Purine analogues. I. The status of Hückel molecular orbital calculations as predictors of proton shifts, basic strengths, and reactivity[1]

BRIAN M. LYNCH,[2] ALLAN J. ROBERTSON,[3] AND JOHN G. K. WEBB[4]
Organic Chemistry Laboratory, Saint Francis Xavier University, Antigonish, Nova Scotia

Received June 17, 1968

A detailed series of molecular orbital calculations based on the HMO method has been made for the various possible ionic species of purine, pyrazolo(3,4-*d*)pyrimidine, v-triazolo(4,5-*d*)pyrimidine, and pyrazolo(3,4-*b*)pyridine. π-Electron densities and localization and delocalization energies for nucleophilic substitution have been derived.

The results are compared with the observed proton chemical shifts in the conjugate acids of these molecules, with the relative rates of nucleophilic piperidinodehalogenations in the neutral molecules, and with the ionization constants.

It is concluded that it is possible to reconcile the calculations with experimental results for the various positions within a six-membered ring, but that positions in six- and five-membered rings cannot be directly compared. The electron densities seem to be of little value in correlating the observed ionization patterns of purines and their analogues.

Canadian Journal of Chemistry, **47**, 1129 (1969)

Introduction

As a consequence of their involvement as determinants of biological processes, the purine components of nucleic acids and their analogues have received much attention from synthetic organic chemists (1, 2) and from quantum chemists (3). However, there is little constructive interaction between the two groups: uncritical applications of molecular orbital (MO) calculations by synthetic chemists (4) often suggest that the calculations have little value, while defences of the value of simple MO treatments (5, 6) often quote irrelevant or incorrect supporting data. In this paper, we seek to show where simple MO calculations on appropriate ionic species of purines and their analogues are of correlative or predictive value, and also to indicate areas where the simple approach gives predictions at variance with experimental facts.

We have chosen three areas where detailed comparisons between theory and experiment are possible: calculated π-electron densities and observed proton chemical shifts (cf. 7, 8); π-electron densities and ionization constants (cf. 9); and localization energies (10) and delocalization energies (11) for nucleophilic substitution as compared with observed activation parameters for piperidinodehalogenations (cf. 12).

Results and Discussion

Molecular Orbital Calculations

The simple Hückel method (HMO) (13) was used, employing a standard matrix diagonalization program with an IBM 1620 40K computer (14, 15). The following electronegativity parameters h (16) were used: carbon and anionic nitrogen, 0; pyridine-type nitrogen, +0.5; pyridinium- and pyrazole-type NH, +1. All resonance integrals were assumed equal to β_{CC}. The output of the calculations consisted of the orbital energies, π-electron densities, and π-bond orders. ω-Technique calculations (17), using $\omega = 1.4$, were performed using the electron densities from the HMO calculations as input.

Localization energies for nucleophilic substitution at the various positions were evaluated by subtraction of the energies of electrons in the occupied orbitals in the residual systems from those in the parent species, and delocalization energies for nucleophilic piperidinodechlorinations were evaluated following Beltrame *et al.* (11).

The results for purine **1** and the purine analogues 1*H*-pyrazolo(3,4-*d*)pyrimidine **2**, 3*H*-v-triazolo(4,5-*d*)pyrimidine **3**, and 1*H*-pyrazolo-

---

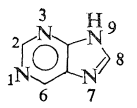
[1]Presented in part at the International Congress of Heterocyclic Chemistry, Albuquerque, New Mexico, June 14, 1967.

[2]Enquiries should be addressed to this author. Address until September 1969: Department of Medical Chemistry, John Curtin School of Medical Research, Australian National University, Box 334, Canberra City, A.C.T. 2601, Australia.

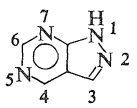
[3]Graduate Research Assistant, 1965–1967. Present address: Cyanamid of Canada Ltd., St. Catharines, Ontario.

[4]Graduate Research Assistant, 1966–1968. Present address: Queen's University, Kingston, Ontario.

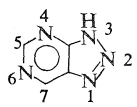
(3,4-*b*)pyridine **4** are summarized in Tables I through III.



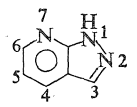
1



2



3



4

π-Electron Densities and Proton Chemical Shifts

Previous work has established qualitative and quantitative correlations between proton chemical shifts and HMO π-electron densities for pyridinium and diazolium ions (7), and for the amino protons in carbocyclic and heterocyclic aromatic amines (8), and Black, Brown, and Heffernan (18) have analyzed the proton chemical shifts of a series of heteroaromatic nitrogen compounds, establishing "empirical" electron density distributions after allowing for the effects of ring currents, lone-pair anisotropy, and non-adjacent charges.

Although the proton and carbon-13 magnetic resonance spectra of purine have received considerable attention (19–22), little success has been achieved in correlating electron densities directly with the proton shifts. In view of the fairly wide range of proton shifts available in the series purine, pyrazolo(3,4-*d*)pyrimidine, and v-triazolo(4,5-*d*-)pyrimidine, and also pyrazolo-(3,4-*b*)pyridine (23), we have attempted such a correlation for the protons in the six-membered rings employing the proton chemical shifts observed in trifluoroacetic acid-*d* (in this solvent, many paramagnetic effects of n–π^* transitions will disappear (cf. 7)), and using ring-current corrections of 0.20 p.p.m. for the influence of the five-membered rings at positions α to them, and a further correction of 0.30 p.p.m. for the influence of a pyridine-type nitrogen in a position *peri* to protons α to a five-membered ring. We have used π-electron densities appropriate to models assuming that protonation has occurred at positions analogous to position-1 of purine (which we expect to be the predominant basic site (cf. 9, 21)).

We find that the chemical shifts of the protons in this series of compounds are linearly related to the calculated electron densities, and the correlation coefficient meets the acceptable standard of $r = 0.96$, set by Jaffe (24): the least-squares regression line is defined by

$$\delta = 14.86 - 6.64q\pi \; (r = 0.972)$$

and the average deviation is ca. 0.1 p.p.m. (see Table IV, and Fig. 1).

Thus, the calculated HMO π-electron densities for assumed models of the conjugate acids of purine and purine analogues are excellent predictors for the proton chemical shifts within the six-membered rings. Since very few points are available for correlations within the five-membered rings, no analysis was made in these instances. We point out, however, that the proton shifts are well downfield from the values predicted from the calculated electron densities using the regression line given above, suggesting that the deshielding effect of the ring current in the six-membered ring on protons in five-membered rings is significantly greater than 0.20 p.p.m. (cf. 18).

MO Quantities and Ionization Behavior

As Clark and Perrin (25) have noted, many difficulties beset quantitative correlations of the base strengths of nitrogen heterocycles with MO quantities, although there are correlations between pK_a and electron density at nitrogen with aza-derivatives of alternant hydrocarbons and with polycyclic aromatic amines (26, 27). Thus, although the total HMO π-electron densities at the nitrogen atoms in the pyrimidine rings of the neutral molecules of pyrazolo(3,4-*d*)pyrimidine, purine, and 3*H*-v-triazolo(4,5-*d*)-pyrimidine decrease in the same order as the basic strengths of these compounds (electron densities: 2.491, 2.480, 2.473 (cf. Table I) pK_a of cations: 2.8, 2.4, ca. −0.1), the absolute changes are very small and may have little significance.

Nakajima and the Pullmans (28, 29) have suggested that correlations between relative SCF-MO ionization potentials for the lone-pair electrons on ring nitrogen atoms and pK_a values, which are observed for other nitrogen heterocyclic compounds, allow predictions of the basic strengths of purines and their analogues. We find that these predictions are of very limited

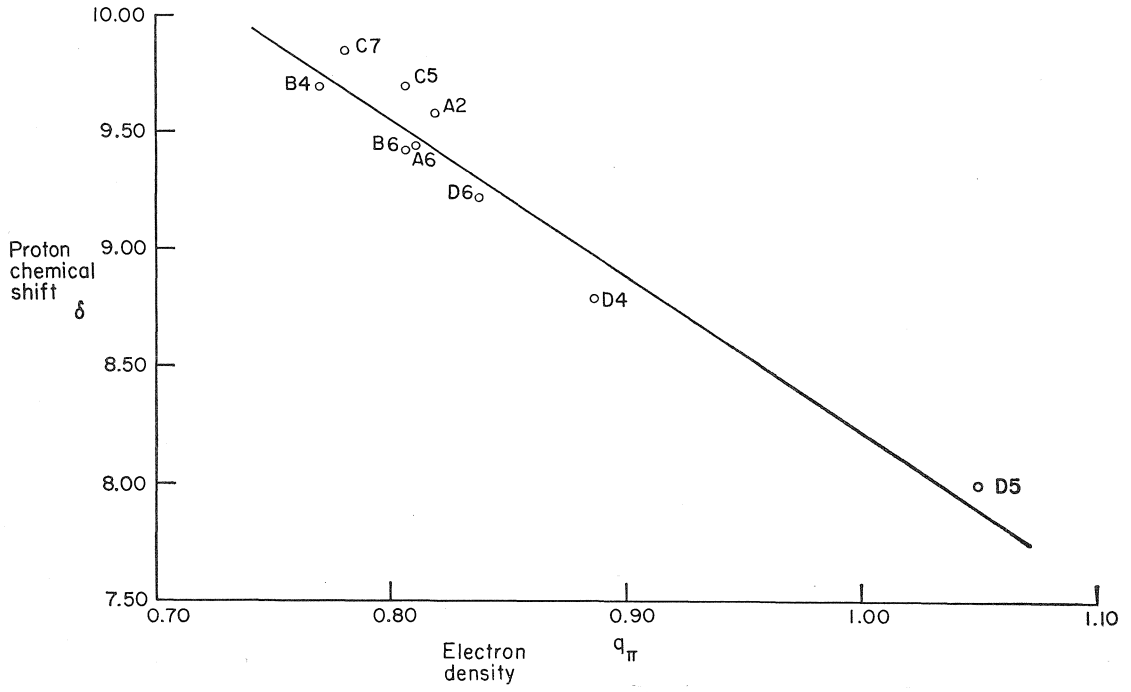


FIG. 1. Plot of π-electron density vs. corrected proton chemical shift. A, Purine; B, 1-methylpyrazolo(3,4-*d*)pyrimidine; C, 3*H*-v-Triazolo(4,5-*d*)pyrimidine; D, 1-methylpyrazolo(3,4-*b*)pyridine. Numerical suffixes refer to positions of protons.

value: although there is a significant correlation between the relative ionization potential and pK_a for purine and some aminopurines ($r = 0.984$) (29), use of the regression line to predict the pK_as of the cations of 4-aminopyrazolo(3,4-*d*)pyrimidine (cf. 9), v-triazolo(4,5-*d*)pyrimidine (30), and its 7-amino derivative results in spectacular failure (the respective values (predicted, found) are: 3.14, 4.59; 1.31, −0.10; 3.05, 2.84). Although the aminopyrazolo pyrimidine and the aminotriazolopyrimidine are predicted to have almost identical pK_a values (their relative ionization potentials are quoted as −1.67 and −1.65 e.V. (29)), the actual values differ by almost two units (see Table V, also (9)).

Further, although it is quite probable that all three basic sites in purine are protonated to some extent (9, 21), the Nakajima–Pullman treatment uses the existence of very small differences in relative ionization potentials to predict preferential basic centers, although as Table V demonstrates, very small differences in relative ionization potentials are accompanied by large variations in actual basic strength.

In view of the failure of the Nakajima–Pullman approach in these examples, we must conclude that calculations in terms of the π-electron approximation do not enable us to predict the base strengths of purines or their analogues (cf. 32).

MO Quantities and Nucleophilic Substitutions

As Pullman (33) has pointed out, π-electron densities in heterocyclic rings often give no indication of the relative reactivities of different positions, and it might be expected that calculations of localization energies would be of greater significance. Pullman and Pullman's calculations of these quantities for the neutral molecules of purine, pyrazolo(3,4-*d*)pyrimidine, and v-triazolo(4,5-*d*)pyrimidine might therefore be expected to be of correlative value for the rates of nucleophilic substitution in such molecules.

One difficulty in a direct correlation has been pointed out by Sutcliffe and Robins (4), since the nature of the reacting species may change with the pH of the reaction medium, and realistic localization energies should take these

TABLE I

Calculated π-electron densities in purine and some analogues

No.	Method of calculation	Input parameters	Corresponding model	π-Electron density, q_π						
				Position						
				1	2	3	6	7	8	9
	A: Purine									
1	HMO	$h_9 = 1$ $h_{1,3,7} = 0.5$	Neutral molecule (9H isomer)	1.233	0.876	1.247	0.884	1.362	0.883	1.539
2	ω-technique	output from No. 1	Neutral molecule (9H isomer)	1.154	0.975	1.154	0.968	1.270	1.013	1.404
3	HMO	$h_{1,9} = 1$ $h_{3,7} = 0.5$	1-Protonated conjugate acid	1.402	0.820	1.248	0.811	1.356	0.878	1.533
				Position						
				1	2	3	4	5	6	7
	B: 1H-Pyrazolo(3,4-d)pyrimidine									
4	HMO	$h_1 = 1$ $h_{2,5,7} = 0.5$	Neutral molecule	1.538	1.211	1.050	0.848	1.239	0.859	1.252
5	ω-technique	output from No. 4	Neutral molecule	1.399	1.177	1.107	0.959	1.155	0.971	1.153
6	HMO	$h_{1,5} = 1$ $h_{2,7} = 0.5$	5-Protonated conjugate acid	1.531	1.209	1.039	0.770	1.407	0.807	1.253
				Position						
				1	2	3	4	5	6	7
	C: 3H-v-Triazolo(4,5-d)pyrimidine									
7	HMO	$h_3 = 1$ $h_{1,2,4,6} = 0.5$	Neutral molecule	1.256	1.095	1.494	1.245	0.860	1.228	0.860
8	ω-technique	output from No. 7	Neutral molecule	1.227	1.132	1.374	1.147	0.966	1.147	0.958
9	HMO	$h_{3,6} = 1$ $h_{1,2,4} = 0.5$	6-Protonated conjugate acid	1.247	1.090	1.490	1.246	0.807	1.397	0.781
	D: 1H-Pyrazolo(3,4-b)pyridine									
10	HMO	$h_1 = 1$ $h_{2,7} = 0.5$	Neutral molecule	1.544	1.213	1.062	0.942	1.046	0.919	1.248
11	HMO	$h_{1,7} = 1$ $h_2 = 0.5$	7-Protonated conjugate acid	1.541	1.216	1.054	0.887	1.050	0.838	1.428

variations into account. Robins and Sutcliffe were able to give excellent qualitative explanations of apparent inconsistencies in the order of reactivity of the 2-, 6-, and 8-positions in chloropurines and their 7- or 9-alkyl derivatives by postulating cation or anion formation at the appropriate pH as reactivity-controlling factors. Recent kinetic studies of nucleophilic substitutions in 9-alkylchloropurines and chloropurines by Barlin and Chapman (12*a*) and by Barlin (12*b*) show that these explanations are well founded.

The localization energies for nucleophilic attack at the 6- and 8-positions in neutral purine were evaluated by Pullman (3, 33), and these results (suggesting virtually equivalent reactivities for these positions) are consistent with Barlin and Chapman's findings for the piperidinodechlorinations of 9-methyl-6- and 8-chloropurines (12*a*). It is likely that this agreement is an artifact of the parameters used in the calculation (the localization energy will depend upon the choice of resonance integral for the C_8—N_9 bond), and direct comparison of localization energies for five- and six-membered rings is a risky procedure. Evidence regarding this point is available from Barlin's study (34) of nucleophilic piperidinodebrominations of

TABLE II

Localization energies for ionic species of purine and some analogues

Species	Position:	Localization energy (−β units) for nucleophilic substitution 2	6	8
A: Purine				
Neutral molecule (9H isomer)		2.269	2.121	2.249
Neutral molecule (7H isomer)			2.138	
1-Protonated conjugate acid		2.146	1.960	2.235
3-Protonated conjugate acid		2.069	2.011	2.222
7-Protonated conjugate acid		2.269	2.136	2.075
Anion		2.300	2.110	2.572
B: 1H-Pyrazolo(3,4-d)pyrimidine				
	Position:	3	4	6
Neutral molecule		2.599	2.049	2.219
5-Protonated conjugate acid		2.464	1.896	2.112
C: 3H-v-Triazolo(4,5-d)pyrimidine				
	Position:		7	
Neutral molecule			2.053	

bromoazoles. There is a reasonable correspondence between calculated HMO localization energies (35) and observed activation energies (the ratio is 0.16 ± 0.02), but the ratio for the analogous 2-substitution in pyridine is 0.11. Further, the localization energy for nucleophilic 4-substitution in 2-methyltetrazole is −2.65β and the activation energy 18.7 Kcal, while the localization energy for 2-substitution in pyridine is −2.35β, and the activation energy 21.8 Kcal.

It is therefore probable that direct comparisons in calculated localization energies for 6- and 8-positions are not meaningful.

The changes in relative localization energies accompanying anion formation (see Table II) are as expected, with the value at the 8-position increasing by 0.3β, with little effect at the 2- and 6-positions. Thus, the experimental activation energies for nucleophilic ethoxydechlorination of 9-methyl chloropurines and for the chloropurines (which will exist entirely as anions under the reaction conditions) differ by 11.5 Kcal for the 2-isomer, by 9.8 Kcal for the 6-isomer, but by 17.2 Kcal for the 8-isomer.

TABLE III

Delocalization energies (11) for nucleophilic piperidinodechlorinations*

Compound	Delocalization energy (units of −β)
2-Chloropurine	1.839
6-Chloropurine	1.734
8-Chloropurine	1.826
4-Chloro-1*H*-pyrazolo(3,4-*d*)pyrimidine	1.689
6-Chloro-1*H*-pyrazolo(3,4-*d*)pyrimidine	1.811
7-Chloro-3*H*-v-triazolo(4,5-*d*)pyrimidine	1.680
5-Chloro-3*H*-v-triazolo(4,5-*d*)pyrimidine	1.807

*The following parameters were employed: $h_{NH} = +1$, $h_N = +\frac{1}{2}$, $h_{Cl} = +1$, h for pseudoatom $= -0.4$. All resonance integrals are equal to β_{CC} except $\beta_{C-Cl} = 0.45\ \beta_{CC}$.

Localization energies corresponding to various protonated species (Table II) are relevant to Robins' findings (cf. 2) of selective nucleophilic replacement of 6,8-dichloropurine at the 8-position in aqueous acid solution (where 7-protonation is postulated), and also of 6-amino-2,8-dichloropurine under similar conditions to yield 6-amino-8-chloro-2-hydroxypurine (where 1-protonation seems probable by analogy with adenine).

Within the pyrimidine ring of purine and its aza-analogues, direct comparison of reactivities towards nucleophilic substitution with calculated localization energies should be acceptable. Barlin and Chapman (12*a*) have examined the kinetics of piperidinodechlorinations of 9-methyl-2- and 6-chloropurines in 99.8% ethanol, using classical titrimetry, and we have examined analogous reactions with 1-substituted 4-chloropyrazolo(3,4-*d*)pyrimidine and 3-phenyl-7-chloro-v-triazolo(4,5-*d*)pyrimidine in 99.9% methanol, where we have used ultraviolet spectrophotometry to follow the rates (36). The

TABLE IV

π-Electron densities and corrected proton chemical shifts

Compound	Position of proton	π-Electron density*	Proton chemical shift (observed) δ obs.	Proton chemical shift (corrected)† δ corr.
Purine	2	0.820	9.57	9.57
Purine	6	0.811	9.92	9.42
1-Methylpyrazolo(3,4-*d*)pyrimidine	4	0.770	9.88	9.68
1-Methylpyrazolo(3,4-*d*)pyrimidine	6	0.807	9.40	9.40
3*H*-v-triazolo(4,5-*d*)pyrimidine	5	0.807	9.68	9.68
3*H*-v-triazolo(4,5-*d*)pyrimidine	7	0.781	10.33	9.83
1-Methylpyrazolo(3,4-*b*)pyridine	4	0.887	8.98	8.78
1-Methylpyrazolo(3,4-*b*)pyridine	5	1.050	7.97	7.97
1-Methylpyrazolo(3,4-*b*)pyridine	6	0.838	9.20	9.20

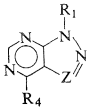
*These refer to protonated species (see Table I).
†See text for details of correction procedure.

solvents should be closely comparable in their effects, and Table VI shows that the effects of *N*-methyl and *N*-phenyl groups are similar. The effects of substituents in the *N*-phenyl moiety, and the rate-retarding influence of a 6-methyl group, are as expected for a normal nucleophilic substitution. These reactions, therefore, provide a series establishing the effects of annelation of five-membered rings on the reactivity of the pyrimidine ring. The enthalpies of activation for these reactions and the free energies of activation were compared with the following HMO quantities for the relevant positions in the neutral molecules of the three annelated pyrimidines: (*a*) the HMO π-electron

TABLE V

Ionization behavior of corresponding pyrazolo(3,4-*d*)pyrimidines* and v-triazolo(4,5-*d*)pyrimidines*

R_1	R_4	Pyrazolopyrimidine	Triazolopyrimidine
H	NH_2	4.59	—
H	$NHCH_3$	4.53	2.84
H	NHC_2H_5	4.60	2.82
H	$N(CH_3)_2$	4.53	2.67
CH_3	NH_2	4.32	2.76
CH_3	$NHCH_3$	4.24	2.73

*Compounds of type  where Z = —CH: pyrazolopyridine) or —N: (triazolopyrimidine).

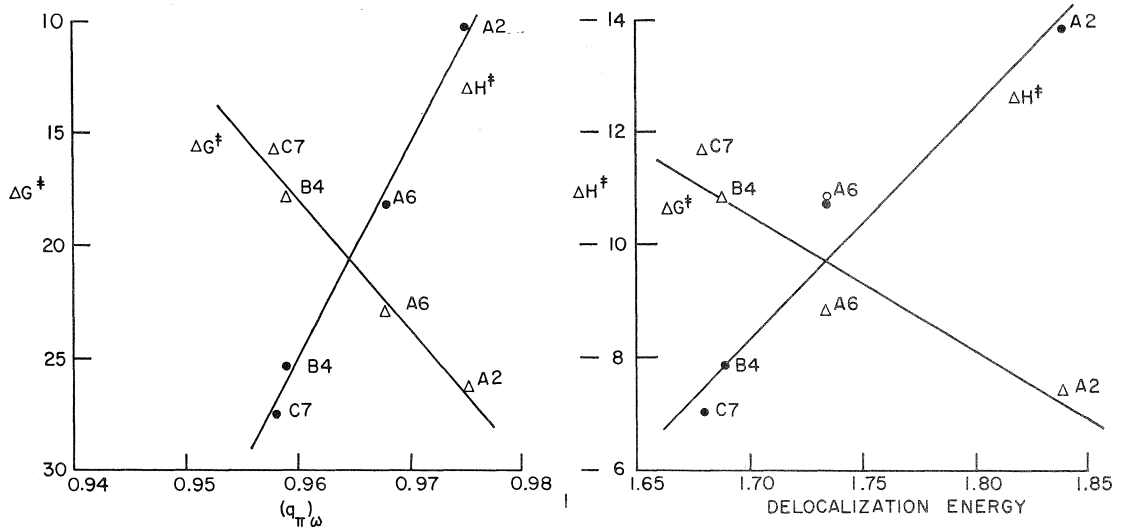


FIG. 2. Plot of activation parameters vs. MO quantities for purines and analogues (see Tables I–III). A, Purine; B, 1-methylpyrazolo(3,4-*d*)pyrimidine; C, 3*H*-v-Triazolo(4,5-*d*)pyrimidine; D, 1-methylpyrazolo(3,4-*b*)pyridine. Numerical suffixes refer to positions of protons.

TABLE VI

Kinetic results for piperidinodehalogenations*

Compound	k_2, $M^{-1}s^{-1}$ (at 25 °C)	$\Delta H^{\neq}$ (kcal·mole^{-1})	$\Delta G^{\neq}$ (kcal·mole^{-1})	$\Delta S^{\neq}$ (cal·deg^{-1}mole^{-1})
Pyrazolo(3,4-d)pyrimidines				
1-Phenyl-4-chloro	4.61×10^{-1}	7.82	17.90	33.8
1-Methyl-4-chloro	3.45×10^{-1}	7.70	18.12	34.9
1-Phenyl-4-bromo	6.14×10^{-1}	7.14	17.71	35.5
1-*p*-Nitrophenyl-4-chloro	8.68×10^{-1}	6.30	17.57	37.6
1-*p*-Tolyl-4-chloro	4.17×10^{-1}	7.87	17.94	33.8
1-Phenyl-4-chloro-6-methyl	2.16×10^{-1}	7.88	18.40	35.2
v-Triazolo(4,5-d)pyrimidine				
3-Phenyl-7-chloro	12.06	7.05	15.96	29.9
Purines†				
9-Methyl-2-chloro	1.6×10^{-5}	13.8	26.4	42.2
9-Methyl-6-chloro	5.0×10^{-3}	10.7	23.0	41.2
9-Methyl-8-chloro	4.0×10^{-3}	9.0	23.1	45.4

*Parameters of activation are calculated at 25 °C.
†From ref. 46.

TABLE VII

Regression equations linking activation parameters to molecular-orbital quantities*

			Slope	Intercept	Correlation coefficient, r
$\Delta G^{\neq}$	vs.	q_π (HMO)	223	−173	0.764
		q_π (ω)	587	−546	0.991
		Localization energy	43.1	−70.7	0.931
		Delocalization energy	61.4	−85.7	0.940
$\Delta H^{\neq}$	vs.	q_π (HMO)	136	−108	0.723
		q_π (ω)	381	−357	0.997
		Localization energy	29.1	−51.9	0.973
		Delocalization energy	41.2	−61.7	0.978

*Activation parameters refer to 1-phenyl-4-chloropyrazolo(3,4-*d*)pyrimidine, 3-phenyl-7-chloro-v-triazolo(4,5-*d*)pyrimidine, 9-methyl-2-chloro- and 9-methyl-6-chloropurine. Slopes are in kcal/electron or kcal/|β|.

densities q_π (HMO), (*b*) ω-technique π-electron densities (Table I), (*c*) localization energies for nucleophilic substitution (Table II), and (*d*) delocalization energies (11) (Table III) for piperidinodechlorination. Least-squares regression lines were drawn relating the experimental energy parameters to these quantities; slopes, intercepts, and correlation coefficients are given in Table VII, and illustrated in Fig. 2. Significant correlations are found between the free energies of activation or enthalpies of activation and the ω-technique electron densities, and also the localization and delocalization energies. The delocalization energy calculations and the ω-technique electron densities would predict the experimentally observed order of reactivities.

We conclude that simple HMO calculations can mirror reality in nucleophilic substitution reactions in purines and their analogues, providing that comparisons of the same fundamental ring system are being made.

Experimental

General

Melting points were determined using a Fisher–Johns block or a Kofler Heizbank, and are uncorrected. Proton magnetic resonance spectra were recorded for dilute solutions (2% w/w) using a Varian A-60A spectrometer; signals are expressed in parts per million downfield from

TABLE VIII

1,4-Substituted pyrazolo(3,4-*d*)pyrimidines

R_1	R_4	Melting point (°C)*	Ultraviolet absorption in methanol (nm)	
			λ_{max}	log ε
CH_3	Cl	96(98)	258	3.71
CH_3	NC_5H_{10}	120	293	4.22
C_6H_5	Cl	126(128)	243	4.51
C_6H_5	Br	135	244	4.50
C_6H_5	NC_5H_{10}	112	243, 300	4.35, 4.28
p-CH_3·C_6H_4	Cl	124	245.5	4.47
p-CH_3·C_6H_4	NC_5H_{10}	118	244, 301	4.49, 4.31
p-NO_2·C_6H_4	Cl	206	224, 263, 310	4.33, 3.93, 4.23
p-NO_2·C_6H_4	NC_5H_{10}	214	281, 327	4.36, 4.30
C_6H_5	Cl (6-CH_3)	89	245	4.50
C_6H_5	NC_5H_{10}(6-CH_3)	139	298	4.25

*Literature values are given in parentheses.

tetramethylsilane, present as internal reference; signal positions were unaffected by fivefold dilution. Trifluoroacetic acid-*d* was supplied by Merck, Sharp, and Dohme, Montreal. Ultraviolet spectroscopic measurements were made using a Beckman DK-2A ratio-recording spectrophotometer (for pK_a determinations) or a Hitachi Perkin–Elmer model 139 spectrophotometer (for kinetic measurements). A Radiometer model 4 pH meter was used for standardization of buffer solutions.

Sources of Materials

1-Methylpyrazolo(3,4-d)pyrimidine, m.p. 125°, was prepared by desulfurization of the corresponding thio-compound using Raney nickel (37).

1-Substituted-4-halopyrazolo(3,4-d)pyrimidines were prepared *via* cyclization of 1-substituted-5-amino-4-pyrazolo-carboxamides to 1-substituted-4-hydroxypyrazolo(3,4-*d*) pyrimidines followed by treatment with the appropriate phosphorus oxyhalide, using the general method developed by Robins and Cheng (37). The corresponding 1-substituted-4-piperidinopyrazolo (3,4-*d*) pyrimidines were prepared by a standard nucleophilic displacement reaction using an excess of piperidine in methanol solvent, following the general conditions used previously (37, 38). The reaction products were crystallized from methanol. Physical constants for these compounds are given in Table VIII. Proton magnetic resonance signals (in two solvents, deuteriochloroform and trifluoroacetic acid-*d*) were completely consistent with the assigned structures (39), and revealed no impurities. The intensity ratios of the various proton signals, established by electronic integration, were also in agreement with the assigned structures. It is considered that the spectroscopic evidence provides more satisfactory confirmation of structures than conventional element analyses, in view of difficulties in analysis often encountered with polyaza-heterocyclic compounds.

3-Phenyl-7-chloro-v-triazolo(4,5-d)pyrimidine was prepared as follows: 4,6-dichloro-5-aminopyrimidine (Aldrich Chemical Co.) (10 g) was dissolved in acetic acid (75 ml), aniline (6.5 g) was added, and the mixture was heated under reflux for 30 min. After cooling, water (100 ml) was added and the reaction mixture was cooled to 0°. A solution of sodium nitrite (10 g) in water (100 ml), also at 0°, was added rapidly (2 min) and the resulting precipitate was collected and twice crystallized from petroleum (b.p. 27–55°), yielding 3-phenyl-7-chloro-v-triazolo(4,5-*d*)pyrimidine (5.0 g), m.p. 117–118° (lit. 118–119° (40)) (ultraviolet absorption in methanol: λ_{max} 235.5 nm, log ε 4.51).

3-Phenyl-7-piperidino-v-triazolo(4,5-d)pyrimidine, m.p. 85° (ultraviolet absorption in methanol: λ_{max} 246, 308.5 nm; log ε 4.35, 4.28), was obtained by displacement of chlorine from the chloro precursor using piperidine in methanol (cf. 37, 38), and was crystallized from methanol.

Substituted-amino-v-triazolo(4,5-d)pyrimidines listed in Table V were provided through the courtesy of the Cancer Chemotherapy National Service Center.

3H-v-triazolo(4,5-d)pyrimidine, m.p. 174°, was prepared from 4,5-diaminopyrimidine and butyl nitrite (Albert (3)). The proton magnetic resonance spectra of the triazolopyrimidines (39) were consistent with their postulated structures.

1-Methyl-pyrazolo(3,4-b)pyridine, m.p. ca. −10°, was prepared by dediazoniation (41) of the diazonium salt from 1-methyl-3-aminopyrazolo (3,4-*b*) pyridine, prepared by treatment of 2-chloronicotinonitrile with methylhydrazine (42). Its proton magnetic resonance spectrum in trifluoroacetic acid-*d* and in deuteriochloroform was in complete accord with the assigned structure (42).

Molecular Orbital Calculations

Detailed results are available on request, as are listings of the standard HMO programs and the ω-technique program (the averaging method suggested by Ettinger (43) was used).

pK_a Determinations of (substituted amino)triazolopyrimidines

A method based on differential ultraviolet spectrophotometry was used (Lynch *et al.* (9)). Measurements were made at 20°. Although the ionization steps of the triazolopyrimidines overlap significantly, the absorption spectra of the neutral and the anionic species are virtually

identical, and differ from those of the cationic species. The spread of pK_a values obtained using buffers of varying pH was 0.20 (i.e. ± 0.10 from the mean). Full details of the ultraviolet absorption spectra of the various ionic species are available on request. The results are assembled in Table V.

Kinetics of Piperidinodehalogenations

The reactions between the various halogen derivatives of pyrazolo(3,4-*d*)pyrimidine and v-triazolo(4,5-*d*)pyrimidine with excess piperidine in methanol (99.9%; Fisher Scientific Co.) were followed by ultraviolet spectrophotometry. The increase in absorbance of the solutions in the region of maximum ultraviolet absorbance of the reaction products was followed as a function of time.

All reactions were carried out in standardized 500 ml flasks immersed in a constant temperature bath regulated to $\pm 0.02°$ using a Bronwill constant temperature circulator–controller. Samples were withdrawn at various times, rapidly diluted to an appropriate concentration and their ultraviolet absorbance was measured. Since all reactions were carried out above 25° and the cell compartment temperature was 20°, cooling accompanied the dilution procedure and reactions were arrested while the absorbance measurements were in progress. For each reaction and reaction temperature, at least ten samples were taken, and in almost all cases reactions were followed to 85% of completion. The "infinite-time" readings (ten half-lives) coincided within 3% with the values expected from the known ultraviolet absorption spectra of the reaction products.

Rate constants were determined from the concentrations of reactants and products (as deduced from their known ultraviolet absorptions) and time measurements using the appropriate second-order reaction expression (12), employing a Fortran II program. The correlation coefficients linking the time and the appropriate term involving the concentrations of the reactants and products exceeded 0.99 in all examples. Typical initial concentrations were: pyrazolopyrimidine, $2 \times 10^{-4} M$; piperidine, $1 \times 10^{-3} M$. Reactions were carried out either in duplicate at three temperatures, or as individual measurements at five temperatures ranging from 25° to 45°.

A Fortran II program was used to establish the activation energies from the linear regression equations linking the logarithms of the rate constants to the reciprocal of the Kelvin temperatures. The correlation coefficients for these regressions exceeded 0.99. The activation parameters $\Delta G^{\neq}$, $\Delta H^{\neq}$, and $\Delta S^{\neq}$ were calculated from the rate constants at 25° and the activation energies, and the values of these parameters, together with the rate constants at 25°, are listed in Table VI. Detailed results, listing the rate constants at various temperatures, are available on request.

Conclusions

Simple HMO calculations are very successful in correlating proton chemical shifts in the conjugate acids of purine and its analogues, and we are currently engaged in further synthetic work to test the scope of these correlations. Further examples of linear relationships linking π-electron densities to corrected chemical shifts have been found for the neutral molecules of several azaindenes prepared in recent years by the research groups of Potts (44) and W. W. Paudler (45) and we hope to present a complete analysis of these results in a later paper in the present series.

In nucleophilic substitution reactions of purine analogues, the correlation coefficients linking activation parameters to the ω-technique electron densities and to the delocalization energies are highly significant, suggesting, for example, that the regression equations could be used with some confidence to predict the rates of nucleophilic piperidinodehalogenations of 1-substituted-6-halopyrazolo(3,4-*d*)pyrimidines and of 3-substituted-5-halo-v-triazolo(4,5-*d*)pyrimidines.

Acknowledgments

We are grateful for support from the National Research Council of Canada through operating and equipment grants to B.M.L., and for a Scholarship to J.G.K.W., and also for grants-in-aid to this university for computing facilities. Supplies of triazolopyrimidines from the Cancer Chemotherapy National Service Center of the National Institutes of Health, Bethesda, Maryland are gratefully acknowledged. It is a pleasure to acknowledge many valuable discussions with Professor Roland K. Robins (University of Utah).

1. R. K. ROBINS. J. Med. Chem. **7**, 186 (1964).
2. R. K. ROBINS. *In* Heterocyclic compounds. Vol. 8. *Edited by* R. C. Elderfield. John Wiley and Sons, Inc. New York. 1967.
3. B. PULLMAN and A. PULLMAN. Quantum biochemistry. John Wiley and Sons, Ltd. London. 1963.
4. E. Y. SUTCLIFFE and R. K. ROBINS. J. Org. Chem. **28**, 1663 (1963).
5. B. PULLMAN. Tetrahedron Letters, 231 (1963).
6. B. PULLMAN. J. Org. Chem. **29**, 508 (1964).
7. B. M. LYNCH and H. J. M. DOU. Tetrahedron Letters, 2627 (1966).
8. B. M. LYNCH, B. C. MACDONALD, and J. G. K. WEBB. Tetrahedron, **24**, 3595 (1968).
9. B. M. LYNCH, R. K. ROBINS, and C. C. CHENG. J. Chem. Soc. 2973 (1958).
10. A. STREITWIESER. Molecular orbital theory for organic chemists. John Wiley and Sons, Inc., New York. 1961. p. 335.
11. P. BELTRAME, P. L. BELTRAME, and M. SIMONETTA. Tetrahedron, **24**, 3043 (1968).
12. (*a*) G. B. BARLIN and N. B. CHAPMAN. J. Chem. Soc. 3017 (1965); (*b*) G. B. BARLIN. J. Chem. Soc. B, 954 (1967).

13. A. STREITWIESER. Molecular orbital theory for organic chemists. John Wiley and Sons, Inc. New York. 1961. Chap. 2.
14. C. L. WILKINS and C. E. KLOPFENSTEIN. J. Chem. Educ. **43**, 10 (1966).
15. B. M. LYNCH and L. POON. Can. J. Chem. **45**, 1431 (1967).
16. A. STREITWIESER. Molecular orbital theory for organic chemists. John Wiley and Sons, Inc. New York. 1961. p. 120.
17. A. STREITWIESER. J. Am. Chem. Soc. **82**, 4123 (1960).
18. P. J. BLACK, R. D. BROWN, and M. L. HEFFERNAN. Australian J. Chem. **20**, 1305 (1967).
19. F. J. BULLOCK and O. JARDETZKY. J. Org. Chem. **29**, 1988 (1964).
20. S. MATSUURA and T. GOTO. Tetrahedron Letters, 1499 (1963).
21. J. M. READ and J. H. GOLDSTEIN. J. Am. Chem. Soc. **87**, 3440 (1965).
22. R. J. PUGMIRE, D. M. GRANT, R. K. ROBINS, and G. W. RHODES. J. Am. Chem. Soc. **87**, 2225 (1965).
23. B. M. LYNCH and J. G. K. WEBB. To be published.
24. H. H. JAFFE. Chem. Rev. **53**, 191 (1953).
25. J. CLARK and D. D. PERRIN. Quart. Rev. London, **18**, 295 (1964).
26. J. J. ELLIOTT and S. F. MASON. J. Chem. Soc. 2352 (1959).
27. H. C. LONGUET-HIGGINS. J. Chem. Phys. **18**, 275 (1950).
28. T. NAKAJIMA and A. PULLMAN. J. Chim. Phys. 793 (1958).
29. T. NAKAJIMA and B. PULLMAN. Bull. Soc. Chim. France, 1502 (1958).
30. A. ALBERT. J. Chem. Soc. B, 427 (1966).
31. D. D. PERRIN. J. Chem. Soc. 5590 (1965).
32. P. G. LYKOS and R. L. MILLER. Tetrahedron Letters, 1743 (1963).
33. B. PULLMAN. J. Chem. Soc. 1621 (1959).
34. G. B. BARLIN. J. Chem. Soc. B, 641 (1967).
35. B. M. LYNCH. To be published.
36. A. J. ROBERTSON. Studies in purine analogs. M.Sc. Thesis. St. Francis Xavier University. Antigonish, Nova Scotia. 1967.
37. R. K. ROBINS and C. C. CHENG. J. Org. Chem. **21**, 1240 (1956).
38. R. K. ROBINS. J. Am. Chem. Soc. **78**, 786 (1956).
39. B. M. LYNCH and A. J. ROBERTSON. To be published.
40. D. J. BROWN and M. N. PADDON-ROW. J. Chem. Soc. C, 1856 (1967).
41. J. F. BUNNETT and H. TAKAYAMA. J. Org. Chem. **33**, 1924 (1968).
42. B. M. LYNCH, M. A. KHAN, and J. G. K. WEBB. To be published.
43. R. ETTINGER. Tetrahedron, **20**, 1579 (1964).
44. K. T. POTTS, H. R. BURTON, T. H. CRAWFORD, and S. W. THOMAS. J. Org. Chem. **31**, 3522 (1966).
45. W. W. PAUDLER and J. E. KUDER. J. Heterocyclic Chem. **3**, 33 (1966).