portion of docusate anion and the negatively-charged aluminum hydroxycarbonate surface.

The adsorption of polyols, although occurring by hydrogen bonding rather than electrostatic attraction (10, 11), was also affected by the pH–ZPC relationship (Fig. 3). The extent of mannitol adsorption by either gel 1 or 2 is much less than was observed for the adsorption of the magnesium cation or the docusate anion, reflecting the weaker adsorption mechanism of hydrogen bonding in comparison to electrostatic attraction. However, the fraction of mannitol adsorbed increased from ~30% at pH conditions below the ZPC to 50% when the pH was above the ZPC. Hydrogen bonding occurs more readily when mannitol serves as the proton donor and the negatively-charged oxygen at the aluminum hydroxycarbonate gel surface serves as the proton acceptor. This condition exists when the pH is above the ZPC and coincides with the region of maximum adsorption of mannitol.

The results of this study suggest that the pH–ZPC relationship will provide a useful guideline for predicting adsorption reactions in the formulation of antacid dosage forms and may also be useful in predicting drug interactions arising from the coadministration of drugs and aluminum hydroxide-containing antacids.

REFERENCES

(1) R. G. Gast, in "Minerals in Soil Environments," J. B. Dixon and S. B. Weed, Eds., Soil Science Society of America, Madison, Wis., 1977, pp. 27–73.

(2) J. R. Feldkamp, D. N. Shah, S. L. Meyer, J. L. White, and S. L. Hem, *J. Pharm. Sci.*, **70**, 638 (1981).

(3) L. I. Osipow, "Surface Chemistry: Theory and Industrial Applications," Reinhold, New York, N.Y. 1962, pp 169.

(4) "The United States Pharmacopeia," 20th rev., United States Pharmacopeial Convention, Inc. Rockville, Md., 1980, p. 1072.

(5) *Ibid.*, p. 262.

(6) J. M. Bailey, *J. Lab. Clin. Med.*, **54**, 158 (1959).

(7) F. Dalang and W. Stumm, in "Colloid Interface Science," (Proc. 50th Int. Conf.), **4**, 157–163 (1976).

(8) R. O. James and T. W. Healy, *J. Colloid Interface Sci.*, **40**, 42 (1972).

(9) "The Merck Index," 9th ed., Merck and Co., Rahway, N.J., 1976, p. 438.

(10) D. N. Shah, J. L. White, and S. L. Hem, *J. Pharm. Sci.*, **70**, 1101 (1981).

(11) S. L. Nail, J. L. White, and S. L. Hem, *ibid.*, **65**, 1195 (1976).

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COMMUNICATIONS

TLC and GLC Determination of Aromatic Amine Impurities in Bulk *p*-Aminobenzoic Acid and in Its Potassium and Sodium Salts

Keyphrases □ *p*-Aminobenzoic acid—aromatic amine impurities determined by GLC and TLC □ TLC—determination of aromatic amine impurities in *p*-aminobenzoic acid □ GLC—determination of aromatic amine impurities in *p*-aminobenzoic acid

To the Editor:

The current USP monograph (1) for *p*-aminobenzoic acid lacks a requirement for limiting the amount of aromatic amine impurities in the finished bulk drug. The carcinogenicity of these possible amine impurities prompted this laboratory to adapt the derivatization and GLC technique of Bruce and Maynard (2) to identify and quantitate these compounds. A TLC confirmatory test using a portion of the underivatized sample solution was also developed.

A finely ground 5-g sample of bulk *p*-aminobenzoic acid or its salts, in a 50-ml stoppered centrifuge tube, was extracted by shaking with 25 ml of benzene for 5 min. After centrifuging, the benzene layer was transferred to a separator and extracted with two 15-ml portions of 2% aqueous sodium bicarbonate. The aqueous layers were discarded, and the benzene layer was dried through anhydrous sodium sulfate.

A 2-ml portion of the extract was derivatized in a separator with 25 μl of heptafluorobutyric anhydride. After 30 min at room temperature, 10 ml of benzene was added, the organic layer was washed with three 10-ml portions of water, and the water was discarded. The benzene layer was diluted to 50 ml with benzene and 5 μl was injected into a gas chromatograph equipped with a 15-μCi ^{63}Ni-electron-capture detector, a 1.8 m × 4-mm i.d. spiral glass tube packed with 6% OV-101 and 9% OV-210 (1:1) coated on acid-washed silanized high-performance flux calcined diatomite support (100–120 mesh). Inlet, column, and detector temperatures were 225, 140, and 325°, respectively. Argon–methane (95:5) at a flow rate of 50 ml/min was used as the carrier gas.

For TLC, 5 ml of the dried benzene sample extract was evaporated to 200 μl, and 20 μl was applied to a silica gel GF plate. After 10-cm development with benzene–ethyl acetate–acetic acid (90:5:5), the plate was sprayed with 1% *p*-dimethylaminobenzaldehyde in ethanol containing 5% HCl.

Table I lists the TLC R_f values for the 10 amines used in this study. The GLC retention times for the corre-

Table I—TLC and GLC Data for 10 Aromatic Amines

Amine	TLC[a] R_f Free Amine	GLC[b] Retention Time, min[c]	Approximate Nanograms for HSD[d]
Aniline[e]	0.07	1.2	0.05
o-Toluidine	0.28	1.7	0.10
p-Toluidine	0.19	2.0	0.10
p-Chloroaniline	0.42	3.1	0.10
2-Methyl-5-chloroaniline	0.67	4.4	0.10
Diphenylamine	0.85	11.9	0.80
2-Chloro-5-nitroaniline	0.74	15.0	0.40
Benzocaine[e]	0.45	16.8	0.40
p-Nitroaniline	0.42	17.9	0.40
2-Methyl-5-nitroaniline	0.55	26.0	0.40

[a] 20 × 20 cm 0.25-mm thick silica gel GF plates, Mallinckrodt Chemical Works, St. Louis, Mo. [b] Tracor 560, Tracor Inc., Austin, Tex. [c] Heptafluorobutyric anhydride derivative. [d] Nanograms for half-scale deflection (HSD) at GLC detector setting of 7 namp, and attenuation at 5. [e] Found in commercial samples.

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sponding heptafluorobutyric anhydride derivatives are also reported with the approximate nanogram amounts of the parent amine needed for half-scale deflection at the electron-capture detector settings.

Eight samples of *p*-aminobenzoic acid, two samples of its sodium salt, and one sample of its potassium salt were analyzed in duplicate. Salt forms from three different lots contained no detectable aromatic amine impurities. Four of the acid samples contained benzocaine at levels of 3–65 ppm, while one sample of the acid contained 25 ppm of aniline. The TLC procedure confirmed the presence of both amine impurities. The derivatized samples were analyzed by GC–mass spectrometry and the mass spectral patterns of the samples matched those of derivatized standards of aniline and benzocaine.

The presence of benzocaine as an impurity was unexpected and cannnot be explained without knowledge of the synthesis process employed by the manufacturer. The aniline found in one sample could have been a contaminant of toluene used as a starting material for the synthesis of the *p*-aminobenzoic acid. The presence of aniline is significant because of its possible harmful effects on the body.

In synthesizing *p*-aminobenzoic acid, the process usually starts with toluene, followed by nitration, reduction, and oxidation reactions. The reported analytical procedure will determine the primary aromatic amine impurities formed in the synthesis of *p*-aminobenzoic acid or its salts. The 10 amines listed in Table I were added at levels of 1–5 ppm to the uncontaminated lots of free acid and recoveries of 90% or better were achieved using the derivatization technique.

(1) "The United States Pharmacopeia," 20th rev., Mack Publishing Co., Easton, Pa, 1980, p. 29.

(2) R. B. Bruce and W. R. Maynard, *Anal. Chem.*, **41**, 977 (1969).

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The Antitumor and Mammalian Xanthine Oxidase Inhibitory Activity of 5-Methyl-6-substituted Pyrrolo(2,3-*d*)pyrimidine-2,4-diones

Keyphrases □ Antitumor agents—potential, 5-methyl-6-substituted pyrrolo(2,3-*d*)pyrimidine-2,4-diones □ Xanthine oxidase—potential inhibitors, 5-methyl-6-substituted pyrrolo(2,3-*d*)pyrimidine-2,4-diones

To the Editor:

Gout is a disease that is a consequence of hyperuricemia. The objective of drug therapy in gout is to ameliorate inflammatory arthritis and to control serum urate concentration to <6 mg/100 ml. The two drug treatments currently used to decrease urate levels are the blocking of uric acid renal tubular reabsorption with probenecid or sulfinpyrazone and to block the enzymatic activity of xanthine oxidase. This enzyme catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid.

Table I—The Xanthine Oxidase Inhibitory Constant (K_i) of 5-Methyl-6-substituted Pyrrolo(2,3-*d*)pyrimidine-2,4-diones and Allopurinol

R[a]	K_i[b], *M*
$-CH_3$	1.5×10^{-5}
$-C_2H_5$	*NA*[c]
$-CH_2CH(CH_3)_2$	*NA*
$-C_6H_5$	4.0×10^{-6}
$-CH_2C_6H_5$	*NA*
$-CH_2C_6H_4$-*p*-OH	*NA*
Allopurinol	8.0×10^{-8}

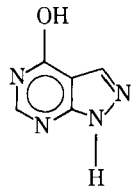
[a] R refers to structure II in the text. [b] Inhibitory constant. [c] No activity.

Allopurinol (I) is used currently as a xanthine oxidase inhibitor. A series of substituted pyrrolo(2,3-*d*)pyrimidine-2,4-diones (II) was synthesized (1) and tested for inhibition of xanthine oxidase activity. A drug as potent as allopurinol would provide an alternative therapy for the gout patient.

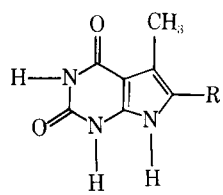
The activity of buttermilk xanthine oxidase (1, 2) was measured *in vitro* by a previous method (2). [The activity of xanthine oxidase from humans is similar to that obtained from cow's milk (2).] The assay was performed at pH 8.0 in 0.1 *M* sodium phosphate buffer. Allopurinol[1], the pyrrolo(2,3-*d*)pyrimidine-2,4-diones, and hypoxanthine[2] were dissolved in 0.1 *M* sodium phosphate buffer, pH 8.0. Uric acid production was monitored at 300 nm in a UV spectrophotometer[3] at 36° after 1 μl (0.035 units) of xanthine oxidase was added to each reaction mixture. The hypoxanthine concentration varied from 0.06 to 0.10 m*M*, and the inhibitor concentration varied from 0 to 5×10^{-2} m*M*. The inhibitor constant (K_i) for each drug was determined by using a Dixon plot where 1/Vr is plotted against inhibitor concentration (3). Allopurinol was used as the standard xanthine oxidase inhibitor.

Table I lists the K_i for allopurinol and the six pyrrolo(2,3-*d*)pyrimidine-2,4-diones that were tested. Only two of the tested compounds showed any *in vitro* inhibition. The phenyl substituted compound had the most activity but it was low compared to allopurinol.

Because of the similarity of these compounds to normal purines, one compound, 5,6-dimethylpyrrolo(2,3-*d*)-pyrimidine-2,4-dione, was tested for *in vivo* activity against two transplantable mouse lymphoid tumor systems: H-5 ascites tumor[4] in A/J male mice[5] and L-1210 leukemia[6] in $B6D2F_1$ male mice[5].



(I)



(II)

[1] Sigma Chemical Co., Saint Louis, MO 63178.
[2] Gilford Model 222A Photometer.
[3] From Buttermilk, grade III.
[4] A gift from Dr. J. Wynn, University of South Carolina.
[5] Jackson Laboratories, Bar Harbor, Ma.
[6] A gift from Dr. C. Bauguess, University of South Carolina.

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