**Table VII—One-Way Analysis of Variance for Each Week**

| Hours | $F$ | Probability[a] |
|---|---|---|
| 0.5 | 0.6561 | 0.5944 |
| 1.0 | 0.2979 | 0.8263 |
| 1.5 | 0.3271 | 0.8058 |
| 2.0 | 0.7519 | 0.5421 |
| 3.0 | 0.5732 | 0.6434 |
| 4.0 | 0.2914 | 0.8308 |
| 6.0 | 1.074 | 0.3967 |
| 8.0 | 1.147 | 0.3697 |
| 12.0 | 1.145 | 0.3707 |

[a] No significance.

**Table VIII—Significant Differences in Drug Elimination at Selected Times Using the Tukey Procedure**

| Hours | Brands A and B | Brands D and A | Brands D and C | Brands B and A | Brands B and C | Brands A and C |
|---|---|---|---|---|---|---|
| 0.5 | *[a] | *[a] | | *[a] | | *[a] |
| 1.0 | | *[a] | | | | |
| 1.5 | | *[a] | | | | |
| 2.0 | | *[a] | | | | |
| 3.0 | | | | | | |
| 4.0 | | *[a] | | | | |

[a] * = significant at the 0.05 level.

0.5, 1, 1.5, 2, and 4 hr and from Brands B and C at 0.5 hr. This result could be expected since only 0.5 min was required for 25% of Brands B and C to be dissolved whereas 30 min was required for 25% of Brand A to be dissolved.

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# Quantitative Structure–Activity Relationships of Purines I: Choice of Parameters and Prediction of pKa Values


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Received August 21, 1980, from the *Laboratory of Medicinal Chemistry and Biology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.* Accepted for publication September 22, 1980.

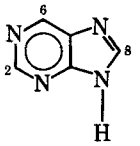


**Abstract** □ Linear free energy relationships were derived for several monosubstituted purines. The derived equations relate the pKa to the Hammett constants $\sigma_m$ and $\sigma_p$. A general linear free energy relationship was derived that permits calculation of the pKa of polysubstituted purines. The results suggest that correlation of biological data with standard parameters is feasible.



**Keyphrases** □ Purines—quantitative structure–activity relationships, prediction of pKa for polysubstituted purines from pKa of monosubstituted purines, linear free energy relationship equations □ Structure–activity relationships, quantitative—monosubstituted and polysubstituted purines, choice of parameters for prediction of pKa □ Models, mathematical—linear free energy relationship derived for calculation of pKa of polysubstituted purines


Purine analogs (I–XLV) comprise an important class of potential anticancer agents. Synthetic, unnatural purines can be administered exogenously and utilized by the intact animal to meet its requirements for nucleotides. These analogs then may produce disturbances that disrupt purine biosynthesis and interconversion or be incorporated directly into RNA and DNA, eventually producing cell death (1). These considerations led to the synthesis (2) and testing of thousands of purine derivatives for their anticancer properties, but only two purine analogs, mercaptopurine (6-MP) and its guanine analog (6-TG), have found general clinical use in the treatment of human cancer (3).




I-XLV


*0022-3549/81/0400-0425$01.00/0*

**Table I—Physicochemical Parameters**

| Substituent | $\pi$ | $\sigma_m$ | $\sigma_p$ | $\mathcal{F}$ | $\mathcal{R}$ | *MR* |
|---|---|---|---|---|---|---|
| H | 0 | 0 | 0 | 0 | 0 | 0.103 |
| $CH_3$ | 0.56 | −0.07 | −0.17 | −0.04 | −0.13 | 0.565 |
| $C_6H_5$ | 1.96 | 0.06 | −0.01 | 0.08 | −0.08 | 2.536 |
| $CF_3$ | 0.88 | 0.43 | 0.54 | 0.38 | 0.19 | 0.502 |
| CN | −0.57 | 0.56 | 0.66 | 0.51 | 0.19 | 0.633 |
| $CH_2OH$ | −1.03 | 0 | 0 | 0 | 0 | 0.719 |
| CHO | −0.65 | 0.35 | 0.42 | 0.31 | 0.13 | 0.688 |
| $COO^-$ | −4.36 | −0.10 | 0 | −0.15 | 0.13 | 0.605 |
| $CO(NHCH_3)$ | −1.27 | 0.35 | 0.36 | 0.34 | 0.05 | 1.457 |
| F | 0.14 | 0.34 | 0.06 | 0.43 | −0.34 | 0.092 |
| Cl | 0.71 | 0.37 | 0.23 | 0.41 | −0.15 | 0.602 |
| $OCH_3$ | −0.02 | 0.12 | −0.27 | 0.26 | −0.51 | 0.787 |
| $OC_2H_5$ | 0.38 | 0.10 | −0.24 | 0.22 | −0.44 | 1.247 |
| $SCH_3$ | 0.61 | 0.15 | 0 | 0.20 | −0.18 | 1.382 |
| $SC_2H_5$ | 1.07 | 0.18 | 0.03 | 0.23 | −0.18 | 1.842 |
| $SO_2CH_3$ | −1.63 | 0.60 | 0.72 | 0.54 | 0.22 | 1.349 |
| $SO_3^-$ | −4.76 | 0.05 | 0.09 | 0.03 | 0.07 | — |
| $NH_2$ | −1.23 | −0.16 | −0.66 | 0.02 | −0.68 | 0.542 |
| $NHCH_3$ | −0.47 | −0.30 | −0.84 | −0.11 | −0.74 | 1.033 |
| $N(CH_3)_2$ | 0.18 | −0.15 | −0.83 | 0.10 | −0.92 | 1.555 |
| $^+N(CH_3)_3$ | −5.96 | 0.88 | 0.82 | 0.89 | 0 | — |
| NHOH | −1.34 | −0.04 | −0.34 | 0.06 | −0.40 | 0.722 |
| $NHCONH_2$ | −1.30 | −0.03 | −0.24 | 0.04 | −0.28 | 1.372 |
| $NHCOOC_2H_5$ | 0.17 | 0.07 | −0.15 | 0.14 | −0.28 | 2.118 |

## BACKGROUND

No detailed quantitative structure–activity study of the action of the purines against cancer has been published (4), perhaps due to the difficulty of establishing a suitable set of physicochemical parameters. In quantitative structure–activity relationship studies, the usual approach is to achieve correlations with parameters that were determined in conventional aromatic (5) (*e.g.*, benzoic acid) or aliphatic (6) systems. Thus, the basic set of parameters consists of hydrophobic constants (log *P* or $\pi$), electronic parameters ($\sigma_m$, $\sigma_p$, $\mathcal{F}$, $\mathcal{R}$), and volume parameters such as Taft's steric parameter ($E_s$) (6) and molar refractivity (*MR*) (4). In treating alicyclic or heterocyclic systems, it is assumed that the Hammett approach can be extended to any rigid system that prevents direct steric interactions (7).

With purine analogs, the situation with respect to hydrophobic and electronic parameters is more complex since positions 2, 6, and 8 are not equivalent and behave differently toward chemicals (2) and enzymes (8). Thus, substituents at positions 2 and 6 are attached to a six-membered, $\pi$-electron-deficient pyrimidine ring, while those at position 8 are located on a $\pi$-electron-excessive imidazole moiety (9). Theoretically, new sets of physicochemical parameters should be determined for each of the three nucleus positions of purine. This task is not impossible but is a major undertaking.

Tomasik *et al.* (10) examined the application of linear free energy relationships to nitrogen heterocyclic systems, including purines. They reported the correlation of a small set of pKa values of monosubstituted purines in terms of $\sigma_m$. The reason this parameter was selected is unknown, and the arbitrary elimination of data points was not explained. In addition, the customary statistical criteria (*e.g.*, *F* statistics) were not given. Perrin (11) reported the prediction of the pKa values of three aminopurines from a Hammett equation for pyridines.

Hammett electronic constants are determined by measuring the pKa values of the appropriately substituted benzoic acids (5). In the present study, this procedure was reversed so that correlations were derived that relate the pKa values of monosubstituted purines to a set of well-established substituent constants. General equations then were derived that permit the prediction of the pKa values of polysubstituted purines. The predicted pKa values from these equations then were compared with experimental values. The results demonstrate the feasibility of deriving quantitative structure–activity relationships for the biological activity of purines.

## EXPERIMENTAL

The parameters employed are given in Table I and were taken from the recent literature (12). The pKa values were collected from several sources (13) and are listed with the references in Table II. The *MR* values were scaled to one-tenth of the original values (14).

Reported pKa values for purines vary extensively. Thus, for adenine (6-aminopurine, XXXIII), reported values for anion formation range from 9.30 (15) to 9.96 (16). Also, some pKa values were measured in aqueous organic solvents (13), which suppress ionization and may cause the pKa values to differ by as much as 0.5 unit (17).

In this study, purines with substituents capable of (thio)lactim–(thio)lactam tautomerization were excluded. The reason was that for the prototropy: RNH—C=X ⇌ RN=C—XH (X = S,O), there are no "true" parameters for the X–H or C=X substituents, and it was felt that use of normal aromatic values for SH and OH was not justified. This ap-

**Table II—Observed and Predicted pKa Values of Monosubstituted Purines**

| Compound | Substituent at Position 2 | 6 | 8 | pKa[a] Obs. | Calc.[b] | \|ΔpKa\| | Reference[c] |
|---|---|---|---|---|---|---|---|
| I | H | H | H | 8.93 | 9.43 (1), 8.85 (3) | 0.50, 0.08 | 22 |
| | | | | 2.52 | 2.82 (2), 1.86 (4) | 0.30, 0.66 | |
| | | | | | 8.43 (5), 8.71 (7) | 0.50, 0.22 | |
| | | | | | 2.61 (6), 2.69 (8) | 0.09, 0.17 | |
| II | $CH_3$ | — | — | 9.10 | 9.65 (1), 9.04 (7) | 0.55, 0.06 | 23 |
| | | | | ND[d] | 3.19 (2), 3.05 (8) | — — | |
| III | — | $CH_3$ | — | 9.02 | 9.19 (3), 9.06 (7) | 0.16, 0.03 | 24–26 |
| | | | | 2.60 | 2.34 (4), 3.05 (8) | 0.26, 0.45 | |
| IV | — | — | $CH_3$ | 9.37 | 8.80 (5), 9.10 (7) | 0.57, 0.27 | 24–26 |
| | | | | 2.85 | 3.08 (6), 3.16 (8) | 0.23, 0.31 | |
| V | $C_6H_5$ | — | — | 9.60 | 9.25 (1), 8.73 (7) | 0.35, 0.87 | 23 |
| | | | | ND | 2.50 (2), — | — — | |
| VI | — | — | $C_6H_5$ | 8.09 | 8.12 (5), 8.37 (7) | 0.03, 0.28 | 24 |
| | | | | 2.68 | 2.63 (6), 2.71 (8) | 0.05, 0.03 | |
| VII | — | $CH_3$ | — | 7.35 | 7.75 (3), 7.60 (7) | 0.40, 0.25 | 25, 26 |
| | | | | ND | 0.31 (4), 0.44 (8) | — — | |
| VIII | — | — | $CF_3$ | 5.10 | 6.17 (5), 6.30 (7) | 1.07, 1.20 | 25, 26 |
| | | | | 1.00 | 1.09 (6), 1.19 (8) | 0.09, 0.19 | |

**Table II**—*Continued*

| Compound | Substituent at Position 2 | 6 | 8 | pKa[a] Obs. | Calc.[b] | \|ΔpKa\| | Reference[c] |
|---|---|---|---|---|---|---|---|
| IX | — | CN | — | 6.88 | 7.50 (3), 7.35 (7) | 0.62, 0.47 | 25, 26 |
| | | | | 0.30 | −0.03 (4), −0.23 (8) | 0.33, 0.53 | |
| X | — | — | $CH_2OH$ | 8.79 | 8.43 (5), 8.71 (7) | 0.36, 0.08 | 27 |
| | | | | 2.62 | 2.61 (6), 2.69 (8) | 0.01, 0.07 | |
| XI | — | CHO | — | 8.80 | 7.99 (3), 7.85 (7) | 0.81, 0.95 | 28 |
| | | | | —[e] | 0.65 (4), 0.86 (8) | — — | |
| XII | — | — | COOH | 9.37 | 8.96 (5), 9.27 (7) | 0.41, 0.10 | 29 |
| | | | | 2.91 | 2.61 (6), 2.69 (8) | 0.30, 0.22 | |
| XIII | — | $CO(NHCH_3)$ | — | 8.90 | 8.12 (3), 7.97 (7) | 0.78, 0.93 | 25, 26 |
| | | | | 1.00 | 0.83 (4), 0.86 (8) | 0.17, 0.14 | |
| XIV | F | — | — | 8.17 | 8.40 (1), 8.59 (7) | 0.23, 0.42 | 30 |
| | | | | ND | 1.02 (2), 0.91 (8) | — — | |
| XV | Cl | — | — | 8.21 | 8.30 (1), 8.27 (7) | 0.09, 0.06 | 31 |
| | | | | 0.69 | 0.86 (2), 0.75 (8) | 0.17, 0.06 | 31 |
| XVI | — | Cl | — | 7.88 | 8.38 (3), 8.24 (7) | 0.50, 0.36 | 31 |
| | | | | 0.45 | 1.20 (4), 0.76 (8) | 0.75, 0.31 | |
| XVII | — | — | Cl | 6.02 | 6.49 (5), 6.64 (7) | 0.47, 0.62 | 31 |
| | | | | 1.77 | 1.96 (6), 2.05 (8) | 0.19, 0.28 | |
| XVIII | $OCH_3$ | — | — | 9.20 | 9.07 (1), 9.23 (7) | 0.13, 0.03 | 24 |
| | | | | 2.44 | 2.18 (2), 2.06 (8) | 0.26, 0.38 | |
| XIX | — | $OCH_3$ | — | 9.16 | 9.40 (3), 9.27 (7) | 0.24, 0.11 | 24 |
| | | | | 2.21 | 2.63 (4), 2.06 (8) | 0.42, 0.15 | |
| XX | — | — | $OCH_3$ | 7.73 | 7.80 (5), 8.04 (7) | 0.07, 0.31 | 32 |
| | | | | 3.14 | 3.36 (6), 3.43 (8) | 0.22, 0.29 | |
| XXI | $OC_2H_5$ | — | — | 9.47 | 9.13 (7), 9.17 (7) | 0.34, 0.30 | 33 |
| | | | | 2.46 | 2.29 (2), 2.16 (8) | 0.17, 0.30 | |
| XXII | — | $OC_2H_5$ | — | 9.52 | 9.34 (3), 9.20 (7) | 0.18, 0.32 | 33 |
| | | | | 2.13 | 2.54 (4), 2.16 (8) | 0.41, 0.03 | |
| XXIII | $SCH_3$ | — | — | 8.91 | 8.98 (1), 8.71 (7) | 0.07, 0.20 | 24 |
| | | | | 1.91 | 2.02 (2), 1.90 (8) | 0.11, 0.01 | |
| XXIV | — | $SCH_3$ | — | 8.75 | 8.85 (3), 8.71 (7) | 0.10, 0.04 | 32, 34 |
| | | | | 1.63 | 1.86 (4), 1.90 (8) | 0.23, 0.22 | |
| XXV | — | — | $SCH_3$ | 7.67 | 7.65 (5), 7.87 (7) | 0.02, 0.20 | 24, 32 |
| | | | | 2.95 | 2.61 (6), 2.69 (8) | 0.34, 0.26 | |
| XXVI | $SC_2H_5$ | — | — | 9.19 | 8.89 (1), 8.65 (7) | 0.30, 0.54 | 35 |
| | | | | ND | 1.87 (2), 1.74 (8) | — — | |
| XXVII | — | $SC_2H_5$ | — | 8.86 | 8.79 (3), 8.65 (7) | 0.07, 0.21 | 35 |
| | | | | 1.72 | 1.77 (4), 1.75 (8) | 0.05, 0.03 | |
| XXVIII | — | — | $SC_2H_5$ | 7.72 | 7.49 (5), 7.70 (7) | 0.23, 0.02 | 35 |
| | | | | 3.04 | 2.52 (6), 2.60 (8) | 0.52, 0.44 | |
| XXIX | — | — | $SO_2CH_3$ | 4.87 | 5.28 (5), 5.35 (7) | 0.41, 0.48 | 36 |
| | | | | 0.42 | 0.59 (6), 0.69 (8) | 0.17, 0.27 | |
| XXX | — | $SO_3H$ | — | 8.56 | 8.67 (3), 8.53 (7) | 0.11, 0.03 | 37 |
| | | | | 1.13 | 1.60 (4), 2.43 (8) | 0.47, 1.30 | |
| XXXI | — | — | $SO_3H$ | 6.93 | 8.17 (5), 8.43 (7) | 1.24, 1.50 | 37 |
| | | | | 2.22 | 2.35 (6), 2.44 (8) | 0.13, 0.22 | |
| XXXII | $NH_2$ | — | — | 9.93 | 9.92 (1), 9.98 (7) | 0.01, 0.05 | 24–26 |
| | | | | 3.80 | 3.66 (2), 3.52 (8) | 0.14, 0.28 | |
| XXXIII | — | $NH_2$ | — | 9.96 | 10.19 (3), 10.07 (7) | 0.23, 0.11 | 16 |
| | | | | 4.22 | 3.75 (4), 3.52 (8) | 0.47, 0.70 | |
| XXXIV | — | — | $NH_2$ | 9.36 | 9.28 (5), 9.60 (7) | 0.08, 0.24 | 24 |
| | | | | 4.68 | 4.46 (6), 4.51 (8) | 0.22, 0.17 | |
| XXXV | $NHCH_3$ | — | — | 10.32 | 10.35 (1), 10.32 (7) | 0.03, 0.00 | 38 |
| | | | | 4.01 | 4.40 (2), 4.25 (8) | 0.39, 0.24 | |
| XXXVI | — | $NHCH_3$ | — | 9.99 | 10.56 (3), 10.44 (7) | 0.57, 0.45 | 24, 39 |
| | | | | 4.18 | 4.26 (4), 4.25 (8) | 0.08, 0.07 | |
| XXXVII | — | — | $NHCH_3$ | 9.56 | 10.01 (5), 10.39 (7) | 0.45, 0.83 | 24 |
| | | | | 4.78 | 4.96 (6), 5.01 (8) | 0.18, 0.23 | |
| XXXVIII | $N(CH_3)_2$ | — | — | 10.22 | 9.89 (1), 10.30 (7) | 0.33, 0.08 | 24 |
| | | | | 4.02 | 3.61 (2), 3.42 (8) | 0.41, 0.55 | |
| XXXIX | — | $N(CH_3)_2$ | — | 10.50 | 10.54 (3), 10.42 (7) | 0.04, 0.08 | 24, 30, 39 |
| | | | | 3.87 | 4.23 (4), 3.47 (8) | 0.36, 0.40 | |
| XL | — | — | $N(CH_3)_2$ | 9.73 | 9.22 (5), 9.55 (7) | 0.51, 0.18 | 24 |
| | | | | 4.80 | 4.93 (6), 4.98 (8) | 0.13, 0.18 | |
| XLI | — | $^+N(CH_3)_3$ | — | 6.85 | 7.18 (3), 7.02 (7) | 0.33, 0.17 | 40 |
| | | | | ND | −0.49 (4), −1.90 (8) | — — | |
| XLII | — | — | $^+N(CH_3)_3$ | 4.88 | 3.81 (5), 3.79 (7) | 1.07, 1.09 | 40 |
| | | | | ND | 0.31 (6), 0.42 (8) | — — | |
| XLIII | — | NHOH | — | 9.83 | 9.54 (3), 9.41 (7) | 0.29, 0.42 | 25, 26 |
| | | | | 3.80 | 2.83 (4), 2.89 (8) | 0.97, 0.91 | |
| XLIV | — | $NHCONH_2$ | — | 9.95 | 9.34 (3), 9.20 (7) | 0.61, 0.75 | 25, 26 |
| | | | | 2.35 | 2.54 (4), 2.84 (8) | 0.19, 0.49 | |
| XLV | — | $NHCOOC_2H_5$ | — | 9.63 | 9.15 (3), 9.02 (7) | 0.48, 0.61 | 25, 26 |
| | | | | 2.40 | 2.29 (4), 2.32 (8) | 0.11, 0.08 | |

[a] Underlined values refer to cations. [b] The equation number is in parentheses. [c] The references refer to the source of the observed values. [d] No data available. [e] Unstable in acid (*cf.*, footnote 1 in text).

proach may narrow the conclusions since many purines possess these substituents. In the case of mercaptopurine and its guanine analog, it was shown that one active form is the 6-methylthio derivative obtained by *in vivo* *S*-methylation (3). Although these methylthio compounds are incapable of thiolactam–thiolactim tautomerization, they are active themselves and were included in this study.

Aminopurines exist mainly in the $NH_2$ form rather than as the imino (NH=) tautomer (18). For this reason, use of established parameters for

**Table III—Equations Correlating pKa Values of Purines with Physicochemical Constants**

| Equation | $n$ | $r$ | $s$ | $F_{1,x}$[a] | Equation | $n$ | $r$ | $s$ | $F_{1,x}$[a] |
|---|---|---|---|---|---|---|---|---|---|
| 2-Monosubstituted purines (anions) | | | | | 8-Monosubstituted purines (cations) | | | | |
| 1. pKa = 9.43(±0.22) − 3.05(±1.10)$\sigma_m$ | 12 | 0.891 | 0.328 | 38.40 | 6. pKa = 2.61(±0.14) − 2.80(±0.32)$\sigma_p$ | 15 | 0.982 | 0.246 | 353.11 |
| 2-Monosubstituted purines (cations) | | | | | Combined equation (anions) | | | | |
| 2. pKa = 2.82(±0.27) − 5.28(±1.33)$\sigma_m$ | 8 | 0.970 | 0.308 | 94.25 | 7. pKa = 8.71(±0.17) − 1.92(±0.79)$\sigma_{p(2)}$ − 2.06(±0.54)$\sigma_{p(6)}$ − 5.59(±0.86)$\sigma_{m(8)}$ | 45 | 0.931 | 0.535 | 89.07 |
| 6-Monosubstituted purines (anions) | | | | | | | | | |
| 3. pKa = 8.85(±0.22) − 2.04(±0.47)$\sigma_p$ | 19 | 0.910 | 0.447 | 81.79 | | | | | |
| 6-Monosubstituted purines (cations) | | | | | Combined equation (cations) | | | | |
| 4. pKa = 1.86(±0.27) − 2.86(±0.65)$\sigma_p$ | 16 | 0.930 | 0.477 | 88.88 | 8. pKa = 2.69(±0.14) − 5.23(±1.48)$\sigma_{m(2)}$ − 5.21(±0.96)$\sigma_{m(6)}$ − 2.77(±0.51)$\sigma_{p(8)}$ | 37 | 0.950 | 0.414 | 100.74 |
| 8-Monosubstituted purines (anions) | | | | | | | | | |
| 5. pKa = 8.43(±0.37) − 5.26(±1.14)$\sigma_m$ | 16 | 0.935 | 0.634 | 97.68 | | | | | |

[a] $p < 0.001$.

**Table IV—Correlation Matrix for Equations 1–6** [a]

| Parameter | $\pi$ for Position | | | $\sigma_m$ for Position | | | $\sigma_p$ for Position | | | $\mathcal{F}$ for Position | | | $\mathcal{R}$ for Position | | | $MR$ for Position | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 6 | 8 | 2 | 6 | 8 | 2 | 6 | 8 | 2 | 6 | 8 | 2 | 6 | 8 | 2 | 6 | 8 |
| $\pi$ | | 1.0 | | | | | | | | | | | | | | | | |
| $\sigma_m$ | 0.22 | 0.12 | 0.08 | | 1.0 | | | | | | | | | | | | | |
| | 0.55 | 0.01 | 0.03 | | | | | | | | | | | | | | | |
| $\sigma_p$ | 0.38 | 0.09 | 0.10 | 0.75 | 0.87 | 0.80 | | 1.0 | | | | | | | | | | |
| | 0.44 | 0.00 | 0.11 | 0.82 | 0.84 | 0.78 | | | | | | | | | | | | |
| $\mathcal{F}$ | 0.07 | 0.12 | 0.05 | 0.85 | 0.93 | 0.91 | 0.36 | 0.64 | 0.53 | | 1.0 | | | | | | | |
| | 0.49 | 0.06 | 0.05 | 0.86 | 0.88 | 0.85 | 0.47 | 0.52 | 0.40 | | | | | | | | | |
| $\mathcal{R}$ | 0.38 | 0.04 | 0.07 | 0.32 | 0.49 | 0.29 | 0.82 | 0.83 | 0.73 | 0.04 | 0.23 | 0.07 | | 1.0 | | | | |
| | 0.25 | 0.04 | 0.37 | 0.50 | 0.51 | 0.36 | 0.88 | 0.88 | 0.82 | 0.15 | 0.18 | 0.06 | | | | | | |
| $MR$ | 0.43 | 0.00 | 0.06 | 0.01 | 0.03 | 0.07 | 0.00 | 0.00 | 0.01 | 0.01 | 0.10 | 0.11 | 0.00 | 0.06 | 0.01 | | 1.0 | |
| | 0.11 | 0.23 | 0.73 | 0.02 | 0.00 | 0.00 | 0.14 | 0.02 | 0.00 | 0.01 | 0.03 | 0.02 | 0.27 | 0.08 | 0.02 | | | |

[a] Underlined values refer to cations.

**Table V—Development of Equations 7 and 8**

| Intercept | $\sigma_{p(2)}$ | $\sigma_{p(6)}$ | $\sigma_{m(8)}$ | $r$ | $s$ | $F_{1,x}$ | $p$ | Equation |
|---|---|---|---|---|---|---|---|---|
| | | | | Equation 7 | | | | |
| 8.46 | −2.25 | — | — | 0.329 | 1.352 | $F_{1,44} = 5.22$ | <0.05 | *a* |
| 8.57 | — | −2.08 | — | 0.440 | 1.286 | $F_{1,44} = 10.30$ | <0.005 | *b* |
| 8.86 | — | — | −5.78 | 0.776 | 0.903 | $F_{1,44} = 65.15$ | <0.001 | *c* |
| 8.83 | — | −2.04 | −5.74 | 0.888 | 0.666 | $F_{1,43}$[a] = 36.93 | <0.001 | *d* |
| 8.71 | −1.92 | −2.06 | −5.59 | 0.931 | 0.535 | $F_{1,42}$[b] = 24.21 | <0.001 | *e* |
| | | | | Equation 8 | | | | |
| 2.60 | −5.19 | — | — | 0.388 | 1.180 | $F_{1,36} = 6.22$ | <0.025 | *a* |
| 2.76 | — | −5.32 | — | 0.614 | 1.011 | $F_{1,36} = 21.23$ | <0.001 | *b* |
| 2.50 | — | — | −2.85 | 0.625 | 1.000 | $F_{1,36} = 22.44$ | <0.001 | *c* |
| 2.67 | — | −5.18 | −2.78 | 0.865 | 0.652 | $F_{1,35}$[a] = 48.44 | <0.001 | *d* |
| 2.69 | −5.23 | −5.21 | −2.77 | 0.950 | 0.414 | $F_{1,34}$[b] = 51.25 | <0.001 | *e* |

[a] This $F$ value is obtained by comparison with Eq. *c*. [b] This $F$ value is obtained by comparison with Eq. *d*.

the amino group was justified. Purine-6-carboxaldehyde was not included in the cation equations since this compound is sensitive to acids and may decompose during pKa determination[1].

In the pKa correlations, all pKa values found in the literature were included without any attempt to eliminate "outliers," *i.e.*, to reject suspect values. The broader scope of the conclusions more than justified the somewhat lower correlation coefficients that were obtained as a consequence.

## RESULTS AND DISCUSSION

The data in Tables I and II were used to develop Eqs. 1–6 (Table III). The observed and predicted pKa values are given in Table II with the cation values being underlined. The correlation matrix for Eqs. 1–6 is given in Table IV.

The negative coefficient of the electronic terms in Eqs. 1–6 reflects the well-known relationship that electron-withdrawing groups at positions 2, 6, and 8 of purines increase the acidity. The absence of steric or hydrophobic terms indicates that ionization of these compounds is due mainly to electronic effects.

Analysis of Eq. 3 reveals that 14 of the 19 compounds undergo anion formation only at the imidazole moiety (I, II, VII, IX, XI, XVI, XIX, XXII, XXIV, XXVII, XXXIII, XXXVI, XXXIX, and XLI) and that five derivatives (XIII, XXX, and XLIII–XLV) may undergo ionization elsewhere. If the correlation is restricted to compounds that undergo anion formation exclusively at the imidazole ring, the equation improves:

$$\text{pKa} = 8.70(\pm 0.22) - 2.04(\pm 0.44)\sigma_p \qquad \text{(Eq. 3}a\text{)}$$

$$n = 14 \quad r = 0.946 \quad s = 0.385 \quad F_{1,13} = 101.26 \quad (p < 0.001)$$

Although Eq. 3*a* has better statistics, Eq. 3 is preferred because of its broader applicability.

Equations 5 and 7 have rather large standard deviations, which may reflect some uncertainties in the pKa measurements.

Because the correlations appeared to be statistically sound for the pKa values of monosubstituted purines, it seemed desirable to combine all of the data into one equation so that the pKa values of the disubstituted and trisubstituted purines could be computed. The procedure used was as follows. Monosubstituted purines were assigned the appropriate substituent constant at the substituted position only, and zeros were assigned to the other positions. With the six parameters ($\pi$, $\sigma_m$, $\sigma_p$, $\mathcal{F}$, $\mathcal{R}$, and $MR$) for the three purine positions, 18 variables are obtained. By

[1] A. Giner-Sorolla, Sloan-Kettering Institute for Cancer Research, Rye, N.Y., personal communication.

**Table VI—Observed and Predicted pKa Values of Disubstituted and Trisubstituted Purines [a]**

| Compound | Substituent at Position 2 | 6 | 8 | pKa [b] Obs. | Calc. | \|ΔpKa\| | Reference [c] |
|---|---|---|---|---|---|---|---|
| I | $CH_3$ | $CH_3$ | $CH_3$ | 9.90 | 9.78 | 0.12 | 41 |
| | | | | 4.49 [b] | 3.89 | 0.60 | |
| II | $CH_3$ | $CH_3$ | $OC_2H_5$ | 8.74 | 8.83 | 0.09 | 42 |
| | | | | 4.71 | 4.08 | 0.63 | |
| III | $CH_3$ | $NHCH_3$ | — | ND [d] | (10.76) | — | 39 |
| | | | | 5.08 | 4.62 | 0.46 | |
| IV | F | $NH_2$ | — | 9.58 | 9.95 | 0.37 | 30 |
| | | | | ND | (1.74) | — | |
| V | F | $N(CH_3)_2$ | — | 9.97 | 10.30 | 0.33 | 30 |
| | | | | ND | (1.69) | — | |
| VI | Cl | Cl | — | 7.06 | 7.79 | 0.73 | 31 |
| | | | | −1.16 | −1.18 | 0.02 | |
| VII | Cl | Cl | Cl | 3.96 | 5.72 | 1.76 | 31 |
| | | | | −3.10 | −1.81 | 1.29 | |
| VIII | $NH_2$ | — | $C_6H_5$ | 9.20 | 9.64 | 0.44 | 35 |
| | | | | 3.98 | 3.55 | 0.43 | |
| IX | $NH_2$ | $NH_2$ | — | 10.77 | 11.34 | 0.57 | 24–26, 39 |
| | | | | 5.09 | 4.36 | 0.73 | |
| X | $NH_2$ | $NH_2$ | $NH_2$ | 10.79 | 12.23 | 1.44 | 24 |
| | | | | 6.23 | 6.18 | 0.05 | |
| XI | $NH_2$ | $CF_3$ | — | 8.87 | 8.87 | 0.00 | 25, 26 |
| | | | | 1.85 | 1.28 | 0.57 | |
| XII | $NH_2$ | — | $CF_3$ | 6.14 | 7.57 | 1.43 | 36 |
| | | | | 2.59 | 2.03 | 0.56 | |
| XIII | $NH_2$ | $CF_3$ | $CF_3$ | 5.02 | 6.46 | 1.44 | 25, 26 |
| | | | | 0.30 | −0.21 | 0.51 | |
| XIV | $NH_2$ | $NH_2$ | $CF_3$ | 7.55 | 8.93 | 1.38 | 25, 26 |
| | | | | 3.68 | 2.86 | 0.82 | |
| XV | $NH_2$ | — | $SCH_3$ | 8.48 | 9.14 | 0.66 | 36 |
| | | | | 4.40 | 3.52 | 0.88 | |
| XVI | $NH_2$ | — | $SO_2CH_3$ | 5.61 | 6.62 | 1.01 | 36 |
| | | | | 2.08 | 1.53 | 0.55 | |
| XVII | $N(CH_3)_2$ | $CH_3$ | — | 10.32 | 10.65 | 0.33 | 35 |
| | | | | 4.14 | 3.83 | 0.31 | |
| XVIII | $N(CH_3)_2$ | $C_2H_5$ | — | 10.73 | 10.61 | 0.12 | 35 |
| | | | | 4.42 | 3.83 | 0.59 | |
| XIX | $N(CH_3)_2$ | $CH_3$ | $CH_3$ | 10.83 | 11.05 | 0.22 | 35 |
| | | | | 4.92 | 4.31 | 0.61 | |
| XX | $N(CH_3)_2$ | $CH_3$ | $C_2H_5$ | 11.11 | 11.05 | 0.06 | 35 |
| | | | | 5.05 | 4.25 | 0.80 | |
| XXI | $N(CH_3)_2$ | $C_2H_5$ | $CH_3$ | ND | (11.00) | — | 35 |
| | | | | 4.90 | 4.31 | 0.59 | |
| XXII | $SCH_3$ | — | $CH_3$ | 9.58 | 9.10 | 0.48 | 41 |
| | | | | 2.83 | 2.37 | 0.46 | |
| XXIII | $SCH_3$ | $C_2H_5$ | — | 9.35 | 9.02 | 0.33 | 35 |
| | | | | 2.50 | 2.27 | 0.23 | |
| XXIV | $SCH_3$ | — | $C_2H_5$ | 9.75 | 9.10 | 0.65 | 35 |
| | | | | 2.80 | 2.32 | 0.48 | |
| XXV | $SCH_3$ | $CH_3$ | $CH_3$ | 9.70 | 9.45 | 0.25 | 41, 43 |
| | | | | 3.04 | 2.74 | 0.30 | |
| XXVI | $SCH_3$ | $CH_3$ | $C_2H_5$ | 9.75 | 9.45 | 0.30 | 35 |
| | | | | 3.08 | 2.68 | 0.40 | |
| XXVII | $SCH_3$ | $C_2H_5$ | $CH_3$ | 9.52 | 9.41 | 0.11 | 35 |
| | | | | 3.05 | 2.74 | 0.31 | |
| XXVIII | $SCH_3$ | $CH_3$ | $CF_3$ | 5.67 | 6.65 | 0.98 | 43 |
| | | | | 1.25 | 0.77 | 0.48 | |
| XXIX | $SCH_3$ | — | $SCH_3$ | 7.73 | 7.87 | 0.14 | 36 |
| | | | | 2.19 | 1.90 | 0.29 | |
| XXX | $SCH_3$ | $CH_3$ | $SCH_3$ | 7.94 | 8.22 | 0.28 | 41 |
| | | | | 2.74 | 2.27 | 0.47 | |
| XXXI | $SC_2H_5$ | $CH_3$ | — | 9.43 | 9.00 | 0.43 | 35 |
| | | | | 2.67 | 2.11 | 0.56 | |
| XXXII | $SC_2H_5$ | — | $CH_3$ | 9.50 | 9.04 | 0.46 | 35 |
| | | | | 2.71 | 2.22 | 0.49 | |
| XXXIII | $SC_2H_5$ | — | $C_2H_5$ | 9.62 | 9.04 | 0.58 | 35 |
| | | | | 2.81 | 2.16 | 0.65 | |

[a] Calculated from Eqs. 7 and 8. [b] Underlined values refer to cations. [c] The references refer to the source of the observed values. [d] No data available.

employing all combinations of these parameters, it is possible to generate 262,143 equations. However, this number of equations can be reduced by generating only equations that are chemically and statistically meaningful. In this instance, only about seven equations have one to seven independent variables. In this study, a stepwise regression procedure (19) generated the desired equations having the best statistics in a minimum of computer time. Equations 7 and 8 are the best combined pKa correlations of the purines; their development is given in Table V.

Equation 7 indicates that the main contribution to the anionic pKa is from the $\sigma_m$ variable at position 8, *i.e.*, at the imidazole moiety where most anion formation occurs.

According to Eq. 8, the main contribution to the cationic pKa is from substituents at positions 2 and 6, *i.e.*, on the pyrimidine ring. This finding does not imply that cation formation necessarily takes place at the pyrimidine moiety. The purine skeleton possesses four heterocyclic nitrogen atoms, each of which is prone to protonation, and little is known about the exact site of protonation of most of these compounds. Furthermore, exocyclic groups such as NHR, SR, and OR may be protonated to some extent.

The observed and predicted pKa values for monosubstituted purines calculated from Eqs. 7 and 8 are given in Table II. It is not surprising that Eq. 7 describes the electronic effects at position 2 in terms of $\sigma_p$ while

Eq. 1 employs $\sigma_m$. Table IV shows a strong intercorrelation between $\sigma_m$ and $\sigma_p$ (arc cos 0.75 = 41°) (20). Similarly, in Eq. 8, $\sigma_m$ replaces $\sigma_p$ of Eq. 4 in the treatment of electronic effects at position 6 (arc cos 0.84 = 33°). Another interesting fact derived from the correlation matrix is considerable autocorrelation of $\pi$, especially at position 2, with $\sigma_m$ and $\sigma_p$. A similar phenomenon was observed for some benzene derivatives (21).

The pKa values of 33 disubstituted and trisubstituted purines were calculated from Eqs. 7 and 8 and compared with measured values reported in the literature (Table VI). The agreement between observed and predicted values is reasonable considering the variation in experimental methods.

Studies are in progress on the correlation of the antitumor potency and toxicity of polysubstituted purines with the structural parameters described in this paper.

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# Clofibrate Microcapsules II: Effect of Wall Thickness on Release Characteristics


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**Abstract** □ The effect of wall thickness on the release characteristics of clofibrate from microcapsules prepared in gelatin–sodium sulfate was investigated. The wall thickness, calculated by recovering the wall material from the microcapsules and using the relationship between two concentric spheres, was related to the surface area of the droplets being encapsulated. Thinner walled microcapsules gave faster release and showed greater deviation from zero-order kinetics but followed the square root of time plots. Microcapsules having thicker walls approximated zero-order release but deviated from the square root of time plots. A theoretical model was developed to explain the release characteristics of the microcapsules. A linear correlation was found between the wall thickness and the *in vitro* $t_{50\%}$ release time.



**Keyphrases** □ Clofibrate—microcapsules, effect of wall thickness on release characteristics □ Microcapsules—clofibrate, effect of wall thickness on release characteristics □ Hypocholesterolemic agents—clofibrate, effect of microcapsule wall thickness on release characteristics


A recent investigation (1) reported the microencapsulation of clofibrate USP, a liquid hypocholesterolemic agent. Prepared by simple coacervation in gelatin–sodium sulfate, the microcapsules were recovered as discrete




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