen-bonded base pairs, the enthalpy of association in the ground state is in the range of 4 – 6 kcal/mol when complexing involves two hydrogen bonds, and about 8 – 11 kcal/mol when three hydrogen bonds are formed [2]. The results obtained in this study for association between 2-AP and 1-CHU (and 1-OT) are therefore consistent with formation of 3 hydrogen bonds, in agreement with the bonding scheme shown in Scheme 1.

It is of interest to compare some of the measured ground state association constants with values obtained by other observers (Table III). Note that, with the two 9-alkylpurine systems, 1-MeB forms stronger associates than 1-cyclohexyl-5,6-dihydrouracil; our qualitative results for the latter (see above) are in accord with this. It will also be seen that, whereas the complexes between 1-MeB and 1-CHU with the two 9-alkylpurine exhibit similar association constants, the binding of 1-CHU with free 2-AP is appreciably stronger than for 1-MeB. Hence the stability of a given complex is dependent on the nature of the hydrogen bonds involved.

Entirely apart from the foregoing, the study of potential complementary base pairing by means of emission spectroscopy is of interest in relation to the behaviour of fluorescent bases following their incorporation into polynucleotide chains or helices. A case in point is 2-AP itself. Poly (2-AP), which is single-stranded, exhibits very low emission, clearly as a result of quenching *via* base stacking; this feeble emission is even further reduced when the polymer is complexed with poly (rU) to form a helical complex *via* base pairing [16], in accord with the present results on complex formation between 2-AP and 1-CHU. It is true, as mentioned above, that most of the natural bases found in nucleic acids exhibit only very low, or virtually no, emission in fluid medium at room temperature. But the growing interest in the synthesis of modified bases with fluorescent properties for use as probes in studies on conformation, interaction with proteins and enzymes, etc., point to the utility of investigating the hydrogen bonding complexing reactions of such bases by emission spectroscopy at the monomer level. It is, in fact, surprising that no attempts appear to have been made to utilize either 2-aminopurine riboside or 2,6-diaminopurine riboside as possible fluorescent analogue probes of adenosine in the CCA termini of tRNA's.

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