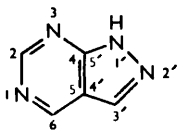
605. *Potential Purine Antagonists. Part X.* Purines and Pyrazolopyrimidines : Comparisons of Ionisation.†*

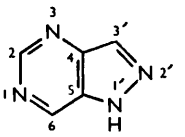
By B. M. Lynch, R. K. Robins, and (in part) C. C. Cheng.

Ionisation constants of 55 pyrazolopyrimidines have been measured, and are compared with the published values [1] for some purines. Differences in basicity, and in acidity, between similarly substituted representatives of these isomeric ring systems are discussed and interpreted. An apparent relation between anti-tumour activity (in experimental animals) and basic strength is noted for a series of amino-substituted pyrazolo(5′ : 4′-4 : 5)-pyrimidines.

Although syntheses of several derivatives of pyrazolo(5′ : 4′-4 : 5)pyrimidine ‡ (I), pyrazolo(4′ : 5′-4 : 5)pyrimidine ‡ (II), and purine (III) have been reported recently,[1,2] and the physical properties of the purines have been investigated extensively,[1] little attention has been given to the physical properties of the pyrazolopyrimidines. Since it has been found that some 6-(substituted amino)-derivatives of (I) show promising anti-tumour activity in mice,[3] we have sought physicochemical correlations with biological activity (compare the relations between basic strength and biological activity observed with the acridines,[4] benzacridines,[5] and sulphonamides [6]), and in the present paper we have measured the basic and the acidic ionisation constants of a series of pyrazolo(5′ : 4′-4 : 5)-pyrimidines. Some substituted purines and pyrazolo(4′ : 5′-4 : 5)pyrimidines were also studied. A preliminary account of this work has already been given.†



(I)



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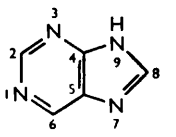


(III)

The ionisation constants were determined by the standard ultraviolet spectrophotometric method.[7] Although the ultraviolet absorption spectra of many of the compounds studied in this paper have been recorded previously,[2,8] these measurements are of little value, since they were made with solutions of arbitrary pH values, so that the recorded spectra refer to mixtures of ions and neutral molecules, uncertain in composition. Consequently, in the present work we measured the light-absorption curves for the various species of each compound, in buffered solutions of pH values at least two units distant from the pK_a of the substance being examined.

A full account of the ultraviolet absorption spectra of the various ionic species of the substituted pyrazolo(5′ : 4′-4 : 5)pyrimidines will be given in a later paper.

* Part IX, *J. Amer. Chem. Soc.*, 1957, **79**, 6407.

† Presented in part at the Amer. Chem. Soc. Meeting, Miami, 1957, Abstracts, p. 7C.

‡ In other papers of this series,[2] Ring Index names (pyrazolo[3 : 4-*d*]pyrimidine and pyrazolo-[4 : 3-*d*]pyrimidine) were used for these ring systems. The numbering system adopted in this paper appears preferable for comparisons with the purines.

[1] (*a*) Albert and Brown, *J.*, 1954, 2060; (*b*) Brown and Mason, *J.*, 1957, 682.

[2] (*a*) Pyrazolo(5′ : 4′-4 : 5)pyrimidines: Robins, *J. Amer. Chem. Soc.*, 1956, **78**, 784; 1957, **79**, 6407; Cheng and Robins, *J. Org. Chem.*, 1956, **21**, 1240; 1958, **23**, 191. (*b*) Pyrazolo(4′ : 5′-4 : 5)pyrimidines: Robins, Furcht, Holum, Grauer, and Jones, *J. Amer. Chem. Soc.*, 1956, **78**, 2418; Robins, Holum, and Furcht, *J. Org. Chem.*, 1956, **21**, 833.

[3] Skipper, Robins, and Thomson, *Proc. Soc. Exp. Biol. N.Y.*, 1955, **89**, 594.

[4] Albert, Rubbo, Goldacre, Davey, and Stone, *Brit. J. Exp. Path.*, 1945, **76**, 160.

[5] Pages-Flon, Buu-Hoï, and Daudel, *Compt. rend.*, 1953, **236**, 2182.

[6] Bell and Roblin, *J. Amer. Chem. Soc.*, 1942, **64**, 2905.

[7] Irvin and Irvin, *ibid.*, 1947, **69**, 1091; cf. Albert and Phillips, *J.*, 1956, 1294.

[8] Falco and Hitchings, *J. Amer. Chem. Soc.*, 1956, **78**, 3143.

Experimental

Sources of Materials.—Except where otherwise stated, the compounds used were prepared as described previously,[2] and were crystallised to constant ultraviolet absorption immediately before use.

pK_a *Determinations.*—Solutions were prepared in a series of buffers, standardised by means of a Beckman Model H-2 glass electrode pH meter. The series decreased in pH down to values where the change in spectrum, corresponding to the ionisation being studied, ceased; similarly, the pH was increased in the alkaline direction. The buffer solutions were 0·01M-glycine (for pH 1·5—3·5), 0·01M-formate (for pH 2·5—3·9), 0·01M-acetate (for pH 3·8—5·7), 0·01M-phosphate (for pH 5·0—7·9 and 10·3—11·3), 0·01M-borate (for pH 8·2—10·0), together with N- (pH 0) and 0·1N-hydrochloric acid (pH 1), and 0·1N- (pH 13) and 0·01N-carbonate-free potassium hydroxide (pH 12), all of which have low ultraviolet absorption.

Measurements were made with a Beckman Model DU ultraviolet spectrophotometer with matched 1-cm. cells. Buffer solutions of the same strengths as above were used as controls.

In wavelength regions selected because of marked differences between the extinction coefficients of the protonated (ε_{MH+}) and unprotonated species (ε_M) (*i.e.*, cation and neutral molecule, or neutral molecule and anion), the extinction coefficients of the sum of the two species (ε) were measured at 1 mμ wavelength intervals; the pH values of the solutions used were chosen so as to correspond to the range from 15% to 85% protonation. The pK_a's were determined by using the following formula:

$$pK_a = pH - \log [(\varepsilon_{MH+} - \varepsilon)/(\varepsilon - \varepsilon_M)],$$

converted into antilogarithms, and averaged. This method requires no hydrolysis corrections,[7] but the usual activity corrections apply.

Results.—The ionisation constants of the various pyrazolo(5′ : 4′-4 : 5)pyrimidines and pyrazolo(4′ : 5′-4 : 5)pyrimidines, measured in water at 20°, are listed in Table 1. Compounds which are effective inhibitors of the mouse carcinoma Ad755 (as reported by Skipper and his co-workers [3,9]) are indicated by asterisks. The pK_a values of several pairs of correspondingly substituted pyrazolo(5′ : 4′-4 : 5)pyrimidines and purines are compared in Table 2. Except where otherwise stated, the values for the purines are taken from the papers of Albert and Brown [1a] and of Brown and Mason.[1b]

Discussion

Previously, it has been assumed [2b] that the pyrazolopyrimidines are weaker bases than the corresponding purines, because of the large differences in basic strengths between the glyoxaline (imidazole) and pyrazole molecules (pK_a's of cations: 7·03 and 2·53 respectively; cf. Albert, Goldacre, and Phillips [10]), and programmes of synthesis [2b] seeking to prepare compounds with basicities similar to those of naturally occurring purines have been based on this assumption.

Inspection of Table 2 reveals, however, that the pyrazolo(5′ : 4′-4 : 5)pyrimidines are both slightly stronger bases (*i.e.*, their neutral molecules accept protons more readily) and slightly weaker acids (*i.e.*, their neutral molecules lose protons less readily) than the corresponding purines. From the small number of examples studied (see Table 1, part b) it appears that the pyrazolo(4′ : 5′-4 : 5)pyrimidines are stronger bases than the corresponding pyrazolo(5′ : 4′-4 : 5)pyrimidines (compare nos. 7 and 51, 48 and 52).

These results are difficult to explain if the sites of proton-acceptance in these molecules are the tertiary nitrogen atoms in the five-membered heterocyclic rings, but the elevation in pK_a on passing from the cations of purines to those of pyrazolo(5′ : 4′-4 : 5)pyrimidines can be explained satisfactorily if a nitrogen atom of the *pyrimidine ring* is the most basic centre in both systems.

If one assumes initially that the most basic centre is the 1-nitrogen atom, the electron density at this atom in a pyrazolo(5′ : 4′-4 : 5)pyrimidine would be expected to be higher than in the corresponding purine, because the electron-attracting (base-weakening) tertiary

[9] Skipper, Robins, Thomson, Cheng, Brockman, and Schabel, *Cancer Res.*, 1957, **17**, 579.
[10] Albert, Goldacre, and Phillips, *J.*, 1948, 2240.

TABLE 1. *Ionisation constants of pyrazolo(5′ : 4′-4 : 5)pyrimidines and pyrazolo-(4′ : 5′-4 : 5)pyrimidines, in water at 20°.*

No.	Substituents		pK_a	Analytical concn. (10^{-4}M)	and wavelength range (mμ)
	(a) *Pyrazolo*(5′ : 4′-4 : 5)*pyrimidines.*				
1	Unsubstituted	cation	2·84 ± 0·10	1·095	250—260
		anion	9·54 ± 0·04		250—260
2	1′-Methyl	cation	2·50 ± 0·02	1·469	260—290
3	1′ : 2-Dimethyl	cation	3·27 ± 0·20	1·414	284—320
4	6-Hydroxy [a]	anion	9·38 ± 0·05	1·503	260—280
5	6-Hydroxy-1′-methyl [a]	anion	9·26 ± 0·03	1·396	276—284
6	6-Methylthio	cation	1·0 ± 0·5	0·640	240—250
		anion	9·65 ± 0·01		244—250
7 *	6-Amino	cation	4·59 ± 0·02	0·772	270—274
		anion	10·84 ± 0·05		274—290
8	2 : 6-Diamino	cation (mono)	5·58 ± 0·07	0·979	250—270
9 *	6-Methylamino	cation	4·53 ± 0·04	0·577	272—280
		anion	10·55 ± 0·10		270—280
10	2 : 6-Bismethylamino	cation (mono)	6·08 ± 0·09	1·070	250—270
11 *	6-Dimethylamino [b]	cation	4·53 ± 0·06	0·631	280—284
		anion	*ca.* 11		280—290
12 *	6-Ethylamino	cation	4·60 ± 0·05	1·265	256—266, 280—290
		anion	10·90 ± 0·04		280—288
13 *	6-Diethylamino [b]	cation	4·71 ± 0·10	0·631	276—296
14 *	6-Propylamino [c]	cation	4·58 ± 0·05	1·118	280—292
15 *	6-*iso*Propylamino	cation	4·62 ± 0·06	1·175	280—292
		anion	10·99 ± 0·10		276—284
16 *	6-Butylamino	cation	4·67 ± 0·06	1·065	274—290
		anion	11·21 ± 0·05		276—286
17 *	6-(3-Methylbutylamino) [d]	cation	4·62 ± 0·10	0·827	276—290
18*	6-(2-Ethylhexyl)amino [d]	cation	4·60 ± 0·05	0·835	276—290
19	6-Anilino	cation	3·92 ± 0·03	1·042	290—310
20 *	6-Benzylamino	cation	4·16 ± 0·06	0·903	276—286, 296—306
		anion	10·93 ± 0·03		276—284
21 *	6-(4-Chlorobenzylamino) [d]	cation	3·96 ± 0·10	0·668	274—286, 296—310
22 *	6-Phenethylamino [d]	cation	4·38 ± 0·08	1·005	276—290
23 *	6-Furfurylamino	cation	4·01 ± 0·05	1·031	270—286, 294—312
24 *	6-Amino-1′-methyl	cation	4·32 ± 0·05	0·693	276—286
25	6-Amino-2-chloro-1′-methyl [e]	cation	2·69 ± 0·19	1·286	276—290
26	6-Amino-2-methoxy-1′-methyl	cation	3·66 ± 0·10	0·876	256—282
27 *	1′-Methyl-6-methylamino	cation	4·24 ± 0·05	1·248	280—290
28 *	6-Dimethylamino-1′-methyl	cation	4·06 ± 0·10	1·157	290—300
29 *	6-Ethylamino-1′-methyl	cation	4·24 ± 0·05	1·171	282—290
30 *	1′-Methyl-6-propylamino	cation	4·25 ± 0·07	1·072	284—300
31 *	1′-Methyl-6-*iso*propylamino	cation	4·22 ± 0·04	1·186	250—260, 286—300
32 *	6-Butylamino-1′-methyl	cation	4·22 ± 0·04	1·054	250—260, 286—300
33 *	6-Hexylamino-1′-methyl	cation	4·24 ± 0·01	0·778	280—300
34	1′-Methyl-6-(1 : 1 : 3 : 3-tetramethylbutylamino)	cation	3·96 ± 0·01	0·778	286—300
35 *	6-(2-Hydroxyethylamino)-1′-methyl	cation	3·86 ± 0·06	1·061	250—260, 286—300
36	6-Anilino-1′-methyl	cation	3·53 ± 0·05	0·920	290—310
37	6-Benzylamino-1′-methyl	cation	3·66 ± 0·15	0·865	284—298
38	6-Furfurylamino-1′-methyl	cation	3·80 ± 0·15	0·872	284—298
39	6-Amino-2-methyl	cation	5·41 ± 0·20	1·381	260—280
		anion	11·30 ± 0·10		290—300
40	2-Methyl-6-methylamino	cation	5·56 ± 0·10	1·326	270—280, 294—304
		anion	11·27 ± 0·15		270—282
41	6-Furfurylamino-2-methyl	cation	4·65 ± 0·05	0·548	290—306
42 *	6-Amino-1′-(2-hydroxyethyl)	cation	4·29 ± 0·02	1·123	270—284
43	6-Amino-1′-phenyl	cation	3·89 ± 0·02	1·150	282—290
44 *	2 : 6-Diamino-1′-phenyl	cation	4·49 ± 0·20	0·383	274—290
45	6-Amino-2-methyl-1′-phenyl	cation	4·52 ± 0·08	0·422	240—250, 276—290
46	6-Amino-1′ : 2-dimethyl	cation	5·00 ± 0·10	1·250	280—290
47	1′ : 2-Dimethyl-6-methylamino	cation	5·00 ± 0·10	1·185	280—290
48	6-Amino-3′-methyl	cation	4·61 ± 0·05	1·304	270—280
		anion	11·11 ± 0·10		296—306
49	6-Methylamino-2-methylthio	cation	3·61 ± 0·05	1·083	280—300
		anion	11·40 ± 0·10		260—280
50	6-Methoxy-1′-methyl	cation	2·51 ± 0·05	1·325	240—250

TABLE 1. (*Continued.*)

No.	Substituents		pK_a	Analytical concn. (10^{-4}M) and wavelength range (mμ)	
	(b) *Pyrazolo*(4′ : 5′ : 4 : 5)*pyrimidines.*				
51	6-Amino	cation	5·00 ± 0·07	1·369	300—320
		anion	10·15 ± 0·10		280—300
52	6-Amino-3′-methyl	cation	5·02 ± 0·05	1·348	312—320
53	6-Benzylamino-3′-methyl	cation	4·95 ± 0·10	0·771	282—298
54	6-Furfurylamino-3′-methyl	cation	4·84 ± 0·06	0·938	284—296, 312—320
55	6-Anilino-3′-methyl	cation	4·61 ± 0·15	0·848	300—310, 332—340

[a] pK_a values of these cations were not determined, as the spectra of the cations and neutral molecules are virtually identical. [b] Decomposition by alkali made it difficult to obtain accurate values for the pK_a's of the anions of these compounds. [c] Unpublished preparation by R. K. R. [d] These compounds were prepared by Mr. C. W. Noell (unpublished work). [e] Unpublished preparation by C. C. C.

TABLE 2. *Comparisons of ionisation between purines and pyrazolo*(5′ : 4′-4 : 5)*pyrimidines.*

Purine derivative		pK_a	Pyrazolo(5′ : 4′-4 : 5)pyrimidine derivative		pK_a
Unsubstituted	cation	2·39	Unsubstituted	cation	2·84
	anion	8·93		anion	9·54
9-Methyl	cation	2·36	1′-Methyl	cation	2·50
6-Hydroxy	anion	8·94	6-Hydroxy	anion	9·54
6-Hydroxy-9-methyl	anion	9·32	6-Hydroxy-1′-methyl	anion	9·26
6-Methylthio	cation	0·0	6-Methylthio	cation	1·0
	anion	8·75		anion	9·65
6-Amino	cation	4·22	6-Amino	cation	4·59
	anion [a]	9·8		anion	10·84
2 : 6-Diamino	cation [b]	5·02 ± 0·10	2 : 6-Diamino	cation	5·58
6-Methylamino	cation	4·18	6-Methylamino	cation	4·53
	anion	9·99		anion	10·55
6-Dimethylamino	cation	3·87	6-Dimethylamino	cation	4·53
	anion	10·5		anion	*ca.* 11
2-Methyl-6-methylamino-	cation [c]	5·08 ± 0·04	2-Methyl-6-methylamino-	cation	5·56

[a] Taylor, *J.*, 1948, 765. [b] Present work: analytical concn., $1·182 \times 10^{-4}$M; analytical wavelength range, 288—306 mμ. Albert and Brown[1a] found 5·09 (potentiometric method). [c] Present work: analytical concn., $1·081 \times 10^{-4}$M; analytical wavelength range, 280—290 mμ. 6-Ethylamino-2-methylpurine cation has pK_a 5·05 ± 0·10 (analytical concn., $0·663 \times 10^{-4}$M, analytical wavelength range 280—290 mμ). For syntheses of these compounds, see Robins, Jones, and Lin, *J. Org. Chem.*, 1956, **21**, 695.

nitrogen atom of the five-membered ring (the 2′-nitrogen atom in the pyrazolopyrimidine, and the 7-nitrogen atom of the purine) is further from, and less effectively conjugated with, the 1-nitrogen atom in the pyrazolo(5′ : 4′-4 : 5)pyrimidine than in the purine, and would therefore have less effect on the basicity of the pyrimidine-nitrogen atoms.

In the purines, the hydrogen atom is shared between the 9- and the 7-nitrogen atom, but, on an average, one of the nitrogen atoms in the glyoxaline ring is exerting a base-weakening influence on the pyrimidine-nitrogen atoms. In the pyrazolo(5′ : 4′-4 : 5)-pyrimidines, tautomeric exchange of a hydrogen atom to the 2′-nitrogen atom is unlikely, since it would involve the loss of the Kekulé resonance in the pyrimidine ring.

These explanations are essentially unaltered if the 3-nitrogen atom is the basic centre; however, for reasons to be mentioned below, we favour the 1-nitrogen atom as the predominant basic centre in both the purines and the pyrazolo(5′ : 4′-4 : 5)pyrimidines.

Some supporting evidence is available for the suggestion that a pyrimidine ring-nitrogen atom is the basic centre in the purines. The ultraviolet absorption spectra of the 7- and the 9-methylpurine cation differ markedly;[11] if cation formation involved the glyoxaline, and not the pyrimidine ring, the spectra should be almost identical. Further, it has been found that a trifluoromethyl substituent in purine is far more base-weakening

[11] Bendich, Russell, and Fox, *J. Amer. Chem. Soc.*, 1954, **76**, 6073.

from the 6- than from the 8-position, once again indicating that the pyrimidine ring is the site of proton capture.[12] Similar effects are noted with methoxy- and methylthio-purines.[1]

However, it is difficult to establish that a particular pyrimidine-nitrogen atom is the predominant basic centre in the purines and pyrazolo(5′ : 4′-4 : 5)pyrimidines; the question could be solved by *X*-ray crystallography (cf. Cochran's study [13] of adenine hydrochloride, which established that the proton is on the 1-nitrogen atom), but this technique is of course applicable only to solids, and it is possible that the position of proton-attachment in the crystal may not be identical with that in solution.

In the absence of direct experimental evidence as to the basic centres, some recent calculations [14-16] of the charge densities in these molecules using the molecular-orbital method are of interest. Mason [14] and Pullman, Pullman, and Berthier [15] have made calculations for the purine molecule, but since the results are at variance with experiment (the calculated charge densities at the various carbon atoms predict an incorrect order of reactivities towards nucleophilic reagents), little reliance can be placed on them. Mason assumed that the 7- and the 9-nitrogen atom have identical electronegativities, and this may offer a partial explanation for the discrepancy between calculation and experiment; Pullman, Pullman, and Berthier give no details of the parameters used in their calculations.

The π-electron densities calculated for the pyrazolopyrimidines [16] are listed in Table 3. The simple LCAO method, neglecting overlap, was used, with the following values for the various parameters:

$$\alpha_O = \alpha;\ \alpha_{-N=} = \alpha + \beta;\ \alpha_{-\overset{|}{N}-} = \alpha + 2\beta;\ \beta_{CO} = \beta_{CN} = \beta$$

The values chosen probably exaggerate the electronegativities of the nitrogen atoms (cf. Brown [17]), *i.e.*, the true charge migrations are smaller than they appear to be, but the relative values should be unaffected by this choice.

TABLE 3. *π-Electron densities in the pyrazolopyrimidines.*

(a) *Pyrazolo*(5′ : 4′-4 : 5)*pyrimidine.*

Position	π-Electron density	Position	π-Electron density
1′	1·71	1	1·34
2′	1·35	2	0·75
3′	0·87	3	1·36
4′ (=5)	1·01	6	0·73
5′ (=4)	0·88		

(b) *Pyrazolo*(4′ : 5′-4 : 5)*pyrimidine.*

Position	π-Electron density	Position	π-Electron density
1′	1·70	1	1·33
2′	1·29	2	0·78
3′	0·90	3	1·35
4′ (=4)	0·92	6	0·76
5′ (=5)	0·97		

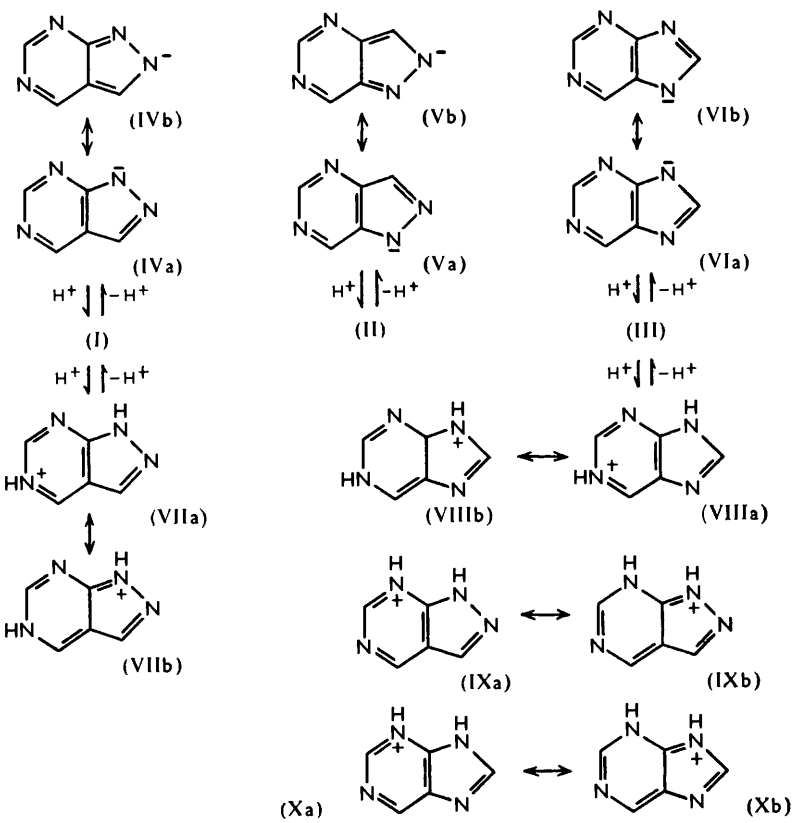
The results are in good agreement with experiment, predicting that 6-substituents would be preferentially attacked by nucleophilic reagents, and can therefore be regarded with some confidence. The π-electron densities are highest at the 3-nitrogen atoms, and it would therefore be expected that these atoms would be the predominant sites of proton-attachment in the pyrazolopyrimidines, in the absence of other factors. However, the π-electron densities in the neutral molecules may not be the controlling factor in determining the preferred sites of protonation; the magnitude of resonance-stabilisation of the various possible cations (over and above that prevailing in the neutral molecules), *i.e.*, the " additional ionic resonance " effect (cf. Albert, Goldacre, and Phillips [10]) may be the most important factor. Thus, protonation at the 1-nitrogen atom in purine or pyrazolo-(5′ : 4′-4 : 5)pyrimidine introduces a base-strengthening additional ionic resonance of a *para*-quinonoid type (structures VIIa, b; VIIIa, b), while protonation at the 3-nitrogen atom would lead to the less effective *ortho*-quinonoid type of additional ionic resonance

[12] Bendich, Giner-Sorolla, and Fox, Ciba Foundation Symposium, " The Chemistry and Biology of Purines," Churchill Ltd., London, 1957, p. 3.
[13] Cochran, *Acta Cryst.*, 1951, **4**, 81.
[14] Mason, ref. 12, p. 72.
[15] Pullman, Pullman, and Berthier, *Compt. rend.*, 1956, **243**, 380.
[16] Unpublished calculations by Dr. A. J. Owen (see Table 3).
[17] Brown, *J.*, 1956, 272.

(structures IXa, b; Xa, b). We suggest, therefore, that the cations of purines and pyrazolo(5′ : 4′-4 : 5)pyrimidines have structures of types VII and VIII. The structures resemble those deduced for the 4-aminopyrimidine cation, where the basic strength, ultraviolet absorption spectra, and ease of methylation at the 1-position, all suggest that protonation occurs at the 1-nitrogen atom, leading to the generally preferred *para*-quinonoid type of additional ionic resonance.[18]

It is, of course, realised that an equilibrium may exist between the various possible cations. By proposing that the 1-nitrogen atoms of purines and pyrazolo(5′ : 4′-4 : 5)-pyrimidines are the basic centres, we imply only that these nitrogen atoms are protonated in the *predominating* cations.[19] It should be pointed out, however, that a nitrogen atom participating in *para*-quinonoid additional ionic resonance will be the predominating basic centre when compared with a corresponding *ortho*-quinonoid system. Relevant examples [10] are: 2-aminopyridine (*ortho*-quinonoid), pK_a 6·86, 4-aminopyridine (*para*-quinonoid), pK_a 9·17; 2-aminoquinoline (*ortho*-quinonoid), pK_a 7·34, 4-aminoquinoline (*para*-quinonoid). pK_a 9·17.

The similar basic strengths of purine, pyrazolo(5′ : 4′-4 : 5)pyrimidine, and 1 : 2 : 3-triazolo(5′ : 4′-4 : 5)pyrimidine (XI) (pK_a of cation, 2·1) [12,20] can be attributed to the

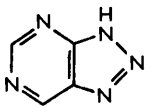


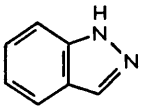
para-quinonoid additional ionic resonance common to the cations derived from all three ring systems. The importance of this effect is well illustrated by comparing the pK_a's of the cations of pyrazolo(5′ : 4′-4 : 5)pyrimidine (2·84) and indazole (XII) (approx. 1·3),[10]

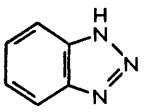
[18] Brown, Hoerger, and Mason, *J.*, 1955, 4035.
[19] Cf. Osborn, Schofield, and Short, *J.*, 1956, 4191.
[20] Felton, " Recent Work on Naturally Occurring Nitrogen Heterocyclic Compounds," *Chem. Soc. Special Publ.*, No. 3, 1955, p. 134.

and the values for the triazolopyrimidine (XI) and benzotriazole (XIII) (1·6).[10] In these examples, replacement of two aromatic carbon atoms by normally base-weakening nitrogen atoms has given rise to increases in basicity. This result is ascribed to the additional ionic resonance in the cations of (I) and (XI), which obviously cannot contribute to basic strength in the indazole and benzotriazole cations.

In the 6-substituted-aminopyrazolo(5′ : 4′-4 : 5)pyrimidines (XIV; R^1 and R^2 = H, alkyl, or aryl) (nos. 7, 9, 11—23), relatively large increases in the pK_a's of the cations over the value for the parent compound (I) (about 2 units of pK) indicate that some further stabilisation of the cations relative to the neutral molecules is present. The increases can be understood readily in terms of protonation at the 1-nitrogen atoms to yield amidine-type structures, once again stabilised by additional ionic resonance, as exemplified


(XI)


(XII)


(XIII)

by the canonical forms (XVa, b, c). Analogous base-strengthening by amino-substituents is observed with the purines.[1a]

The differential effects of methyl groups in the pyrimidine and the pyrazole ring of pyrazolo(5′ : 4′-4 : 5)pyrimidines lend further supporting evidence to the suggestion that the site of protonation is in the pyrimidine ring. Insertion of a methyl group into a nitrogen-heterocyclic molecule generally gives rise to an appreciable base-strengthening effect (from 0·3 to 1 unit of pK) if it is α or γ to the basic centre (*e.g.*, the quinoline [21] and acridine [22] series). 6-Amino-3′-methylpyrazolo(5′ : 4′-4 : 5)pyrimidine (no. 48) has virtually the same basic strength as the 6-amino-compound (no. 7), but 6-amino- and 6-methylamino-2-methylpyrazolo(5′ : 4′-4 : 5)pyrimidines (nos. 39 and 40), and the 6-alkylamino-2-methylpurines (Table 2) show large elevations in pK_a over the corresponding compounds lacking a methyl group in the pyrimidine ring (compare also nos. 2 and 3, 23 and 41, 43 and 45), indicating very strongly that protonation occurs in the pyrimidine ring in both the pyrazolo(5′ : 4′-4 : 5)pyrimidines and the purines.

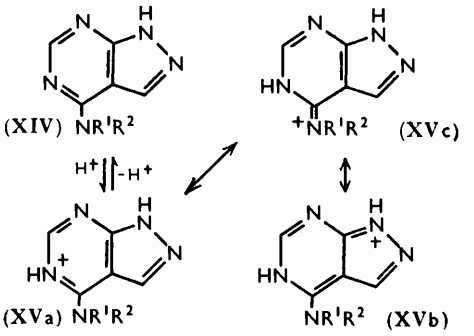
The basicities of the various alkylamino- and arylamino-substituted pyrazolo(5′ : 4′-4 : 5)pyrimidines are more or less as expected, and where comparisons can be made, follow similar sequences to those recorded for the purines.[1a] Mono- and di-alkylation of the amino-group does not greatly affect the basic strength, while the 6-anilino-, 6-benzylamino-, and 6-phenethylamino-compounds (nos. 19, 20, and 22) reveal the decreased base-weakening effect of a phenyl group as it is further separated from the basic centre. Further substitution by amino-groups increases the base-strengthening effect (as compared with the parent compound), as a result of the obvious additional resonance-stabilisation of the cations (the new amino-group provides a further seat for the positive charge). A 2-amino-substituent has approximately the same base-strengthening effect as a 2-methyl group (compare nos. 8 and 39, 10 and 40, 44 and 45), and an analogous relationship holds for suitably substituted purines (Table 2).

Chloro- and methylthio-groups are base-weakening, as expected from their effects in benzenoid systems (compare nos. 24 and 25, 1 and 6, 9 and 49), while a 6-methoxy-substituent seems to have little effect (nos. 2 and 50); a 2-methoxy-substituent is, surprisingly, appreciably base-weakening (nos. 24 and 26).

Substitution of the 1′-hydrogen atom in pyrazolo(5′ : 4′-4 : 5)pyrimidines by a methyl group results in a small decrease in basic strength (*ca.* 0·3—0·5 unit of pK) for many compounds (compare nos. 1 and 2, 7 and 24, 9 and 27, 11 and 28, 12 and 29, 14 and 30, 15 and 31, 16 and 32, 23 and 38, 19 and 36, 20 and 37, 39 and 46, 40 and 47). It is possible

[21] Felsing and Biggs, *J. Amer. Chem. Soc.*, 1933, **55**, 3624.
[22] Albert and Goldacre, *J.*, 1946, 706.

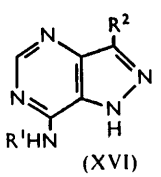
that this base-weakening is a result of the lowering of the resonance energy of the cations, because of the non-equivalence (lack of symmetry) of the 1- and 1′-nitrogen atoms in the cations derived from the methylated compounds [compare the similar (small) base-weakening effects arising from *N*-alkylation of guanidine [23]]. Steric interference with the coplanarity of the pyrimidine and pyrazole rings, leading to less effective conjugation,



could also explain these observations, and Stuart–Briegleb models show that there is interference between the 3-nitrogen atom and a 1′-methyl group in the 1′-methylpyrazolo-(5′ : 4′-4 : 5)pyrimidines. The basic strengths of two of the 6-alkylamino-1′-methyl-pyrazolo(5′ : 4′-4 : 5)pyrimidines [no. 34, 1′-methyl-6-(1 : 1 : 3 : 3-tetramethylbutylamino-) pyrazolo(5′ : 4′-4 : 5)pyrimidine, and no. 35, 6-(2-hydroxyethylamino)-1′-methylpyrazolo-(5′ : 4′-4 : 5)pyrimidine] are appreciably lower than would be expected by comparison with the other compounds in this series. The decreased basic strength of no. 34 can be explained by steric interference to protonation at the 1-nitrogen atom, caused by the highly branched 6-alkylamino-group, and that of no. 35 in terms of intramolecular hydrogen-bonding between the 1-nitrogen atom and the hydroxy-group of the 6-(2-hydroxyethylamino)-substituent. Base-weakening due to intramolecular hydrogen-bonding has been noted previously.[10,19,24]

Introduction of 1′-phenyl groups into the pyrazolo(5′ : 4′-4 : 5)pyrimidines leads to decreased basic strengths (compare nos. 7 and 43, 8 and 44, 39 and 45), readily explained by the electron-attracting effect of such groups.

The 6-aminopyrazolo(4′ : 5′-4 : 5)pyrimidines (XVI; R^1 = H, alkyl, or Ph, R^2 = H or Me) are stronger bases than the corresponding purines and pyrazolo(5′ : 4′-4 : 5)pyrimidines (compare the following pK_a's for the appropriate cations: no. 51, 5·00; adenine, 4·22; no. 7, 4·59; also nos. 52, 5·02, and 48, 4·61), perhaps indicating that the site of protonation in the pyrazolo(4′ : 5′-4 : 5)pyrimidines differs from that in the purines and pyrazolo(5′ : 4′-4 : 5)pyrimidines. The similarities in basic strength between nos. 51 and 52 indicate, however, that the pyrazole ring does not accept a proton. Close resemblance between the ultraviolet absorption spectra of the cations of purines [25] and pyrazolo(5′ : 4′-4 : 5)-pyrimidines,[26] and the marked difference between the spectra of these two systems and the corresponding pyrazolo(4′ : 5′-4 : 5)pyrimidines,[26] support the suggestion that the pattern of protonation in the latter compounds is different from that observed with the purines and pyrazolo(5′ : 4′-4 : 5)pyrimidines.


(XVI)

Turning now to a discussion of the pK_a values of the neutral molecules of the pyrazolo-pyrimidines and purines (*i.e.*, their relative acidic strengths), it is relatively easy to explain the greater acid strengths of the purines when compared with the corresponding pyrazolo-pyrimidines (cf. Table 2). In the purines, the negative charge of the anion may be shared

[23] Angyal and Warburton, *J.*, 1951, 2492.
[24] Short, *J.*, 1952, 4584.
[25] Mason, *J.*, 1954, 2071.
[26] Lynch, unpublished results.

between the two glyoxaline-nitrogen atoms without losing the Kekulé resonance associated with the pyrimidine ring (structures VIa, b), but in the pyrazolo-(5′ : 4′-4 : 5)- and -(4′ : 5′-4 : 5)-pyrimidines, structures where the 2′-nitrogen atoms carry the negative charge (*e.g.*, IVb, Vb) cannot maintain the aromatic character of the pyrimidine ring. The effects of substituents on acid strengths in the pyrazolo(5′ : 4′-4 : 5)pyrimidines are more or less as expected; amino-groups and methyl groups are acid-weakening; however, methylthio-substitution causes slight acid-weakening, in contrast to observations in the purine series [1a] (cf. nos. 1 and 6, 9 and 49).

Introduction of a hydroxyl group (leading to the possibility of tautomerism to an amide) into the pyrazolo(5′ : 4′-4 : 5)pyrimidine molecule (*e.g.*, no. 4) makes feasible the formation of several different anions. As Albert and Brown [1a] found with the corresponding purine, it is difficult to determine which hydrogen atom ionises preferentially, since the pK_a's of the neutral molecules of pyrazolo(5′ : 4′-4 : 5)pyrimidine, its 6-hydroxy-derivative (no. 4), and 1′-methyl-6-hydroxy-derivative (no. 5) are all closely similar.

The anti-tumour activity of several 6-(substituted amino)pyrazolo(5′ : 4′-4 : 5)-pyrimidines has been reported previously; [3,9] the " starred " compounds listed in Table 1 are all strong inhibitors of growth of the mouse Adenocarcinoma 755, *i.e.*, at dose levels which are not appreciably toxic (less than 10% mortality) they effect reduction in tumour weight to a level less than 20% of that in untreated control animals. Details of the biological testing of the various pyrazolo(5′ : 4′-4 : 5)pyrimidines and pyrazolo(4′ : 5′-4 : 5)-pyrimidines listed in Table 1 are given in the recent paper by Skipper and his co-workers.[9] Reference to Table 1 shows that anti-tumour activity (as measured by inhibition of Adeno-carcinoma 755) is virtually confined to compounds whose cations have pK_a's between 4·0 and 4·7. It seems, therefore, that one important factor in determining anti-tumour activity in these compounds is the basic strength, which must approximate to that of adenine; of course, other factors are obviously of importance, *e.g.*, methyl groups in the 2- or 3′-position result in complete loss of activity, and none of the pyrazolo(4′ : 5′-4 : 5)-pyrimidines are active, although no. 55 falls within the prescribed limits of basicity.

There is abundant evidence of competition between adenine and 6-aminopyrazolo-(5′ : 4′-4 : 5)pyrimidine in biological systems: 6-aminopyrazolo(5′ : 4′-4 : 5)pyrimidine is an antimetabolite of adenine in *Neurospora crassa* [27] and *Lactobacillus arabinosus*; [9] also, damage produced by 6-aminopyrazolo(5′ : 4′-4 : 5)pyrimidine in tissue culture experiments on the Hela strain of human cervical carcinoma is relieved by flavine-adenine dinucleotide.[28]

On the basis of this information, it seems probable that the 6-(substituted amino)-pyrazolo(5′ : 4′-4 : 5)pyrimidines owe their biological activity to interference with the utilisation of adenine or some adenine derivative, but far more experimental work is necessary before the mechanism of action of these purine analogues can be established. The present work merely indicates an association between basic strength and biological activity which may merit further investigation, and we hope to extend our studies to other purine analogues with similar basic strengths to the 6-substituted-amino-pyrazolo-5′ : 4′-4 : 5)pyrimidines.

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[27] Fuerst, Somers, and Hsu, *J. Bact.*, 1956, **72**, 387.
[28] Hsu, personal communication.