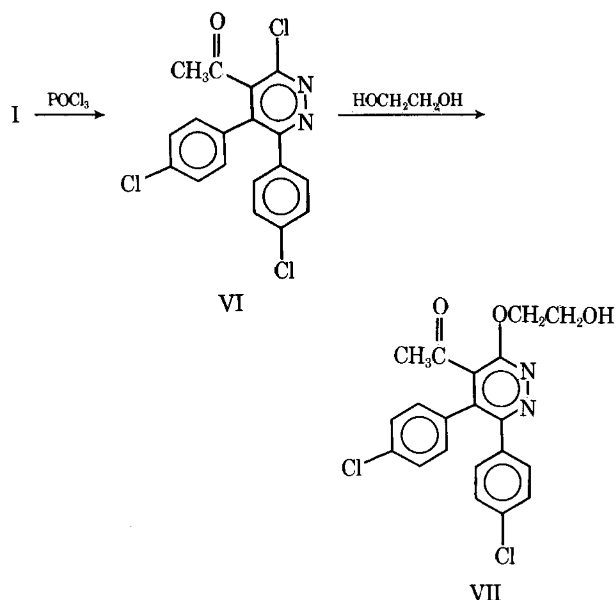

Scheme II

purification of I and probably is entrapped in the crystalline product. This solvent entrapment by the pyridazinones is observed frequently. As with III and IV, the synthetic and isolated samples of V were identical in the two TLC systems and had identical NMR and IR spectra. Of particular note was the downfield shift of the methylene protons between the two pyridazinone rings (Table I).

This initial investigation did not result in the isolation of any product of the *O*-alkylation of I by ethylene carbonate. A synthesis for the *O*-alkylated compound, VII, was developed following the route shown in Scheme II. However, this compound was not resolved from II by the chromatographic systems outlined in Fig. 1. Using an authentic sample of VII, a TLC and HPLC system was developed that separated VII from I and II. With a mobile phase of hexane–methylene chloride–2-propanol (16:3:1), the capacity factors for II and VII were 3.7 and 4.4, respectively. This system indicated that VII was present in II at a level of 0.1%. This conclusion was supported by the spiking of the II sample with synthetic VII.

The biological activities of III–V and VII were compared with that of II. As shown in Table III, both IV and V lacked antihypertensive activity and were not acutely toxic. The formate ester, III, had antihypertensive activity similar to that of II. This activity also was observed for other aliphatic and aromatic esters of II (4). Compound VII had slight, although not statistically significant, antihypertensive activity. In addition, VII appeared to be more toxic than II.

REFERENCES

(1) J. C. Reepmeyer and R. D. Kirchhoefer, *J. Pharm. Sci.*, **68**, 1167 (1979).

(2) R. D. Kirchhoefer, J. C. Reepmeyer, and W. E. Juhl, *ibid.*, **69**, 550 (1980).

(3) R. Buchman, J. A. Scozzie, Z. S. Ariyan, R. D. Heilman, D. J. Rippin, W. J. Pyne, L. J. Powers, and R. J. Matthews, *J. Med. Chem.*, **23**, 1398 (1980).

(4) S. W. Fogt, J. A. Scozzie, R. D. Heilman, and L. J. Powers, *ibid.*, **23**, 1445 (1980).

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Comparative Bioavailability of Three Commercial Acetaminophen Tablets

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Received June 4, 1979, from the *Division of Pharmaceutics, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Brooklyn, NY 11201.* Accepted for publication August 5, 1980.

Abstract ☐ Four different acetaminophen products (three tablets and one liquid) were evaluated for their *in vitro* properties and *in vivo* comparative bioavailability. The *in vivo* properties included assay, hardness, thickness, friability, weight variation, content uniformity, disintegration, and dissolution. A statistically significant variation was observed in friability, disintegration, and dissolution. The dissolution rates were determined in 0.1 *N* HCl under sink conditions, and the $T_{50\%}$ value for Brand A was 50 min while the values for Brands B and C were 1 min. The *in vivo* evaluation was completed in four subjects with a urinary excretion experiment using a crossover design. The calculated elimination half-lives were 4.12, 2.77, 3.14, and 2 hr for Brands A, B, and C and the standard, respectively. The relative bioavailabilities (with respect to solution) were 82, 87, and 92% for Brands A, B, and C, respectively. The mean amount excreted with Brand A was less than the reference at all time points, although it was not significant. Comparison of the *in vitro* and *in vivo* data for the three tablets indicated that the rate and amount of acetaminophen excreted may be related to the dissolution rate.

Keyphrases ☐ Acetaminophen—bioavailability, *in vitro* and *in vivo* properties of three commercial tablets compared ☐ Bioavailability—acetaminophen, *in vitro* and *in vivo* properties of three commercial tablets compared ☐ Tablets—acetaminophen, bioavailability, *in vitro* and *in vivo* properties of three commercial tablets compared

The popularity of acetaminophen, a nonsalicylate and analgesic/antipyretic, as an aspirin substitute has increased to the point that the drug is now available from many sources in several dosage forms in the United States. However, of the more than 30 manufacturers and distributors of acetaminophen tablets, only four companies provided bioavailability data in a recent survey (1, 2).

Mattok *et al.* (3, 4) and McGilveray *et al.* (5) studied the physiological availability of different commercial dosage forms and found no significant differences among the formulations in blood level or urinary excretion parameters. However, other investigators (6, 7) reported differences in blood and plasma levels of acetaminophen after administration of various formulations. Therefore, this study evaluated three commercially available[1] acetaminophen products (A, B, and C) and a reference solution[2] (D), utilizing urinary excretion data, and correlated these data with several physicochemical and manufacturing parameters.

[1] Brand A was acetaminophen tablets USP, lot 027791, Interstate Drug Exchange, Plainview, NY 11805; Brand B was Tylenol tablets, lot 2751, McNeil Laboratories, Fort Washington, PA 19034; and Brand C was Datril tablets, lot DA853401, MNFL, Bristol Myers Co., New York, NY 10022.

[2] Brand D was acetaminophen powder, McNeil Laboratories, Fort Washington, PA 19034.

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Table I—Manufacturing Parameters of the Brands of Acetaminophen Tablets

Parameter	Brand A	Brand B	Brand C
Weight, g			
Mean	397.60	434.2	479.1
SD	8.52	4.36	6.60
CV	2.00	1.00	1.37
Hardness, kg			
Mean	3.1	6.7	9.3
SD	0.43	0.78	0.82
Thickness, mm			
Mean	5.00	4.9	5.00
SD	0.08	0.03	0.03
Friability	—	1.93	1.01
Content uniformity, %			
Mean	99.36	102.00	99.00
SD	3.00	1.99	3.05

EXPERIMENTAL

***In Vivo* Study**—Four healthy volunteers, one female and three males, 24–35 years old, were the subjects. The treatments consisted of three brands of acetaminophen tablets acquired by normal channels of drug distribution. A 650-mg dose of acetaminophen (two 325-mg tablets) was administered to each subject. The control was 650 mg of pure acetaminophen in 100 ml of water acidified with 0.1 *N* HCl in which the acetaminophen was completely dissolved.

The subjects received each of the four treatments (A–D) in a completely randomized Latin square design. A 1-week period was employed between each dosage interval to allow for complete drug elimination. Urine voids were collected predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hr postadministration. Urine samples were collected in 500-ml plastic graduated cylinders. The volume was measured and recorded, and an aliquot was frozen until it was assayed.

Spectrophotometric Determination of Acetaminophen in Urine—The method used for the determination of acetaminophen in urine was a modified form of the spectrophotometric assay developed by Plakogiannis and Saad (8, 9) for plasma acetaminophen. To 2 ml of urine in a screw-capped, 20-ml centrifuge tube was added 4 ml of 3 *N* HCl, and the sample was diluted with distilled water to 10 ml. The tube was centrifuged at 5000 rpm for 1 hr, and the clear supernate was treated with ether (4 ml) two to three times. The *p*-aminophenol then was extracted from ether with 10 ml of 1 *N* HCl. Five milliliters of 5% vanillin in isopropanol was added to 2 ml of the acidic extract; the resulting yellow color was measured at 395 nm. Concentrations were determined from a previously constructed standard curve.

***In Vitro* Assessment of Dosage Forms**—Weight variation tests were performed on 20 tablets of each brand according to the USP XIX method (10) for determining permissible limits of weight variation. Although hardness variation is not specified for acetaminophen tablets, the ability of the tablets to withstand physical abuse was determined[3]. Their variation in thickness and their friability[4] also were determined.

Disintegration times were determined using an apparatus conforming to the USP XIX (11) specifications and six tablets of each brand. The disintegration times were determined in purified water, simulated gastric fluid[5] without enzymes (pH 1.2), and simulated intestinal fluid[6] (pH 7.5) maintained at 37 ± 1° as the immersion fluid.

Dissolution rates were determined in 0.1 *N* HCl in a basket stirrer assembly meeting the USP XIX (11) requirements and equipped with Pyrex beakers. The stirrer speed was 85 rpm. The whole assembly was immersed in a suitable water bath that kept the water constantly in motion and held the temperature at 37 ± 0.5°. At time zero, one tablet was introduced into the dissolution medium; at the same time, 5.0 ml of the dissolution medium was withdrawn and replaced with 5 ml of the appropriate fluid.

[3] Stokes–Monsanto tester, Erweka Chemical and Pharmaceutical Industry Co., New York, N.Y.
[4] Roche friabilator, Erweka Chemical and Pharmaceutical Industry Co., New York, N.Y.
[5] The simulated gastric fluid was prepared by adding 2 g of sodium chloride to 7.0 ml of hydrochloric acid and bringing the solution to 1000 ml with distilled water.
[6] The simulated intestinal fluid was made by dissolving monobasic potassium phosphate (6.8 g) in 250 ml of water. Then 190 ml of 0.2 *N* NaOH and 400 ml of water were added. The resulting solution was adjusted with 0.2 *N* NaOH to pH 7.5 ± 1 and diluted with water to 1000 ml.

Table II—Disintegration Data [a] of the Three Brands of Acetaminophen Tablets

Brand	Water	Gastric Fluid	Intestinal Fluid
A	22	>30	5.1
B	0.28	0.33	3.0
C	2.9	1.9	0.28

[a] Each value is expressed in minutes and is the average of six tablets.

Table III—Percentage of Drug Liberated at Different Dissolution Times [a]

Brand	$T_{25\%}$	$T_{50\%}$	$T_{75\%}$
A	30.0 ± 3.27	50 ± 0.00	85 ± 6.8
B	0.5 ± 0.00	1 ± 1.73	3 ± 0.00
C	0.5 ± 1.35	1 ± 2.70	3 ± 2.65

[a] The values are expressed in minutes and include the standard deviation.

The samples were filtered and placed in a 30-ml bottle, which was immersed in ice to slow hydrolysis. In the same manner, samples were taken every 0.5 min for 7 min for Brands B and C. For Brand A, the samples were analyzed every 5 min for up to 90 min. The reproducibility of dissolution was confirmed with three sets of tablets from each lot, and all analyses were performed in triplicate. The samples were analyzed spectrophotometrically.

Tablet Assay—The analysis was done according to the method of Plakogiannis and Saad (8, 9). Twenty acetaminophen tablets were weighed and pulverized, and an accurate weight of the powder sample corresponding to 200 mg of acetaminophen, based on the label claim, was transferred to a suitable container. Then 50 ml of 4 *N* HCl was added. The solution was shaken mechanically for 20 min, filtered into a 100-ml volumetric flask, and diluted to volume with 4 *N* HCl. The flask contents were mixed well, and 1 ml was transferred to a test tube. Then 5 ml of 4 *N* HCl was added, and the tube was heated for 1 hr in a water bath. The tubes were allowed to cool, and the contents were transferred quantitatively to a 50-ml volumetric flask. The amount of *p*-aminophenol was calculated from a calibration curve and multiplied by a factor of 1.385.

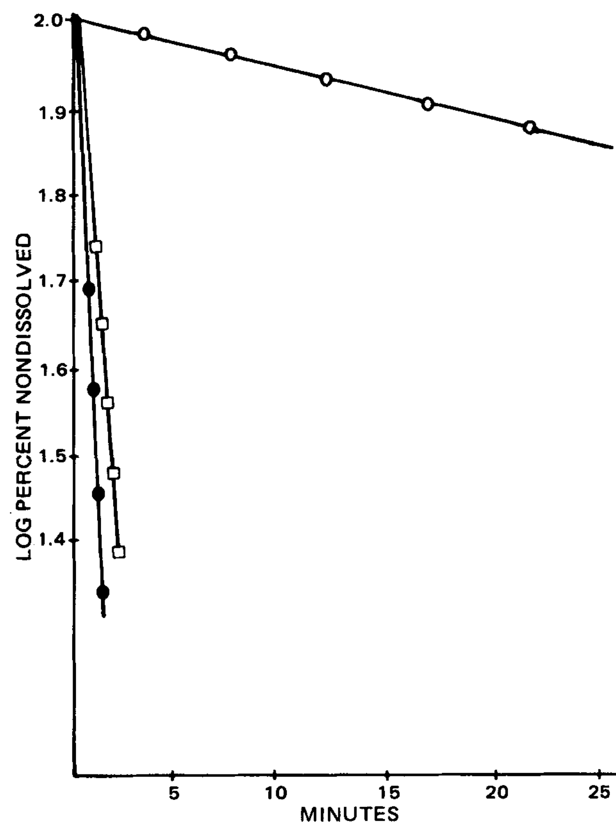

Figure 1—*In vitro dissolution of three formulations of acetaminophen tablets. Key:* ○, *Brand A;* ●, *Brand B; and* □, *Brand C.*

Table IV—Cumulative Percent Urinary Excretion of Four Brands of Acetaminophen as a Function of Time [a]

Hours	Brand A	Brand B	Brand C	Brand D
0.5	3.69 (1.2)	11.24 (2.60)	13.38 (1.33)	17.71 (3.03)
1.0	16.67 (3.92)	27.76 (2.88)	23.19 (2.67)	28.65 (3.32)
1.5	25.48 (6.31)	32.25 (4.66)	32.77 (2.96)	39.26 (3.33)
2.0	36.41 (7.47)	41.42 (5.10)	40.61 (3.32)	48.36 (3.85)
3.0	40.59 (6.95)	55.26 (6.93)	54.34 (4.65)	62.65 (3.78)
4.0	52.77 (4.07)	65.80 (7.18)	63.30 (5.30)	72.53 (3.84)
6.0	81.46 (5.45)	78.13 (7.20)	77.38 (5.15)	85.34 (2.89)
8.0	91.50 (4.99)	87.30 (4.47)	85.65 (4.27)	92.30 (2.88)
12.0	96.65 (1.81)	92.85 (2.19)	94.23 (3.31)	97.07 (1.07)

[a] Each value is the average percent excreted for four subjects. The values in parentheses are the relative standard deviations.

Table V—Repeated-Measures Analysis of Variance for *In Vivo* Data (0–12 hr)

Source	*df*	Mean Square	*F*	Probability
Drug	3	0.04130	3.08	0.0831
Error	9	0.01342		
Time	8	0.52573	384.40[a]	0.0000
Error	24	0.00137		
Drug–time interaction	24	0.00062	0.75	0.7771
Error	72	0.00083		

[a] Significance level = $p < 0.001$.

Table VI—Repeated-Measures Analysis of Variance for *In Vivo* Data (0–4 hr)

Source	*df*	Mean Square	*F*	Probability
Drug	3	0.02052	5.34[a]	0.0218
Error	9	0.00384		
Time	5	0.14788	112.97[b]	0.0000
Error	15	0.00131		
Drug–time interaction	15	0.0057	1.40	0.1911
Error	45	0.00040		

[a] Significance level = $p < 0.05$. [b] Significance level = $p < 0.001$.

RESULTS AND DISCUSSION

The manufacturing parameters for all three brands are given in Table I. All brands passed the weight variation test as specified in USP XIX. Furthermore, the coefficient of variation of weight was within the standards reported by Pietra and Setnikav (12). The resistance of the tablet to chipping, abrasion or breakage, transportation, and handling before use depends on its hardness. According to King (13), a hardness of 4 kg is the minimum for a satisfactory tablet, but Brand A had a hardness of <4 kg (Table I).

Although standards for uniformity in thickness have not been established by the USP, King (13) postulated that a difference in thickness of ±5% may be allowed, depending on the tablet. The average thicknesses (±*SD*) of the acetaminophen tablets were 5.026 ± 0.08, 4.90 ± 0.03, and 5.067 ± 0.03 mm for Brands A, B, and C, respectively (Table I), which fall within the limits set forth by King (13). Official limits for friability also have not been established; however, according to Gunsel and Kanig (14), a weight loss of <0.8% is considered satisfactory. Only Brand C was close to this unofficial limit (Table I). Since most of the Brand A tablets lost their edges and showed capping, their friability values were not calculated.

The disintegration tests, as specified in USP XIX (11), were performed on all of the brands. The average disintegration times were determined in water, simulated gastric fluid (without enzymes), and intestinal fluids (without enzymes) (Table II). The disintegration time of Brand A in the gastric fluid was longer than the times of the other two lots; furthermore, small tablet cores were observed in the basket after 30 min.

The dissolution behavior of the three brands is shown in Fig. 1 and Table III. Figure 1 was constructed by plotting the log concentration of undissolved drug *versus* time. This profile indicates that dissolution is associated with apparent first-order kinetics and occurs under sink conditions (15, 16). The release constants in 0.1 *N* HCl were determined

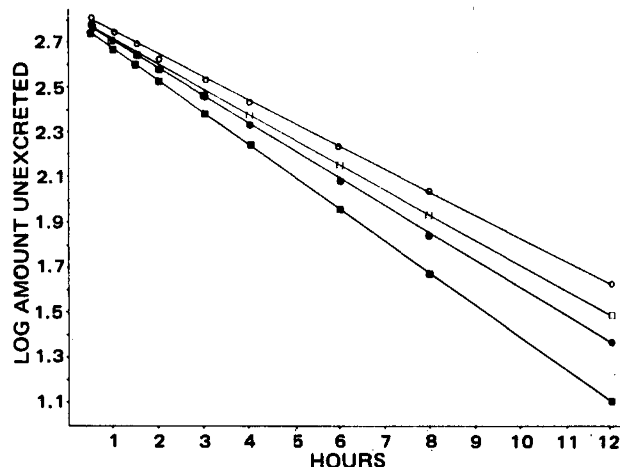

Figure 2—*Amount of unexcreted acetaminophen from four products. Key:* ○, *Brand A;* ●, *Brand B;* □, *Brand C; and* ■, *Brand D.*

from the slope of the lines and were 0.1, 0.74, and 0.62/min for Brands A, B, and C, respectively. Brand A tablets had the slowest dissolution rate. In fact, 90 min was required for 80% of the drug from Brand A to be released, while only 1 min was required for 99.8 and 100% of the drug to be released from Brands B and C, respectively. The slow dissolution rate of Brand A is also evident from the $T_{50\%}$ value, which was 50 min; it was only 1 min for Brands B and C. Brand A also exhibited the longest disintegration time.

Since acetaminophen is eliminated by first-order kinetics (17), some pharmacokinetic parameters were calculated by plotting the log concentration of the excreted drug *versus* time. The elimination constant (K_e) and the elimination half-life were calculated from the resultant slopes (Fig. 2). The mean half-lives were: Brand A, 4.12 hr with a range of 3.36–4.80 hr; Brand B, 2.77 hr with a range of 2.42–3.17 hr; Brand C, 3.12 hr with a range of 1.90–3.70 hr; and Brand D, 2.00 hr with a range of 1.51–2.86 hr. Nelson and Morioka (18) reported a mean half-life of 1.95 ± 0.23 hr, while Cummings *et al.* (17) and McGilveray *et al.* (5) reported values of 2.2 and 3.14 hr, respectively. Hence, Brand A had the longest half-life, which is consistent with its performance with respect to disintegration and dissolution; that is, the relatively short elimination half-life of acetaminophen is distorted by the long absorption half-life of Brand A.

Table IV illustrates the cumulative amount of drug excreted in the urine for each dosage form as a function of time. These data were analyzed using analysis of variance and covariance, including repeated measures. Table V shows that the calculated *F* values (0–12 hr) of 3.08 for the drug and 0.75 for the drug–time interaction were not significant ($p = 0.001$) across the elapsed time. However, the sample times showed a significant difference ($F = 384.40$). Therefore, the data at sample times from 0 to 4 hr were analyzed (Table VI). These data show that the calculated *F* values of 5.34 for the drug ($p = 0.05$) and of 112.97 for time ($p = 0.001$) were significant.

Furthermore, an analysis of variance, one for each week, was performed (one-way analysis of variance) (Table VII); there was no effect at any one of the nine times. These results indicate that absorption is dissolution rate controlled and agree with the suggestion of Levy and Yacobi (19) that urine collections be made at <3 hr to detect a meaningful difference.

Significant difference ($p = 0.05$) in drug elimination at selected times between brand interactions was observed by utilizing the Tukey procedure (Table VIII). Brand A was significantly different from Brand D at

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.

REFERENCES

(1) *J. Am. Pharm. Assoc.*, **NS 13**, 278 (1973).

(2) *Ibid.*, **NS 17**, 517 (1977).

(3) G. L. Mattok, I. J. McGilveray, and D. Cook, *Can. J. Pharm. Sci.*, **6**, 35 (1971).

(4) G. L. Mattok, I. J. McGilveray, and C. A. Mainville, *J. Pharm. Sci.*, **60**, 561 (1971).

(5) I. J. McGilveray, G. L. Mattok, J. R. Fooks, N. Jordan, and D. Cook, *Can. J. Pharm. Sci.*, **6**, 39 (1971).

(6) J. R. Gwilt, A. Robertson, L. Goldman, and A. W. Blanchard, *J. Pharm. Pharmacol.*, **15**, 445 (1963).

(7) L. F. Prescott, *Clin. Pharmacol. Ther.*, **10**, 383 (1969).

(8) F. M. Plakogiannis and A. M. Saad, *J. Pharm. Sci.*, **64**, 1547 (1975).

(9) *Ibid.*, **67**, 561 (1978).

(10) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 671.

(11) *Ibid.*, pp. 650, 651.

(12) J. Pietra and J. Setnikar, *J. Pharm. Sci.*, **59**, 530 (1970).

(13) R. E. King, in "Remington's Pharmaceutical Sciences," 15th ed., Mack Publishing Co., Easton, Pa., 1976, p. 1576.

(14) W. C. Gunsel and J. L. Kanig, in "The Theory and Practice of Industrial Pharmacy," 2nd ed., L. Lachman, H. A. Lieberman, and J. L. Kanig, Eds., Lea & Febiger, Philadelphia, Pa., 1976, p. 348.

(15) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **56**, 1238 (1967).

(16) J. G. Wagner, *ibid.*, **58**, 1253 (1969).

(17) A. J. Cummings, M. L. King, and B. K. Martin, *Br. J. Pharmacol. Chemother.*, **29**, 150 (1967).

(18) E. Nelson and T. Morioka, *J. Pharm. Sci.*, **52**, 864 (1963).

(19) G. Levy and A. Yacobi, *J. Clin. Pharmacol.*, **15**, 525 (1975).

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

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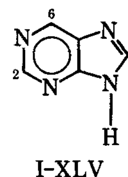
Received August 21, 1980, from the *Laboratory of Medicinal Chemistry and Biology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.* Accepted for publication September 22, 1980.

Abstract ☐ Linear free energy relationships were derived for several monosubstituted purines. The derived equations relate the pKa to the Hammett constants σ_m and σ_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

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