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Heteroassociation between Substituted Purine Bases and Free Purine in Aqueous Solution

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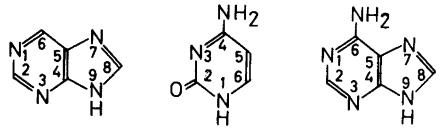
The equilibrium constants for the association of 22 purine derivatives with free purine in aqueous solution have been determined by distributing the solutes between dichlorometane and aqueous purine solutions. The results obtained with alkyl substituted purines are compared with the data of their self-association, and some evidence for the contribution of hydrophobic bonding in the latter process has been presented. The effects of polar substituents on the association tendency have been correlated with the polarizability of the solutes. Besides polarizability, electron donation or withdrawal by polar groups are suggested to affect markedly the association. The influences of the carbon bonded substituents have been shown to be relatively insensitive to the site of the group.

Nucleic acid bases and their derivatives exhibit a marked tendency to associate in aqueous solution.[1–10] The so-called isodesmic model, according to which vertical stacking of several bases occurs in a noncooperative manner,[1] has been widely adopted as an adequate description of the phenomenon. Dipole-induced dipole interactions between the solute molecules have been suggested to constitute the driving force for the association.[11] However, the properties of the solvent also play an important role in the base-stacking, since addition of organic solvents in aqueous solutions results in a marked dissociation of the stacks.[12–14] A few lines of evidence suggest that hydrophobic bonding may also enhance the association process.[3,5,9,15–18]

We have previously studied the thermodynamics of the self-association of alkyl substituted purines and suggested that both dipole-induced dipole interactions and hydrophobic bonding contribute.[18] One of the aims of the present study is to examine the association of the same compounds with the unsubstituted purine. The equilibrium data obtained are compared with those observed earlier for the self-association in order to elucidate further the role of the hydrophobic bonding in the stacking phenomenon. Secondly, the influences of heteroatom substituents on the stacking ability are determined to clarify the relationship between the association tendency and polarizability of the purine derivatives. Finally, the effect of the polar and steric nature of the N9 bonded group is considered.



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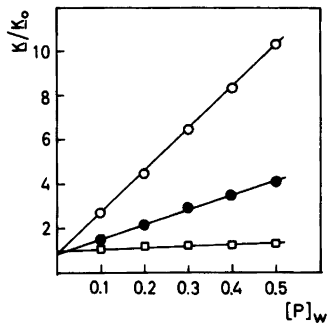

Fig. 1. Distribution of 6-dimethylamino-9-ethoxymethylpurine (○), 9-ethoxymethyladenine (●) and 1-(1-ethoxyethyl)cytosine (□) between aqueous purine solutions and dichloromethane at 298.2 K. $[P]_w$ is the concentration of purine in the aqueous phase, and K and K_o are the distribution coefficients in the presence and absence of purine, respectively.

RESULTS AND DISCUSSION

The heteroaromatic compounds studied were derivatives of purine, cytosine and adenine, the structures of which are indicated below. Fig. 1 shows, as illustrative examples, the distribution of 1-(1-ethoxyethyl)cytosine, 9-ethoxymethyladenine and 6-dimethylamino-9-ethoxymethylpurine between aqueous purine solutions and dichloromethane. The distribution coefficients of 9-ethoxymethyladenine and its N^6,N^6-dimethyl derivative are markedly increased with the increasing purine concentration, whereas the distribution of 1-(1-ethoxyethyl)cytosine is not significantly affected by purine. The differences in the distribution behavior parallel with the known association tendencies of the corresponding nucleosides,[1,3,10,16,19–21] and most probably result from the molecular interactions between the solutes and free purine in the aqueous phase.

Association of a solute, S, with purine, P, in aqueous solution is treated in the following as formation of a 1:1 complex. The association constant, $K_{ass.}$, is thus expressed by eqn. (1),

$$K_{ass.}=\frac{[SP]_w}{[S]_w[P]_w} \tag{1}$$

where $[S]_w$, $[P]_w$ and $[SP]_w$ refer to the equilibrium concentrations of the solute, purine, and their associate, respectively. It should be noted, however, that, owing to the self-association, a considerable proportion of the purine molecules is present as dimers and trimers in the concentration range 0.1–0.5 mol dm^{-3}.[2] The self-association of the solutes is negligible, since the experimental data refer to solute concentrations less than 2×10^{-3} mol dm^{-3}. For the same reason heteroassociates containing more than one solute molecule may be ignored.

The concentration quantities in eqn. (1) can be calculated on the basis of the results of the distribution measurements as follows. The concentration of purine in the organic phase remains always less than 3×10^{-3} mol dm^{-3} (distribution coefficient 170), *i.e.* of the same order as that of the solute. At such low concentrations the interactions between purine and the solute may be ignored. For comparison, 6-dimethylamino-9-ethoxymethylpurine, one of the most efficiently associating species, increased at the concentration of 2.5×10^{-2} mol dm^{-3} the solubility of purine in dichloromethane by less than 20 %. Accordingly, the presence of low concentrations of purine in the organic phase does not affect the distribution of monomeric solute, and $[S]_w$ in aqueous purine solutions can be calculated by eqn. (2).

$$[S]_w=K_o[S]_{org.} \tag{2}$$

Table 1. Equilibrium constants for the association of substituted purine and pyrimidine bases with free purine in aqueous solution at 298.2 K.

Compound	$K_{ass.}$/dm^3 mol^{-1}	b[a]	r[b]
1. 9-Methylpurine	2.9±0.2	1.1±0.1	0.993
2. 2,9-Dimethylpurine	6.7 0.4	1.2 0.1	0.993
3. 6,9-Dimethylpurine	8.3 0.5	1.1 0.2	0.995
4. 8,9-Dimethylpurine	5.6 0.2	1.2 0.1	0.997
5. 6,8,9-Trimethylpurine	13.2 0.6	1.1 0.2	0.996
6. 9-Ethylpurine	3.2 0.2	1.2 0.1	0.996
7. 9-Isopropylpurine	3.3 0.1	1.1 0.1	0.9997
8. 9-Methyladenine	11.7 0.9	1.0 0.3	0.991
9. 2,9-Dimethyladenine	17.2 0.6	0.9 0.2	0.998
10. 8,9-Dimethyladenine	17.7 0.6	0.9 0.2	0.998
11. 6-Dimethylamino-9-methylpurine	47 2	−0.3 0.3	0.998
12. 2-Amino-9-methylpurine	8.6 0.6	1.0 0.2	0.993
13. 8-Amino-9-methylpurine	10.7 1.1	0.7 0.4	0.98
14. 2-Methoxy-9-methylpurine	7.5 0.3	1.1 0.1	0.998
15. 8-Methoxy-9-methylpurine	6.2 0.3	1.2 0.1	0.996
16. 9-Methyl-2-methylthiopurine	13.6 0.6	0.7 0.2	0.997
17. 9-Methyl-8-methylthiopurine	13.3 0.3	0.9 0.1	0.9991
18. 2-Chloro-9-methylpurine	4.5 0.6	1.2 0.2	0.98
19. 9-Ethoxymethyladenine	6.8 0.2	0.8 0.1	0.998
20. 9-(1-Isobutoxyethyl)adenine	6.2 0.2	0.8 0.1	0.998
21. 9-(2-Tetrahydropyranyl)adenine	9.0 0.4	0.9 0.2	0.996
22. 6-Dimethylamino-9-ethoxymethylpurine	19.3 0.2	0.8 0.1	0.9998
23. 1-(1-Ethoxyethyl)cytosine	0.5 0.1	1.1 0.1	0.96

[a] Intercept for eqn. (5) by the least-squares method. [b] Correlation coefficient for eqn. (5).

Here K_o is the distribution coefficient of the solute in the absence of purine, and $[S]_{org.}$ is its concentration in the dichloromethane phase equilibrated with the aqueous purine solution under consideration. The concentration of the associated species may thus be calculated from eqn. (3), where $[S_{tot.}]_w$ denotes the total concentration of the solute in the aqueous purine solution.

$$[SP]_w=[S_{tot.}]_w-K_o[S]_{org.} \tag{3}$$

Substitution of eqns. (2) and (3) in eqn. (1) gives eqn. (4), which can be transformed to eqn. (5), representing a straight line, the slope of which equals to $K_{ass.}$.

$$K_{ass.}=\frac{[S_{tot.}]_w-K_o[S]_{org.}}{K_o[S]_{org.}[P]_w}=\frac{K-K_o}{K_o[P]_w} \tag{4}$$

$$\frac{K}{K_o}=K_{ass.}[P]_w+1 \tag{5}$$

In eqns. (4) and (5) K stands for the distribution coefficient of the solute, when the concentration of purine in the aqueous phase is $[P]_w$.

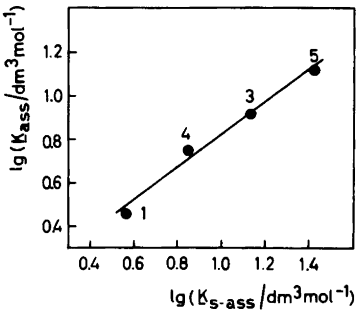

Fig. 2. Comparison of the effects that carbon bonded methyl groups exert on the self-association of 9-methylpurine ($K_{s\text{-}ass.}$) and its association with purine ($K_{ass.}$) in aqueous solution at 298.2 K. The enumeration of the compounds refers to Table 1.

Table 1 summarizes the association constants obtained by eqn. (5) for the compounds studied. The satisfactory linearity of the plots over the whole concentration range studied ($0.1 \leq [P]_w \leq 0.5$ mol dm^{-3}) and the intercepts close to unity lend additional support to the reliability of the experimental method.

We have shown previously that carbon bonded alkyl groups enhance the self-association of 9-methylpurine by making the association enthalpy more negative.[18] Most probably the increased polarizability of the π-electron system facilitates the base-stacking.[3,11] In contrast, the increasing size of the N9 bonded alkyl group stabilizes the associates by reducing the negative entropy contribution.[18] The latter influence has been attributed to the hydrophobic interactions. As seen from Fig. 2, carbon bonded methyl groups enhance the association with purine to the same extent as the self-association. This is expected, if the increased polarizability is regarded as the main stabilizing factor. In contrast, replacing the methyl group in position 9 with ethyl or isopropyl group appears to enforce the self-association to a larger extent than the association with purine (for the self-association K=3.7, 5.1 and 5.7, respectively[18]). A possible explanation is that hydrophobic bonding may take place between two alkylpurines, but not when one of the interacting species is unsubstituted purine.

Besides carbon bonded alkyl groups, several polar substituents have been shown to enhance the stacking of nucleosides and related compounds.[2,7–9,11] The increasing association has been repeatedly attributed to the increased polarizability of the π-electron system.[3,8,11] In Fig. 3 the association constants determined in the present work for a variety of 2-, 6- and 8-substituted 9-methylpurines are plotted against the polarizabilities obtained by the usual additivity rules.[22] All the substituents studied increase both the association efficiency and the polarizability of the parent compound. However, the correlation between these two quantities is a very rough one. As seen from the logarithmic acidity constants indicated in Fig. 3, all the compounds exhibiting positive deviations from the correlation line are more basic than those exhibiting negative deviations. The basicity is in turn related to the electron density of the purine ring, though it measures the electron density at the site of the attachment of the proton rather than that in the whole aromatic system. Accordingly, it seems attractive to assume that electron donation or withdrawal by a polar substituent affects the association ability of 9-methylpurine by increasing or decreasing the π-electron density. The latter suggestion is by no means aimed to argue against the importance of polarizability as a factor affecting the stacking efficiency of purine derivatives, but only to give a possible explanation for the relatively poor correlation between the association constants and the polarizability.

The ability of a carbon bonded substituent to enhance the base-stacking does not markedly depend on its site in the purine ring (Table 1). Methyl and methoxy groups exert

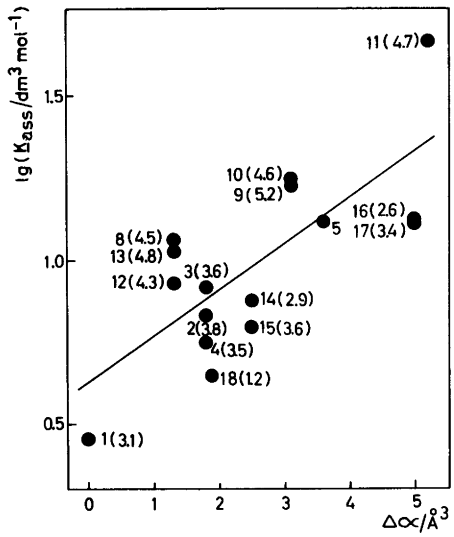

Fig. 3. Correlation between the equilibrium constants, $K_{ass.}$, for the association of substituted 9-methylpurines with free purine and the polarizabilities of the same compounds. $\Delta\alpha$ is the polarizability subtracted by that of 9-methylpurine. The values in the parentheses are the logarithmic protonation constants of the compounds in 1 mol dm^{-3} aqueous sodium perchlorate at 298.2 K (Ref. 24 and unpublished results). The enumeration of the compounds refers to Table 1.

slightly larger effects in the pyrimidine than in the imidazole ring. However, in the case of amino group C8 bonded substituent appears to stabilize the associates more markedly than C2 bonded and almost to the same extent as C6 bonded. With the methylthio group the influences in positions 2 and 8 are equal within the limits of experimental errors. Consequently, no clearcut correlation appears to exist between the site of the substituent and its effect on base-stacking.

The data in Table 1 reveal that replacing the N9 bonded methyl group with ethoxymethyl group reduces the association with free purine by a factor of 2. Two tentative explanations can be given. Either the electronegative ethoxy group decreases the electron density in the purine ring, or the increased size of the N9 substituent retards the stacking sterically. The fact that the influences of 1-isobutoxyethyl and 2-tetrahydropyranyl groups are similar to that of ethoxymethyl group makes the former alternative more attractive. Both of these groups are considerably bulkier than ethoxymethyl group, whereas the polar effects of all three groups may be expected to be approximately the same. It should also be noted that replacing of 9-methyl group with larger ethyl or isopropyl groups does not result in destacking. In other words, the influences of the 9-substituents lend mild additional support to the importance of polar effects as a factor affecting the stacking of purine derivatives.

EXPERIMENTAL

Materials. Dichloromethane, employed in the equilibrations, was an analytical grade reagent of Merck. Purine was purchased from Sigma and used as received.

The preparation and characterization of 9-alkylpurines,[18,23] di- and trimethylpurines,[18,24] and 1-(1-ethoxyethyl)cytosine[25] have been described earlier. The other compounds were synthesized by conventional methods as indicated in the following. The ^1H and ^{13}C NMR chemical shifts of the products are listed in Tables 2 and 3, respectively. The homogeneity of the compounds was checked by LC as described in the context of the distribution measurements.

Table 2. ^{1}H NMR chemical shifts for the compounds prepared.[a]

Compound[b]	δ(H2)	δ(H6)	δ(H8)	δ(N9-R)	Others	
8.[c]	s8.07		s8.01	s3.73	δ(C6-NH_2)	s7.10
	(s8.17)[d]		(s8.13)			(s7.17)
9.[c]			s7.90	s3.68	δ(C2-CH_3)	s2.40; δ(C6-NH_2) s7.02
10.[c]	s8.08			s3.62	δ(C8-CH_3)	s2.49; δ(C6-NH_2) s6.92
11.[e]	s8.35		s7.69	s3.78	δ(C6-N(CH_3)$_2$)	s3.53
12.[c]		s8.58	s8.02	s3.65	δ(C2-NH_2)	s6.40
13.[c]	s8.47	s8.36		s3.52	δ(C8-NH_2)	s7.15
14.[c]		s8.87	s8.32	s3.75	δ(C2-OCH_3)	s3.98
		(s8.79)[f]	(s8.26)	(s3.72)		(s3.93)
15.[c]	s8.68	s8.72		s3.57	δ(C8-OCH_3)	s4.20
	(s8.75)[g]	(s8.80)		(s3.60)		(s4.23)
16.[c]		s8.93	s8.38	s3.80	δ(C2-SCH_3)	s2.60
		(s8.90)[h]	(s7.95)	(s3.85)		(s2.70)
17.[c]	s8.77	s8.89		s3.67	δ(C8-SCH_3)	s2.78
	(s8.82)[h]	(s8.90)		(s3.95)		(s2.80)
18.[c]		s9.07	s8.60	s3.83		
		(s8.90)[i]	(s8.56)	(s3.89)		
19.[c]	s8.18		s8.11	tl.03; q3.48; s5.46	δ(C6-NH_2)	s7.20
20.[e]	s8.30		s7.96	d0.83; d2.90; m3.2; q5.90	δ(C6-NH_2)	s6.35
21.[e]	s8.27		s7.93	ml.5–2.2; m3.5–4.2; m5.7	δ(C6-NH_2)	s6.12
22.[e]	s8.33		s7.83	tl.21; q3.56; s5.58	δ(C6-N(CH_3)$_2$)	s3.56

[a] As ppm from TMS. [b] For the enumeration of the compounds see Table 1. [c] In DMSO-d_6. [d] For 9-ethyladenine.[41] [e] In $CDCl_3$. [f] *Ref.* 42. [g] In $CDCl_3$.[37] [h] In $CDCl_3$.[43] [i] In D_2O.[44]

Table 3. ^{13}C NMR chemical shifts for the compounds prepared.[a]

Compound[b]	δ(C2)	δ(C4)	δ(C5)	δ(C6)	δ(C8)	δ(N9-R)	Others	
8.[c]	152.3	149.8	118.6	155.8	141.3	29.2		
	(152.5)[d]	(149.9)	(118.7)	(155.9)	(141.4)	(29.3)		
9.[c]	160.7	150.3	116.4	155.0	141.3	29.5	$\delta(C2\text{-}CH_3)$	25.1
10.[c]	151.4	150.7	117.6	154.6	148.9	28.1	$\delta(C8\text{-}CH_3)$	13.3
	(151.1)[e]	(149.5)	(117.9)	(155.2)	(148.8)			
11.[c]	152.4	151.0	120.1	155.0	138.7	29.6	$\delta(C6\text{-}N(CH_3)_2)$	38.5
12.[c]	160.5	153.4	126.8	148.7	143.3	28.8		
	(160.6)[g]	(154.9)	(124.8)	(150.8)	(142.6)			
13.[c]	147.7	152.8	134.6	138.4	156.6	27.0		
14.[c]	161.1	153.6	129.7	149.0	146.2	29.1	$\delta(C2\text{-}OCH_3)$	54.7
15.[c]	150.2	152.0	131.7	142.6	159.0	26.8	$\delta(C8\text{-}OCH_3)$	57.4
16.[c]	164.3	152.4	130.8	147.7	146.4	29.2	$\delta(C2\text{-}SCH_3)$	13.9
17.[c]	151.1	153.9	134.1	144.0	158.9	28.8	$\delta(C8\text{-}SCH_3)$	14.0
	(151.3)[h]	(153.9)	(134.4)	(144.4)	(157.7)	(28.3)		(13.9)
18.[c]	153.4	152.7	132.9	149.4	148.7	29.7		
19.[c]	153.5	150.6	119.4	155.7	140.7	14.8; 65.4; 72.6		
20.[c]	153.0	150.0	119.5	155.8	138.0	19.2; 22.6; 28.3; 76.0; 81.3		
21.[c]	153.1	149.3	119.5	156.0	138.2	22.8; 24.9; 31.9; 68.7; 81.9		
22.[c]	152.8	151.0	119.9	155.0	138.3	14.8; 65.1; 72.4	$\delta(C6\text{-}N(CH_3)_2)$	38.5

[a] As ppm from TMS. [b] For the enumeration of the compounds see Table 1. [c] In DMSO-d_6. [d] *Ref.* 45. [e] For 8-methyladenosine.[46] [f] In $CDCl_3$. [g] For 2-amino-9-(β-D-ribofuranosyl)purine.[47] [h] In liquid ammonia.[48]

9-Methyladenine was prepared by methylating adenine (Sigma) with methyl iodide according to Hedayatullah.[26] The product was purified by repeated crystallizations from methanol and vacuum sublimed. M.p. 301 °C (*lit.*[27] 301–302 °C). Calculated for $C_6H_7N_5$: C 48.32 %, H 4.73 %, N 46.95 %. Found: C 48.39 %, H 4.69 %, N 46.83 %.

2,9-Dimethyladenine was synthesized similarly from 2-methyladenine obtained by the method of Taylor *et al.*[28] M.p. 238 °C (*lit.*[29] 238 °C). Calculated for $C_7H_9N_5$: C 51.52 %, H 5.56 %, N 42.92 %. Found: C 51.68 %, H 5.50 %, N 42.72 %.

8,9-Dimethyladenine was synthesized similarly from 8-methyladenine prepared as described earlier.[30] M.p. 275–276 °C. Calculated as for 2,9-dimethyladenine. Found: C 51.53 %, H 5.49 %, N 42.83 %.

6-Dimethylamino-9-methylpurine was obtained by methylating the free base (Sigma) with methyl iodide in acetone. The experimental conditions and the separation of the isomers were analogous to those described for 9-methylpurine.[23] The product was crystallized from *n*-hexane. M.p. 115–116 °C (*lit.*[31] 120 °C). Calculated for $C_8H_{11}N_5$: C 54.21 %, H 6.26 %. Found: C 54.47 %, H 6.28 %.

9-Ethoxymethyladenine was prepared by treating adenine in DMF with an equal amount of chloromethyl ethyl ether (0.5 h, 100 °C). Excess of triethylamine was added to neutralize the released hydrogen chloride. The cooled solution was filtrated and evaporated to dryness under reduced pressure. The product was separated from the 1-, 3-, and 7-isomers, formed as by-products, by repeated crystallizations from methanol. M.p. 167–170 °C. Calculated for $C_8H_{11}N_5O$: C 49.73 %, H 5.74 %, N 36.25 %. Found: C 49.04 %, 5.46 %, N 36.98 %.

9-(1-Isobutoxyethyl)adenine was prepared analogously using 1-chloroethyl isobutyl ether as alkylating agent. The product was crystallized from carbon tetrachloride. M.p. 147–150 °C. Calculated for $C_{11}H_{17}N_5O$: C 56.15 %, H 7.28 %, N 29.76 %. Found: C 56.12 %, H 7.14 %, N 29.68 %.

9-(2-Tetrahydropyranyl)adenine was prepared by the method presented previously for the corresponding purine derivative.[24] The product was crystallized from chloroform. M.p. 170–174 °C (lit.[32] 186–188 °C). Calculated for $C_{10}H_{13}N_5O$: C 55.03 %, H 6.00 %, N 32.09 %. Found: C 54.97 %, H 6.03 %, N 31.86 %.

9-Dimethylamino-9-ethoxymethylpurine was prepared as described for 9-ethoxymethyladenine. The other isomers were not detected. The product was crystallized from carbon tetrachloride. M.p. 79–81 °C. Calculated for $C_{10}H_{15}N_5O$: C 54.29 %, H 6.83 %, N 31.65 %. Found: C 54.22 %, H 6.77 %, N 31.49 %.

2-Amino-9-methylpurine was obtained by methylating 2-aminopurine and separating the isomers as described for 9-methylpurine.[23] 2-Aminopurine was prepared by reducing commercial 2-amino-6-thiopurine (Sigma) with Raney nickel.[33] The product was purified by vacuum sublimation and crystallization from isobutyl methyl ketone. M.p. 240–245 °C. Calculated for $C_6H_7N_5$: C 48.32 %, H 4.73 %, N 46.95 %. Found: C 48.15 %, H 4.73 %, N 46.48 %.

8-Amino-9-methylpurine was prepared by treating 9-methyl-8-methylthiopurine with concentrated aqueous ammonia for 24 h at 150 °C. The preparation of the latter compound is described below. The product was crystallized from water. M.p. 300 °C. Calculated as for 2-amino-9-methylpurine. Found: C 48.41 %, H 4.70 %, N 46.84 %.

2-Chloro-9-methylpurine was obtained by methylating 2-chloropurine and separating the isomers as described for 9-methylpurine.[23] 2-Chloropurine was prepared by cyclizing commercial 2-chloro-4,5-diaminopyridine (Sigma) acording to Montgomery.[34] The product was crystallized from methanol. M.p. 131 °C (lit.[35] 132.0–133.5 °C). Calculated for $C_6H_5ClN_4$: C 42.71 %, H 2.99 %, Cl 21.03 %, N 33.23 %. Found: C 42.72 %, H 2.89 %, Cl 20.84 %, N 33.08 %.

2-Methoxy-9-methylpurine was prepared by refluxing 2-chloro-9-methylpurine in methanolic sodium methoxide.[36] The product was crystallized from *n*-hexane. M.p. 141–142 °C (lit. [37] 140.5–142.5 °C). Calculated for $C_7H_8N_4O$: C 51.21 %, H 4.91 %, N 34.13 %. Found: C 51.14 %, H 4.77 %, N 34.31 %.

8-Methoxy-9-methylpurine was prepared by treating 8-chloro-9-methylpurine with methanolic sodium methoxide at room temperature.[36] The latter compound was obtained by methylating 8-chloropurine [38] and separating the isomers as described for 9-methylpurine.[23] The product was crystallized from *n*-hexane. M.p. 140–142 °C (*lit.*[37] 149–151 °C). Calculated as for 2-methoxy-9-methylpurine. Found: C 51.21 %, H 4.95 %, N 34.28 %.

9-Methyl-2-methylthiopurine was obtained by methylating 2-methylthiopurine and separating the isomers as described for 9-methylpurine.[23] 2-Methylthiopurine was synthesized according to Albert and Brown.[39] The product was crystallized from ethanol. M.p. 132–133 °C (*lit.*[40] 131–132 °C). Calculated for $C_7H_8N_4S$: C 46.65 %, H 4.47 %, N 31.08 %, S 17.79 %. Found: C 46.51 %, H 4.41 %, N 31.08 %, S 17.87 %.

9-Methyl-8-methylthiopurine was prepared similarly from 8-methylthiopurine. The starting material was synthesized from 4,5-diaminopyrimidine (Sigma) *via* 8-thiopurine.[35,39] The product was crystallized from ethanol. M.p. 147–149 °C (*lit.*[40] 151 °C). Calculated as for 9-methyl-2-methylthiopurine. Found: C 46.71 %, H 4.41 %, N 31.24 %, S 17.73 %.

Distribution measurements. The equilibrations of the substituted purine bases between aqueous solutions of free purine and dichloromethane were carried out in stoppered tubes (10 cm^3) at 298.2 K. About 5 μmol of the compound studied was added into a tube containing 3 cm^3 of aqueous and organic phases. The tubes were shaken from time to time during 2–5 days and the phases were allowed to separate for one day. The concentration of the solute in both phases was determined by LC. The determination was repeated on the next day to ascertain that the equilibrium had settled. The analyses were performed on a Varian Aerograph 5020 apparatus equipped with a variable wavelength UV-detector and a commercial Hibar column (250×4 mm) packed with LiChrosorb RP-18 (10 μm). Isocratic elution (1 cm^3 min^{-1}) with a mixture of acetonitrile and acetic acid buffer (pH 4.4) was employed throughout. The composition of the eluant was adjusted so that the difference between the retention times of purine and the solute was about two minutes. Peak heights were employed as the measure of the concentrations.

The solubility of purine in dichloromethane solutions of 6-dimethylamino-9-ethoxymethylpurine was determined analogously after the removal of the solid particles by centrifugation.

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REFERENCES

1. Ts'O, P.O.P., Melvin, I.S. and Olson, A.C. *J. Am. Chem. Soc. 85* (1963) 1289.
2. Ts'O, P.O.P. and Chan, S.I. *J. Am. Chem. Soc. 86* (1964) 4176.
3. Broom, A.D., Schweitzer, M.P. and Ts'O, P.O.P. *J. Am. Chem. Soc. 89* (1967) 3612.
4. Gill, S.J., Downing, M. and Sheats, G.F. *Biochemistry 6* (1967) 272.
5. Helmkamp, G.K. and Kondo, N.S. *Biochim. Biophys. Acta 157* (1968) 242.
6. Solie, T.N. and Schellman, J.A. *J. Mol. Biol. 33* (1968) 61.
7. Nakano, N.I. and Igarashi, S.J. *Biochemistry 9* (1970) 577.
8. Schimmak, W., Sapper, H. and Lohman, W. *Biophys. Struct. Mechanism 1* (1975) 311.
9. Plesiewicz, E., Stepien, E., Bolewska, K. and Wierzchowski, K.L. *Biophys. Chem. 4* (1976) 131.
10. Scheller, K.H., Hofstetter, F., Mitchell, P.R., Prijs, B. and Sigel, H. *J. Am. Chem. Soc. 103* (1981) 247.
11. Lawaczeck, R. and Wagner, K.G. *Biopolymers 13* (1974) 2003.
12. Chan, S.I., Schweitzer, M.P., Ts'O, P.O.P. and Helmkamp, G.K. *J. Am. Chem. Soc. 86* (1964) 4182.
13. Marenchic, M.G. and Sturtevant, J.M. *J. Phys. Chem. 77* (1973) 544.
14. Sapper, H. and Lohman, W. *Biophys. Struct. Mechanism 4* (1978) 327.
15. Crothers, D.M. and Ratner, D.I. *Biochemistry 7* (1968) 1823.
16. Plesiewicz, E., Stepien, E., Bolewska, K. and Wierzchowski, K.L. *Nucl. Acids Res. 3* (1976) 1295.
17. Bascones, A., Peral, F. and Gallego, E. *An. Quim. 77* (1981) 105.
18. Lönnberg, H., Ylikoski, J. and Vesala, A. *J. Chem. Soc. Faraday Trans. 1, 80* (1984) 2439.
19. Bretz, R., Lustig, A. and Schwartz, G. *Biophys Chem. 1* (1974) 237.
20. Vickers, L.P. and Ackers, G.K. *Arch. Biochem. Biophys. 174* (1976) 747.

21. Heyn, M.P., Nicola, C.U. and Schwartz, G. *J. Phys. Chem. 81* (1977) 1611.
22. Denbigh, K.G. *Trans. Faraday Soc. 36* (1940) 936.
23. Arpalahti, J. and Lönnberg, H. *Acta Chem. Scand. B 36* (1982) 545.
24. Arpalahti, J. and Lönnberg, H. *Inorg. Chim. Acta 78* (1983) 63.
25. Lönnberg, H. *Tetrahedron 38* (1982) 1517.
26. Hedayatullah, M. *J. Heterocycl. Chem. 19* (1982) 249.
27. Myers, F.C. and Zelenick, L. *J. Org. Chem. 28* (1963) 2087.
28. Taylor, E.C., Vogl, O. and Cheng, C.C. *J. Am. Chem. Soc. 81* (1959) 2442.
29. Baddiley, J., Lythgoe, B. and Todd, A.R. *J. Chem. Soc.* (1944) 318.
30. Koppel, H.C. and Robins, R.K. *J. Org. Chem. 23* (1958) 1457.
31. Robins, R.K. and Lin, H.H. *J. Am. Chem. Soc. 79* (1957) 490.
32. Robins, R.K., Godefroi, E.F., Taylor, E.C., Lewis, L.R. and Jackson, A. *J. Am. Chem. Soc. 83* (1961) 2574.
33. Prasad, R.N., Noell, C.W. and Robins, R.K. *J. Am. Chem. Soc.* (1959) 193.
34. Montgomery, J.A. *J. Am. Chem. Soc. 78* (1956) 1928.
35. Barlin, G.B. and Chapman, N.B. *J. Chem. Soc.* (1965) 3017.
36. Barlin, G.B. *J. Chem. Soc. B* (1967) 954.
37. Badgar, R.J. and Barlin, G.B. *J. Chem. Soc. Perkin Trans. 2* (1976) 1176.
38. Beaman, A.G. and Robins, R.K. *J. Appl. Chem. 12* (1962) 432.
39. Albert, A. and Brown, D.J. *J. Chem. Soc.* (1954) 2060.
40. Brown, D.J. and Food, P.W. *J. Chem. Soc. C* (1969) 2620.
41. Fort, R.C., Jr. and Lindstrom, T.R. *J. Phys. Chem. 75* (1971) 3963.
42. Kos, N.J. and van der Plas, H.C. *J. Org. Chem. 46* (1981) 5000.
43. Stanovnik, B., Tisler, M., Hribar, A., Barlin, G.B. and Brown, D.J. *Aust. J. Chem. 34* (1981) 1729.
44. Barlin, G.B. and Young, A.C. *J. Chem. Soc. B* (1971) 821.
45. Chenon, M.T., Pugmire, R.J., Grant, D.M., Panzica, R.P. and Townsend, L.B. *J. Am. Chem. Soc. 97* (1975) 4627.
46. Uesugi, S. and Ikehara, M. *J. Am. Chem. Soc. 99* (1977) 3250.
47. Jones, A.J., Grant, D.M., Winkley, M.W. and Robins, R.K. *J. Am. Chem. Soc. 92* (1970) 4079.
48. Kos, N.J., van der Plas, H.C. and Blees, W.J.F. *J. Org. Chem. 48* (1983) 850.

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