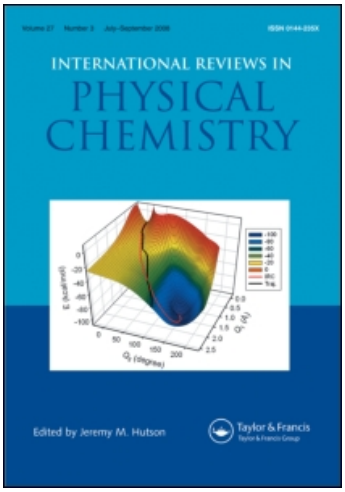

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Hydrogen bond association constants of some nucleoside base pairs formed by an adenosine derivative and anticancer agents

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Hydrogen bond association constants of some nucleoside base pairs formed by an adenosine derivative and anticancer agents†

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Base-pair and trimer formation between certain adenosine derivatives and anticancer agents has been studied by Fourier-transform infrared spectroscopy by determining hydrogen-bond association constants. The results indicate that the anticancer agents studied have a preference for Hoogsteen over Watson–Crick type base-pair formation and that they have an appreciable tendency for trimer formation. This might be the way in which they interfere with site recognition and hinder unwarranted protein formation.

1. Introduction

It has been known for several years (Engel and von Hippel 1974, Watanabe *et al.* 1981, Iwahashi *et al.* 1982) that, in organic solvents, adenine and thymine or uracil derivatives form Watson–Crick and Hoogsteen-type pairs in various proportions. Typically the pairs formed by 9-ethyladenine and uracil derivatives are 70 per cent Hoogsteen and 30 per cent Watson–Crick (Iwahashi *et al.* 1982). Equilibrium constants for dimers and trimers formed by adenine and uracil derivatives were determined in some cases (Nagel and Hanlon 1972, Watanabe *et al.* 1981, Iwahashi *et al.* 1982).

The aim of the present work has been to determine the association constants for a series of base pairs in which one of the partners is an anticancer agent. Some base pairs of this kind have been examined previously (Kyogoku *et al.* 1967 a, Iwahashi and Kyogoku 1977). In this paper a systematic investigation of the equilibrium constants of Watson–Crick and Hoogsteen dimers and trimers involving adenosine derivatives and anticancer agents is reported.

According to N.M.R. studies by Iwahashi *et al.* (1982), 2-chloro-9-ethyladenine forms Hoogsteen-type complexes in the proportion of 93 per cent, whereas 8-bromo-2′,3′,5′-tri-*O*-acetyladenosine forms complexes of the Watson–Crick type, also to 93 per cent.

Figure 1 shows the chemical structures of the compounds dealt with in this paper. 9-ethyladenine, 9-cyclohexyladenine and 2′,3′,5′-tri-*O*-acetyladenosine were chosen as adenine derivatives. Their association with the anticancer agents ftorafur, 5-bromo-3′,5′-di-*O*-acetyl 2′-deoxyuridine, 5-iodo-3′,5′-di-*O*-acetyl-2′-deoxyuridine and 6-aza-2′,3′,5′-tri-*O*-acetyluridine was studied by infrared spectroscopy.

2. Experimental

Our samples of 1-cyclohexylthymine (T), 9-ethyladenine (A), 2-chloro-9-ethyladenine (Cl^2A) and 9-cyclohexyladenine (CA) came from VEGA biochemicals. 8-bromo-2′,3′,5′-tri-*O*-acetyladenosine (Br^8Ado), 2′3,5′-tri-*O*-acetyladenosine (Ado),

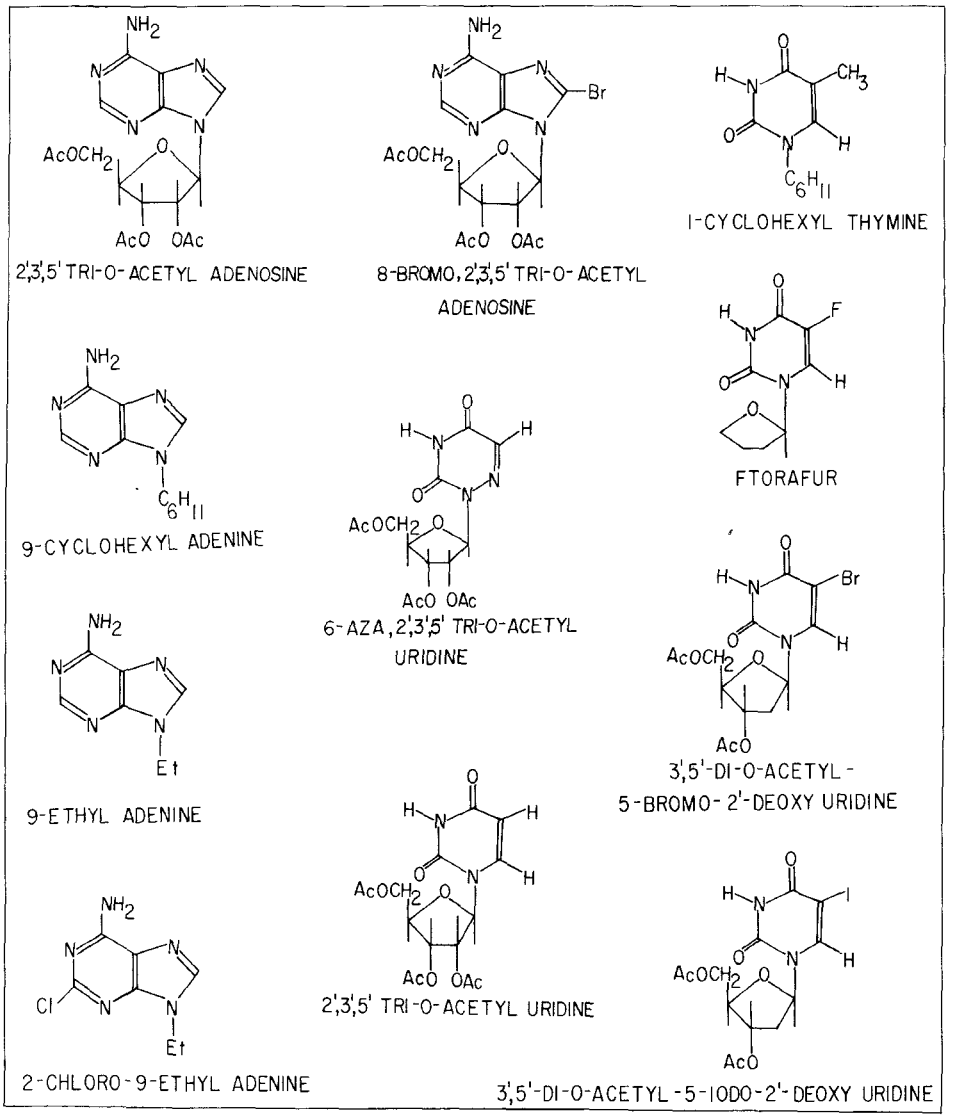

Figure 1. Structures of the compounds studied in the present publication.

2'3'5'-tri-*O*-acetyluridine (Ur), ftorafur (F^5U), 5-bromo-3',5'-di-*O*-acetyl-2'-deoxyuridine (Br^5dUr) and 6-aza-2',3',5'-tri-*O*-acetyluridine (N^6Ur) were Sigma Chemical products. 5-iodo-3',5'-diacetyl-2'-deoxyuridine (I^5dUr) was supplied by the Chemical Dynamics Corporation. They were high purity samples and were used without further purification.

The spectra were recorded on a Nicolet Model 5DXB Fourier transform infrared spectrometer with 2 cm^{-1} resolution; 0·101 cm sodium chloride cells were used. For the calculation of equilibrium constants the concentration of the anticancer agent has been varied from zero to 50 mM and the concentration of the adenosine derivative was kept constant at 20 mM.

The following equilibria were considered:

$$\begin{aligned} A+A &\rightleftharpoons A_2, & K_A \\ T+T &\rightleftharpoons T_2, & K_T \\ A+T &\rightleftharpoons AT, & K_{AT} \\ AT+T &\rightleftharpoons TAT, & K_{TAT} \end{aligned}$$

where A and T stand for adenosine and thymine (or uracil) derivative respectively. It is the absorbance of the band due to the free antisymmetric NH_2 stretching vibration of the adenine derivative which was used for the calculation of the equilibrium constants. The procedure has been outlined in detail in our previous publications. (Buchet *et al.* 1984, Buchet and Sandorfy 1984 b).

Figure 2 shows the various modes of association of the AT dimers. Unfortunately it is not possible to determine the equilibrium constants for one given type of dimer by I.R. spectroscopy. Therefore the constants which were computed relate to the four dimers given in figure 2 in a global way. However, as mentioned above, for the complexes involving 2-chloro-9-ethyladenine they provide approximate values for the Hoogsteen and reversed Hoogsteen dimers and for those involving 8-bromo-2',3',5'-tri-*O*-acetyladenosine for the Watson–Crick and reversed Watson–Crick dimers.

Similarly, our trimerization constants are overall values for all possible trimers formed by one adenosine and two thymine or uracil derivatives.

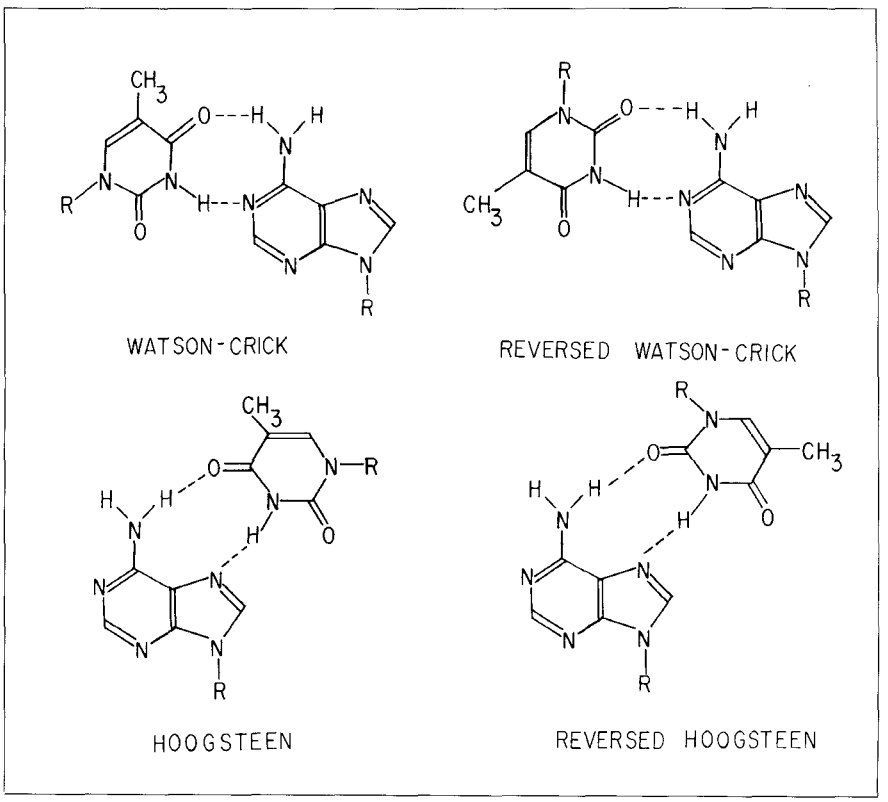

Figure 2. Structures of Watson–Crick and Hoogsteen-type base pairs.

3. Results

In figure 3 a part of the I.R. spectrum of a 40-mM solution in $CDCl_3$ of Ado is shown. The assignment of the bands is the same as for 9-ethyladenine (Kyogoku *et al.* 1967 b). The free and associated asymmetric NH_2 stretching bands are at 3527 and 3489 cm^{-1} respectively. The free and associated symmetrical NH_2 stretching vibrations give bands at 3414 and 3316 cm^{-1}. Other association bands are seen at lower frequencies. (The band at 3156 cm^{-1} is a combination band of $CDCl_3$). The presence of the association bands shows that at the given concentration Ado is partly self-associated. The self-association constant of Ado, determined by proton-N.M.R. spectroscopy is $1{\cdot}6 \pm 0{\cdot}2\ M^{-1}$ (Watanabe *et al.* 1981). In the calculations the absorbance of the 3527 cm^{-1} band was used. While the band is to some extent overlapped by the 3489 cm^{-1} band our results are in good agreement with the ^1H-N.M.R. results of Watanabe *et al.* (1981).

The other spectrum in figure 3 belongs to a mixture of 40 mM of Ado and 40 mM of N^6Ur. As is seen, the intensity of the association bands increased and that of the free bands decreased. (The free NH band of N^6Ur which is at 3372 cm^{-1} is hidden by the broad symmetric band of the associated NH_2 group of 3343 cm^{-1}.) This clearly demonstrates the fact that Ado is associated with N^6Ur. Also the NH_2 bands at 3316

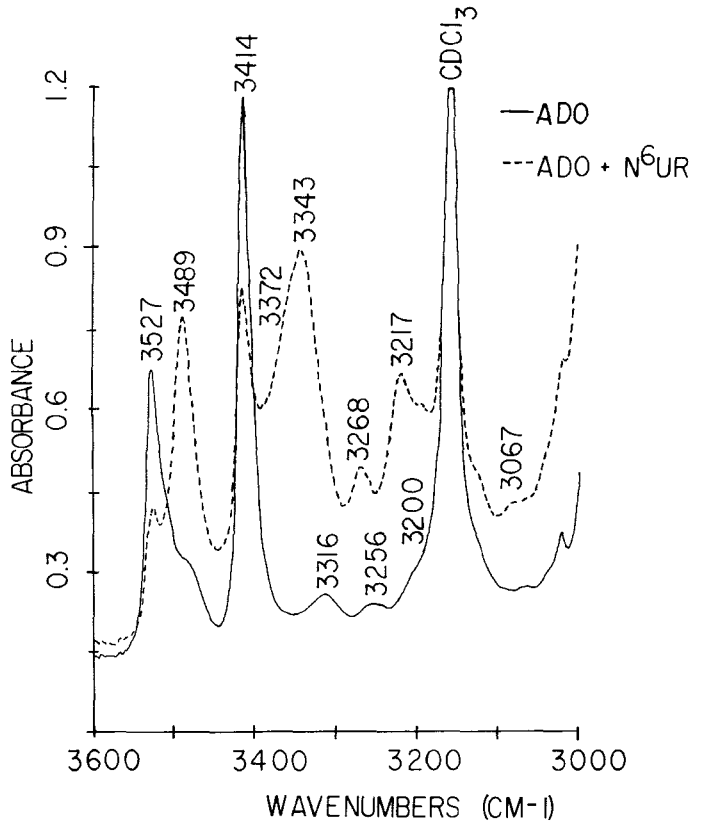

Figure 3. A part of the infrared spectrum of 2′,3′,5′-tri-*O*-acetyladenosine (Ado) (solid line) and a solution of 40 mM of Ado and 40 mM of 6-aza-2′,3′,5′-tri-*O*-acetyluridine (N^6Ur) (dashed line). Solvent $CDCl_3$.

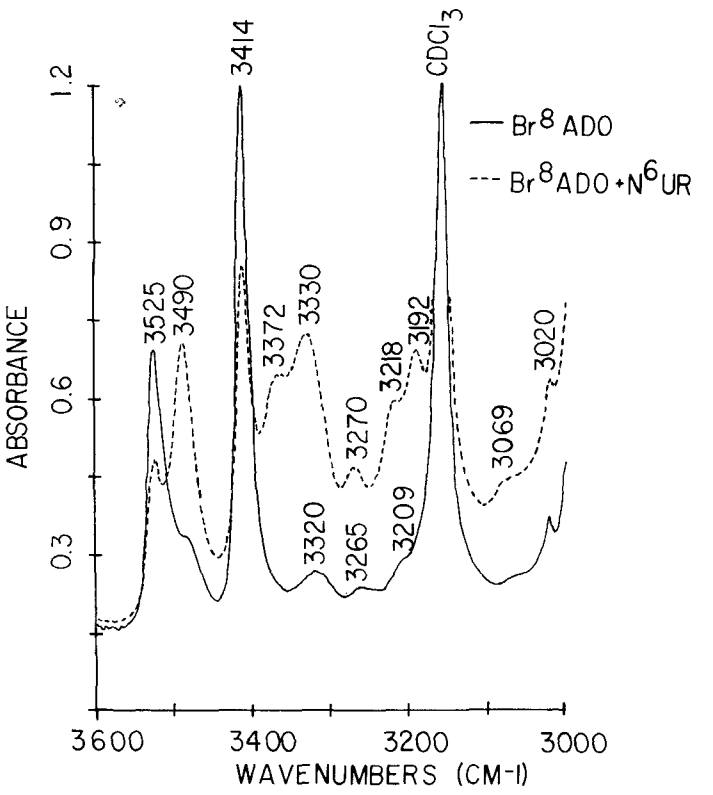

Figure 4. A part of the infrared spectrum of 2',3',5'-tri-O-acetyladenosine (Ado) (solid line) and a solution of 40 mM of Ado and 40 mM of 8-bromo-2',3',5'-tri-*O*-acetyl-adenosine (dashed line). Solvent, $CDCl_3$.

and 3256 cm^{-1} of Ado have shifted to 3343 and 3268 cm^{-1} respectively and a new band appears at 3217 cm^{-1}. N^6Ur itself has its free NH band at 3372 cm^{-1} and its diffuse association band at about 3200 cm^{-1}. The constant for self-association of N^6Ur is $7 \pm 0{\cdot}5\ M^{-1}$ (Buchet *et al.* 1984 a).

In the case of the mixture Ado : N^6Ur, base pairs of the Watson–Crick, reversed Watson–Crick, Hoogsteen and reversed Hoogsteen types are present. It is of interest to compare its spectrum to the spectrum of Br^8Ado : N^6Ur. As mentioned above Br^8 : Ado in organic solvents gives 93 per cent of Watson–Crick + reversed Watson–Crick pairs with thymine derivatives (Iwahashi *et al.* 1982); the bromine atom at the 8 position almost completely prevents the formation of Hoogsteen pairs. Figure 4 shows the spectra of Br^8Ado and that of a mixture of Br^8Ado and N^6Ur (40 mM for each component in $CDCl_3$). Looking at the relative intensities of the free and the association bands in figures 3 and 4 it appears clearly that the mixture Br^8Ado : N^6Ur is *less* associated than the mixture Ado : N^6Ur. Similar results have been obtained with the adenine derivatives A and Cl^2A.

Table 1 gives the equilibrium constants for the self-associated species which are involved. The equilibrium constants for the self-association of A,T (Kyogoku *et al.* 1967 a), Ado, Br^8Ado (Watanabe *et al.* 1981) F^5U and N^6Ur (Buchet *et al.* 1984 a) have been taken from these publications. These are needed for the calculation of the

Table 1. Equilibrium constants for self-associated complexes obtained by I.R. spectra. Solvent, $CDCl_3$; $T = 21 \pm 1°C$.

Compound	$K(M^{-1})$	$\varepsilon(l^{-1}M^{-1})$	Band used (cm^{-1})
CA	$1 \pm 0{\cdot}5$	98 ± 5	3526
Cl^2A	3 ± 2	142 ± 10	3526
Br^5dUr	$8 \pm 2{\cdot}8$	147 ± 6	3385
I^5dUr	$10 \pm 3{\cdot}7$	179 ± 9	3385

Table 2. Equilibrium constants for complexes of adenosine and uridine derivatives. Solvent, $CDCl_3$; $T = 21 \pm 1°C$.

Complex	$K_A(M^{-1})$	$K_T(M^{-1})$	$K_{AT}(M^{-1})$	$K_{TAT}(M^{-1})$	ε $(M^{-1}cm^{-1})$	Band used (cm^{-1})
Br^8Ado:T	$2 \pm 0{\cdot}5$	4 ± 1	20 ± 1		117 ± 2	3525
Br^8Ado:Ur†		$7 \pm 0{\cdot}5$	$27 \pm 3{\cdot}4$		121 ± 2	
Br^8Ado:F^5U†		$7 \pm 0{\cdot}5$	33 ± 5		116 ± 2	
Br^8Ado:I^5dUr		$10 \pm 3{\cdot}7$	49 ± 4		139 ± 3	
Br^8Ado:Br^5dUr		$8 \pm 2{\cdot}8$	50 ± 4		128 ± 1	
Br^8Ado:N^6Ur†		$7 \pm 0{\cdot}5$	66 ± 5		124 ± 1	
Cl^2A:T	3 ± 2	4 ± 1	70 ± 10		139 ± 4	3526
Cl^2A:Ur		$7 \pm 0{\cdot}5$	72 ± 8		136 ± 3	
Cl^2A:F^5U		$7 \pm 0{\cdot}5$	125 ± 8		140 ± 3	
Cl^2A:I^5dUr		$10 \pm 3{\cdot}7$	131 ± 5		140 ± 2	
Cl^2A:Br^5dUr		$8 \pm 2{\cdot}8$	140 ± 12		147 ± 6	
Cl^2A:N^6Ur		$7 \pm 0{\cdot}5$	160 ± 15		142 ± 10	
Ado:T	$1{\cdot}6 \pm 0{\cdot}5$	4 ± 1	47 ± 5	8 ± 5	138 ± 2	3527
Ado:Ur		$7 \pm 0{\cdot}5$	77 ± 10	12 ± 4	120 ± 3	
Ado:F^5U		$7 \pm 0{\cdot}5$	102 ± 10	21 ± 8	135 ± 5	
Ado:I^5dUr		$10 \pm 3{\cdot}7$	143 ± 15	27 ± 3	143 ± 1	
Ado:Br^5dUr		$8 \pm 2{\cdot}8$	151 ± 8	30 ± 2	139 ± 1	
Ado:N^6Ur		$7 \pm 0{\cdot}5$	181 ± 40	26 ± 6	129 ± 3	
A:T	$3{\cdot}2 \pm 0{\cdot}3$	4 ± 1	75 ± 5	9 ± 1	136 ± 2	3527
A:Ur		$7 \pm 0{\cdot}5$	77 ± 8	28 ± 7	141 ± 10	
A:F^5U		$7 \pm 0{\cdot}5$	145 ± 19	34 ± 5	115 ± 2	
A:I^5dUr		$10 \pm 3{\cdot}7$	152 ± 18	25 ± 6	136 ± 2	
A:Br^5dUr		$8 \pm 2{\cdot}8$	173 ± 24	40 ± 16	128 ± 3	
A:N^6Ur		$7 \pm 0{\cdot}5$	204 ± 33	51 ± 8	120 ± 3	
CA:T	$1 \pm 0{\cdot}5$	4 ± 1	75 ± 5	12 ± 1	116 ± 2	3526
CA:Ur		$7 \pm 0{\cdot}5$	82 ± 9	13 ± 2	104 ± 2	
CA:F^5U		$7 \pm 0{\cdot}5$	146 ± 36	19 ± 5	94 ± 4	
CA:Br^5dUr		$10 \pm 3{\cdot}7$	187 ± 60	29 ± 9	100 ± 3	
CA:Br^5dUr		$8 \pm 2{\cdot}8$	248 ± 50	32 ± 6	98 ± 2	
CA:N^6Ur		$7 \pm 0{\cdot}5$	266 ± 67	23 ± 5	99 ± 2	

† Values obtained in our previous work (Buchet *et al.* 1984a).

constants K_{AT} and K_{TAT}. Table 2 contains the equilibrium constants for the pairs formed by our adenosine derivatives and the thymine or uracil derivatives which were used. The N.M.R. values were, for Ado : Ur, $K_T = 5{\cdot}3 \pm 0{\cdot}2\ M^{-1}$; $K_{AT} = 83 \pm 6\ M^{-1}$; $K_{TAT} = 6 \pm 2\ M^{-1}$. Our I.R. values are in reasonable agreement with these.

Next we compare the results listed in table 2 containing the equilibrium constants for complexes of Br^8Ado with thymine derivatives. As indicated above, the H-bonds with Br^8Ado are weaker than with Ado. This is in conformity with Kyogoku's results (Iwahashi *et al.* 1982) who has shown that Br^8Ado prevents the formation of Hoogsteen-type pairs while both Watson–Crick and Hoogsteen-type pairs are possible with Ado. In addition we have shown previously (Buchet *et al.* 1984) that the bromine in Br^8Ado does not hinder significantly the formation of Watson–Crick-type pairs. So Br^8Ado complexes with anticancer agents or uracil derivatives can be considered a good model for the latter.

In general, Cl^2A forms weaker H-bonds than A. The chlorine in position 2 does not affect significantly the formation of Hoogsteen pairs. Ado forms weaker H-bonds than A which is probably due to the effect of the ribose and acetyl groups on these derivatives. In order to ascertain if the ribose group is in itself sufficient to weaken the H-bonds a series of constants were determined using CA, which does not have the acetyl groups. The results were very similar to those using A. Thus the acetyl groups are seen to have little effect on base-pair formation.

4. Discussion

The tendency to base-pair formation as measured by the association constants is in the order:

$$A:T \leqslant A:Ur < A:F^5U < A:I^5dUr < A:Br^5dUr < A:N^6Ur$$

and similarly for Ado, Br^8Ado and Cl^2A. Our results agree with previous qualitative results for similar systems by Katz (1969), Miller and Sobell (1967) and Kyogoku *et al.* (1967 a). However, for A and CA our K_{AT} constants are somewhat lower than those of the latter authors; this is a consequence of our taking account of the contribution of trimer formation (K_{TAT}). A part of the decrease in the intensity of the free NH_2 bands is due to the latter. For the Ado : Ur complex which forms weaker H-bonds than A or CA our results are practically identical with those obtained by Watanabe *et al.* (1981) Complexes of Watson–Crick or Hoogsteen type involving anticancer agents have not been studied before except for the case of 1-cyclohexyl-5-bromo-uracil (Iwahashi *et al.* 1982).

Table 3. Estimated populations of different species in equimolar (40 mM) mixtures of 2′3′5′-tri-*O*-acetyladenosine and thymine derivatives.

Mixture	A (per cent)	A_2 (per cent)	T (per cent)	T_2 (per cent)	AT Watson–Crick (per cent)	AT Hoogsteen (per cent)	TAT (per cent)
Ado : T	51	2	42	3	15	25	6
Ado : Ur	44	1	33	3	15	31	7
Ado : F^5U	41	1	28	2	14	32	11
Ado : I^5dUr	37	1	23	2	16	33	12
Ado : Br^5dUr	36	1	22	2	15	34	13
Ado : N^6Ur	34	1	21	1	18	34	12

The main result is that for all the complexes formed by adenine derivatives (except Br^8Ado) with thymine or uracil derivatives the proportion of Hoogsteen pairs is at least twice higher than the proportion of Watson–Crick pairs. This is in agreement with previous N.M.R. results by Iwahashi *et al.* (1982) for similar systems. The proportion of trimers is, in all cases, less than that of Watson–Crick pairs. Table 3 shows the proportions of the various species.

5. Conclusions

The principal result of this work is that the anticancer agents (F^5Ur, I^5dUr, Br^5dUr and N^6Ur) tend to form Hoogsteen, rather than Watson–Crick, pairs with adenine derivatives except when site 8 on adenine is blocked. Consequently, one might speculate that if these anticancer agents enter into contact with DNA they will replace some of the thymines or uracils and form Hoogsteen-type base pairs with some adenines. Now, as has been seen, the H-bonds formed in this way are stronger than the normal A-T H-bonds. Also, they have a higher tendency to trimer formation. Thus Hoogsteen pair and trimer formation by anticancer agents might be the way in which they can intervene in site recognition processes. One might further speculate that they might bind to RNA and hinder unwarranted protein production.

Acknowledgments

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References

BUCHET, R., BEAUVAIS, L., and SANDORFY, C., 1984, *J. Biomol. Struct. Dyn.*, **2**, 221.
BUCHET, R., and SANDORFY, C., 1984 b, *J. phys. Chem.*, **88**, 3274.
ENGEL, J. D., and VON HIPPEL, P. H., 1974, *Biochemistry*, **13**, 4143.
IWAHASHI, H., and KYOGOKU, Y., 1977, *J. Am. chem. Soc.*, **99**, 7761.
IWAHASHI, H., SUGETA, H., and KYOGOKU, Y., 1982, *Biochemistry*, **21**, 631.
KATZ, L., 1969, *J. molec. Biol.*, **44**, 279.
KYOGOKU, Y., LORD, R. C., and RICH, A., 1967 a, *Proc. natn. Acad. Sci. U.S.A.*, **57**, 250.
KYOGOKU, Y., LORD, R. C., and RICH, A., 1967 b, *J. Am. chem. Soc.*, **89**, 496.
MILLER, G. H., and SOBELL, H. H., 1967, *J. molec. Biol.*, **24**, 345.
NAGEL, G. M., and HANLON, S., 1972, *Biochemistry*, **11**, 823.
WATANABE, M., SUGETA, H., IWAHASHI, H., KYOGOHU, Y., and KAINOSHO, M., 1981, *Eur. J. Biochem.*, **117**, 553.