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}

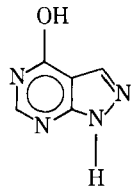
[a] R refers to structure II in the text. [b] Inhibitory constant. [c] No activity.

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.

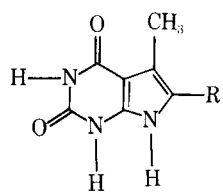
The activity of buttermilk xanthine oxidase (1, 2) was measured *in vitro* by a previous method (2). [The activity of xanthine oxidase from humans is similar to that obtained from cow's milk (2).] The assay was performed at pH 8.0 in 0.1 *M* sodium phosphate buffer. Allopurinol[1], the pyrrolo(2,3-*d*)pyrimidine-2,4-diones, 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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Ascites fluid (0.1 ml) from a tumor-bearing mouse and containing 10^5 cells[7] was injected intraperitoneally on day zero. Each experimental group contained six male mice (20–25 g) (4). The drug was injected intraperitoneally on days 1, 5, and 9 at a 120-mg/kg dose. The T/C was 85.4% for the L-1210 leukemia and 83.5% for the H-5 ascites tumor. A 360-mg/kg dose at day 1 or 40 mg/kg on days 1–9 in the L-1210 tumor also gave a T/C of 85.4%. A T/C value ≤85% indicates that the compound is probably producing a toxic response (4). This compound did not cause any deaths in control A/J or $B6D2F_1$ mice at the doses used, nor did it cause any visible toxicity, such as weight loss.

The probable toxic response of this compound in tumor-bearing mice hindered the testing of other substituted pyrrolo(2,3-*d*)pyrimidine-2,4-diones for antitumor activity.

(1) S. R. Etson, R. J. Mattson, and J. W. Sowell, Sr., *J. Heterocycl. Chem.*, **16**, 929 (1979).
(2) F. Bergman and S. Dikstein, *J. Biol. Chem.*, **223**, 765, (1956).
(3) H. N. Christensen and G. A. Palmer, "Enzyme Kinetics," 2nd ed., W. B. Saunders, Philadelphia, Pa., 1974, p. 82.
(4) B. J. Abbott, *Cancer Chemother. Rep. Part 3*, **3**, 2 (1972).

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[7] Cells were counted on an improved Neubauer ultraplane spot lite counting chamber (Scientific Products).

Studies on Herbal Remedies I: Analysis of Herbal Smoking Preparations Alleged to Contain Lettuce (*Lactuca sativa* L.) and Other Natural Products

Keyphrases □ Pharmacognosy—analysis of smoking preparations alleged to contain lettuce (*Lactuca sativa* L.) □ Psychotropics—alleged, analysis of lettuce (*Lactuca sativa* L.) in smoking preparations

To the Editor:

In this communication we would like to draw attention to the availability in health food stores and other outlets of three so-called "narcotic substitute" smoking products[1] that allegedly contain distillates of lettuce. Preparation 1 ("Hashish") and preparation 2 ("Opium") are recommended by their manufacturer for legal, social smoking, while preparation 3 ("Hash Oil") is suggested for application to marijuana cigarettes to enhance potency and taste. According to the package inscriptions, all three preparations contain "*Lactuca sativa*" (Garden Lettuce) and "*Turnera diffusa*" (Damiana) distillates. In addition, preparation 1 is claimed to be fortified with African Yohimbe bark, and preparation 2 with *Lactuca virosa* (Wild Lettuce) and *Nepeta cataria* (Catnip) distillates, as well as Chinese ginseng root. Ingredient proportions are not stated on the package labels.

It was suggested to us[2] that the smoking of preparation 2, without additives, may have been responsible for producing apparent psychotropic effects experienced by two teenagers. In an attempt to provide a rationale for this observation, the literature was searched for any ethnomedical and biological activities of Garden and Wild Lettuce and Damiana, and to whether or not any of the known constituents of these plants belong to classes of psychotropic substances.

Extracts of both *L. sativa* and *L. virosa* have been claimed to possess narcotic properties (1, 2), while the latter plant has been associated with hypnotic and sedative effects (3). Experimentally, extracts of *L. sativa* have exhibited *in vitro* antitubercular properties (4) and hypotensive activity in dogs (5). *T. diffusa* extracts reputedly show mild stimulant (2), purgative (3), and aphrodisiac (1, 3) activities.

An early study indicated that *L. sativa* and *L. virosa* contain a mydriatic alkaloid, which was identified as the tropane derivative hyoscyamine on the basis of the melting point of its aurochloride (6). The presence of a mydriatic alkaloid, although disputed, was presumably confirmed in later studies on *L. virosa* (7, 8). *L. virosa* has been shown to contain *N*-methyl-β-phenethylamine (9), and *T. diffusa* has been stated to contain the xanthine derivative, caffeine (10). Tropane alkaloids, phenethylamines, and xanthines were recently classified, respectively, as deliriant psychodysleptics, visionary psychodysleptics, and excitatory psychoanaleptics (11).

All three preparations were examined phytochemically to determine if any of these amines could be detected. Three extraction procedures, namely, a general alkaloidal method (12), and methods for the specific extraction of phenethylamines (13) and caffeine (14), were applied to 2-g portions of each product. No alkaloidal spots corresponding to reference hyoscyamine, *N*-methyl-β-phenethylamine[3], or caffeine were detected by TLC on silica gel using several solvent systems (15). Dragendorff's reagent, as well as other reagents useful for the detection of phenethylamines (ninhydrin) (15) and xanthines (iodine and ferric chloride) (15) were used for plate visualization.

Therefore, it appears that any psychotropic effects experienced by the smoking of these lettuce-Damiana distillates are not due to the presence of tropane alkaloids, phenethylamines, or xanthine bases.

(1) "The Dispensatory of the United States of America," 19th ed., H. C. Wood, J. P. Remington, and S. P. Sadtler, Eds., J. B. Lippincott, Philadelphia, Pa., 1907, pp. 686, 1471.
(2) R. K. Seigel, *J. Am. Med. Assoc.*, **236**, 473 (1976).
(3) "Extra Pharmacopoeia (Martindale)," 25th ed., R. G. Todd, Ed., Pharmaceutical Press, London, U.K., 1967, pp. 1518, 1531.
(4) F. K. Fitzpatrick, *Antibiot. Chemother.*, **4**, 528 (1954).
(5) L. Sollero, O. M. da Fonseca, W. B. Mors, A. A. de Figueiredo, and N. Sharapin, *Ciênc. Cult. (Sao Paulo)*, **20**, 33 (1968).
(6) T. S. Dymond, *J. Chem. Soc.*, **61**, 90 (1892).
(7) E. M. Farr and R. Wright, *Pharm. J.*, **72**, 186 (1904).
(8) Editorial, *ibid.*, **72**, 195 (1904).
(9) P. Marquardt, H.-G. Classen, and K.-A. Schumacher,

[1] Preparations 1–3 described in this report are Lettucenes 1–3, Woodley Herber, Okemos, MI 48864.

[2] Our attention was drawn to this problem by C. R. Sherwood, A. J. Canfield Co., Chicago, IL 60619.

[3] We are grateful to Prof. J. L. McLaughlin, Purdue University, W. Lafayette, Ind., for a reference sample of *N*-methyl-β-phenethylamine hydrochloride.

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