United States Patent  [19]


[54] XANTHINE OXIDASE INHIBITORS

[75] Inventors: Darrell E. O'Brien, Mission Viejo; Roland K. Robbins; Lionel N. Simon, both of Santa Ana, all of Calif.


[22] Filed: Feb. 11, 1974

[21] Appl. No.: 440,989

Related U.S. Application Data


[52] U.S. Cl. .............................. 260/256.4 F; 260/154; 424/226;
[51] Int. Cl. .............................. C07D 239/00
[58] Field of Search .......................... 260/256.4 F

[56] References Cited

UNITED STATES PATENTS
2,553,500 5/1951 Harsh .................................. 260/256.4 F
3,244,717 4/1966 Wagner .................................. 260/256.4 F

FOREIGN PATENTS OR APPLICATIONS
375,947 6/1962 Japan .................................. 260/256.4 F
76,677 10/1970 Germany .................................. 260/256.4 F

Primary Examiner—Donald G. Daus
Assistant Examiner—James H. Turnipseed
Attorney, Agent, or Firm—William E. Thomson;
William C. Steffen; Kay H. Boswell

[57] ABSTRACT

Compounds of the following structure are disclosed which are effective inhibitors of the enzyme xanthine oxidase:

\[
\begin{align*}
\text{in which } Y_1 \text{ and } Y_2 \text{ are } C \text{ or } N; \\
R_1 \text{ is } H \text{ or an alkali metal or ammonium; } \\
R_2 \text{ is } H, \text{CH}_3, \text{a halogen, phenylazo or NO}_2; \\
R_3 \text{ is } \text{OR}_1, \text{H, or a halogen; } \\
\text{and } R_4 \text{ is } H, \text{NO}_2 \text{ or a halogen. When } Y_1 \text{ is } C, Y_2 \text{ is } N \text{ and when } Y_1 \text{ is } N, Y_2 \text{ is } C.
\end{align*}
\]

10 Claims, No Drawings
XANTHINE OXIDASE INHIBITORS

This is a continuation, of application Ser. No. 172,196, filed Aug. 16, 1971 now abandoned.

BACKGROUND OF THE INVENTION

It is now well established that the enzyme xanthine oxidase is implicated in the production of uric acid by the body, converting hypoxanthine into xanthine and xanthine, in turn, into uric acid. Under normal conditions, uric acid (2,6,8-trioxypurine) is found in the body in only small amounts, a concentration in the blood on the order of about one to about 3 micrograms per 100 milliliters. Under certain pathological conditions, however, as for example gout, the concentration of uric acid increases significantly.

Gout, of course, is metabolic disturbance in the body resulting from an overproduction of uric acid, chronic hyperuricemia (elevated blood uric acid), and progressive accumulation of uric acid in the tissues. The body may also progressively lose its capacity to excrete uric acid and is, therefore, in a constant state of uric acid imbalance, accumulating a greater and greater excess. Its concentration in the blood is high, and, because of its low solubility, it tends to precipitate and form deposits at various sites where the blood flow is least active, particularly joints and cartilaginous tissues.

One approach to the control of gout commonly used in the past has been the prescription of drugs which tended to prevent the accumulation of uric acid in the body and thus diminish the likelihood of acute recurrences. Such drugs are identified as "uricosuric agents" and promote the excretion of uric acid in the urine. Examples of such drugs include p-dipropylsulfamyl benzoic acid and sulfipyrazone. These drugs cannot, however, be administered in conjunction with aspirin or any other salicylate, which might be given to relieve pain, because the agents and salicylates are mutually antagonistic, i.e., each tends to offset the action of the other.

A second approach to the treatment of gout which has become popular is the use of the drug allopurinol, which blocks the production of uric acid by the body by inhibiting the enzyme xanthine oxidase, which, as noted previously, is responsible for converting hypoxanthine into xanthine and xanthine into uric acid. While allopurinol is effective to inhibit the enzyme xanthine oxidase, nevertheless there are disadvantages which limit its suitability.

First, the toxicity is higher than desirable, having a lethal dosage level, LD50 (the dose required to kill 50% of a group of mice in 2 weeks when injected into the intraperitoneal cavity) on the order of 150 milligrams per kilogram of body weight. Moreover, allopurinol is gradually metabolized in vivo to 4,6-dihydroxy pyrazolo [3,4-d]pyrimidine, which is not as inhibitor as is allopurinol. In addition to the foregoing disadvantages, allopurinol, because of its chemical nature, must compete with xanthine to occupy a place on the enzyme xanthine oxidase in order to inhibit the enzyme and thus prevent the formation of uric acid by the body, which likewise limits its efficiency. It is also known that acute attacks of gouty arthritis occur in the early treatment with allopurinol. It is accordingly necessary to give colchicine during the initial period of therapy to prevent such acute attacks. There have also been reports of the development of a pruritic rash in some patients and of the occasional occurrence of drowsiness when allopurinol is administered. In view of the foregoing, it is apparent that xanthine oxidase inhibitors which are acceptable toxicity and at the same time possess increased inhibition efficiency as compared to allopurinol are highly desirable.

SUMMARY OF THE INVENTION

The present invention thus relates to xanthine oxidase inhibitors comprising imidazo [1,2-a] and pyrazolo [1,5-a] pyrimidine compounds of the following general structure:

\[
\begin{align*}
Y_1 & \text{ and } Y_2 \text{ are carbon or nitrogen; } R_1 \text{ is } H \text{ or an alkali metal or ammonium; } R_2 \text{ is } H, \text{ CH}_3, \text{ a halogen, phenylazol or NO}_2; \\
R_3 & \text{ is } OR_1, \text{ H, or a halogen; and } R_4 \text{ is } H \text{ NO}_2 \text{ or a halogen. When } Y_1 \text{ is carbon, } Y_2 \text{ is nitrogen, thereby forming the pyrazolo compounds, and when } Y_1 \text{ is nitrogen, } Y_2 \text{ is carbon, thus providing the imidazo compounds.}
\end{align*}
\]

DETAILED DESCRIPTION OF THE INVENTION

The xanthine oxidase inhibitors of this invention are represented by the foregoing structure. As will be seen from the illustrative examples which follow, such compounds, especially compounds in which \( R_3 \) is \( H \) or phenylazol, demonstrate inhibitory activity significantly greater than that of allopurinol. Moreover, such compounds are stable to enzymatic oxidation and also are noncompetitive inhibitors of the enzyme xanthine oxidase.

The method of preparing the compounds of the present invention will be described in detail in certain of the illustrative examples. In general, an efficient and comparatively straightforward process is used for the preparation of such compounds with the precise process varying depending upon whether an imidazo or pyrazolo compound is produced.

The imidazo pyrimidines are preferably prepared from an imidazole derivative such as aminoimidazolidinum sulfate, rather than by the more common synthetic procedure for the preparation of imidazo pyrimidines involving the condensation of aminopyrimidines with an \( \alpha \)-halocarboxyl compound, which reaction is limited by the nature of the substituents on the pyrimidine starting material. For example, although reaction of 2-amino-4,6-dimethyl-pyrimidine with \( \alpha \)-haloacetaldehyde yields satisfactory quantities of 5,7-dimethyl imidazol[1,2-a]pyrimidine, when 2-amino-4,6-dihydroxy-pyrimidine is reacted with \( \alpha \)-haloacetaldehyde, only polymeric materials are obtained. This is believed to be due to the fact that pyrimidines, with three
strong electron donating substituents in the 2, 4, and 6 positions, readily undergo electrophilic attack at the aromatic 5 position of the pyrimidine ring. Consequently, as indicated above, the imidazopyrimidines of the present invention are produced from an imidazole derivative. 2-aminoimidazolium sulfate may be condensed with diethylmalonate in the presence of sodium ethoxide in ethanol with acidification of the alcoholic mixture with ethanoic hydrogen chloride to produce the intermediate 2-amino-1(ethoxymalonyl) imidazole. When this compound is heated at 135°C, it cyclizes to 5,7-dihydroxyimidazo[1,2, a]pyrimidine, which may be readily purified by recrystallization from water. Alternatively, the imidazo[1,2,a]pyrimidine compound may be prepared directly from the imidazole derivative 2-aminoimidazolium sulfate without isolation of the intermediate compound if the crude sodium salt which separates from the initial reaction mixture does not come in contact with hot water (in excess of 70°C).

It will, of course, be understood that other of the described 5,7-imidazopyrimidines of the present invention can be prepared by the above-described process if appropriate materials are used in place of diethylmalonate. For the sake of brevity of disclosure, however, the preparation of such compounds will not be specified in greater detail at this point.

Treatment of the 5,7-dihydroxy imidazo[1,2,a]-pyrimidine with an aqueous solution of benzene diazonium chloride produces the corresponding 6-phenylazo derivative. This compound may also be produced by base catalyzed condensation of 2-aminoimidazolium sulfate with diethyl phenylazomalonate. If the 5,7-dihydroxy imidazo[1,2,a]pyrimidine compound is treated with bromine in glacial acetic acid, 6-bromo, 5,7-dihydroximidazo[1,2,a]pyrimidine is produced. Similarly, the 6-nitro derivative may be prepared by base catalyzed condensation of the sulfate with diethyl nitromalonate.

The pyrazolo[1,5,a]pyrimidines described in this invention are preferably prepared by the following procedure. The treatment of 3-amino pyrazole with diethylmalonate in the presence of sodium ethoxide affords the disodium salt of 5,7-dihydroxy pyrazolo[1,5,a]pyrimidine. This salt, when dissolved in water and treated with hydrochloric acid, yields 5,7-dihydroxy pyrazolo[1,5,a]pyrimidine. In an analogous manner, the condensation of 3-amino-4-chloropyrazole, 3-amino-4-bromopyrazole, and 3-amino-4-nitropyrazole with diethylmalonate in the presence of sodium ethoxide affords 3-chloro, 3-bromo, and 3-nitro derivatives of 5,7-dihydroxy pyrazolo[1,5,a]pyrimidine.

The condensation of 3-amino pyrazole with methyl diethylmalonate in the presence of sodium ethoxide affords 5,7-dihydroxy-6-methylpyrazolo[1,5,a]pyrimidine. Similarly, the condensation of 2-phenylazo diethylmalonate with 3-amino pyrazole in the presence of sodium ethoxide yields 5,7-dihydroxy-6-phenylazopyrazolo[1,5,a]pyrimidine.

Refluxing a solution of 5,7-dihydroxy pyrazolo[1,5,a]pyrimidine in phosphorus oxychloride affords 5,7-dichloropyrazolo[1,5,a]pyrimidine (I). The treatment of (I) with 2.5 N sodium hydroxide solution yields 5-chloro-7-hydroxy pyrazolo[1,5,a]pyrimidine (II). The reductive dehalogenation of (II) affords 7-hydroxy pyrazolo[1,5,a]pyrimidine (III). This product is identical in all respects to the product obtained by the condensation of 3-amino pyrazole with the sodium salt of ethyl malonaldehyde ester in the presence of sodium ethoxide. (reaction IV).

The invention will be better understood by reference to the following specific but illustrative examples. The ultraviolet spectra was recorded on a Cary-15 spectrophotometer. Proton magnetic resonance studies were carried out with a Hitachi Perkin-Elmer R–20A spectrometer with DSS as internal reference.

**EXAMPLE I**

Preparation of Imidazo[1,2,a]pyrimidine-5,7-diol.

(Method A)

A solution of sodium ethoxide was prepared by dissolving sodium [8.1g (0.352 formula weights)] in 200 ml of absolute ethanol. Diethylmalonate [27.4g (0.171 mole)] and 2-amino imidazole hemisulfate [22.2g (0.168 mole)] were added to the sodium ethoxide solution. The resulting solution was stirred and warmed slowly to reflux. After reflux was obtained a sodium salt began to separate from the solution. The mixture was stirred and heated at reflux for 8 hours. After cooling to room temperature, the sodium salt was separated by filtration, washed with ethanol, and air dried. The sodium salt was dissolved in 100 ml of water (30°C), and the product precipitated from the solution by the addition of 6% hydrochloric acid until a pH of 1–2 was obtained. The mixture was cooled to 5°C and the product was separated by filtration, washed with ice water, and dried at 100°C. Recrystallization from water afforded 11.9g (47%) of analytically pure product that had a Mp>360°C; λ max (pH 1) 216nm (ε 22,200) and 263nm (λs 7,900); λ max (pH 11) 223nm (ε19,500) and 265nm (ε 7,600).

Anal. calcd. for C9H10N2O5: C, 47.7; H 3.31; N, 27.8. Found: C, 47.8; H, 3.31; N, 27.7.
EXAMPLE II
Preparation of Imidazo [1,2a]-pyrimidine-5,7-diol (Method B)

A. 2-Amino-1-(ethoxymalonyl)imidazole — A solution of sodium ethoxide was prepared by dissolving sodium [2.3 g (0.0375 formula weights)] in 150 ml of absolute ethanol. Diethylmalonate [8.8 g, 55 m moles] and 2-aminoimidazole, hemisulfate [6.7 g, 50 m moles] were added to the sodium ethoxide solution. The solution was stirred and slowly warmed to reflux, at which time a sodium salt began to precipitate. The mixture was refluxed for 6 hours and then 50 ml of anhydrous ethanolic hydrogen chloride was added dropwise. After the addition was complete the mixture was refluxed for 20 minutes, filtered, and the filtrate evaporated to dryness. The gumy residue was dissolved in 30 ml of water and the pH of this solution brought to 6 by the addition of saturated sodium carbonate solution to afford a light yellow product. The product was separated by filtration, washed with water, and dried at 80°C. Recrystallization of this product from water afforded 3.35 g (34%) of analytically pure product that had a melting point of 175°C (slowly melts and resolidifies).

Anal. calc'd. for C11H8N2O2: C, 50.8; H, 3.58; N, 21.3.
Found: C, 48.7; H, 3.69; N, 21.4.

B. Imidazo [1,2a] pyrimidine-5,7-diol — 2-amino-1-(ethoxymalonyl) imidazole [1.97 g, 10 m moles] was heated at 200°C for 45 minutes. The residue after cooling was recrystallized from water to afford 1.13 g (75%) of analytically pure imidazo [1,2a] pyrimidine-5,7-diol; Mp 360°C. The ultraviolet absorption spectra of this product is identical to the ultraviolet absorption spectra of the product obtained by Method A.

Anal. calc'd. for C11H8N2O2: C, 47.4; H, 3.31; N, 27.8.
Found: C, 47.4; H, 3.43; N, 27.5.

EXAMPLE III
Preparation of 6-Compromimidazo [1,2a] pyrimidine-5,7-diol.

Bromine [0.89 g (0.00375 formula weights)] in 10 ml of acetic acid was added dropwise to a solution of imidazo [1,2a] pyrimidine-5,7-diol [0.58 g, 3.3 m moles] in 20 ml of acetic acid with good stirring at room temperature. After the addition was complete, the mixture was stirred at room temperature for 3 hours. The solid was separated by filtration, washed with ethanol 2(25 ml), and dried to afford 0.68 g (90%) of product. Recrystallization from water afforded in analytically pure sample that decomposed above 225°C. λmax (pH 4) 220 nm (ε, 27,100) and 276 nm (ε, 9,900) λmax (pH 11) 226 nm (ε,3,600) and 274 nm (ε, 7,800).

Anal. calc'd. for C11H8N2OBr: C, 31.3; H, 1.69; N, 18.3.
Found: C, 31.2; H, 1.68; N, 18.0.

EXAMPLE IV
Preparation of 6-Methyleneimidazo [1,2a] pyrimidine-5,7-diol.

A solution of sodium ethoxide was prepared by dissolving sodium [1.84 g (0.08 formula weights)] in 100 ml of absolute ethanol. Diethyl methyl malonate [6.96 g, 40 m moles] and 2-aminimidazole, hemisulfate [5.28 g, 40 m moles] were added to the sodium ethoxide solution. The mixture was refluxed with stirring for 8 hours, and then evaporated to dryness at reduced pressure. The residue was dissolved in 100 ml of water (30°C) and the pH adjusted to 2 by adding 6N hydrochloric acid. The product was separated by filtration, washed with cold water, and recrystallized from water to yield 2.74 g (40%) of analytically pure product that had a melting point of 310-2°C (dec.); λmax (pH 1) 218 nm (ε, 28,800) and 275 nm (ε, 11,900); λmax (pH 11) 223 nm (ε, 28,000) and 276 nm (ε, 11,700).

Anal. calc'd. for C14H12N2O3: C, 50.9; H, 4.24; N, 25.4.
Found: C, 50.9; H, 4.38; N, 24.9.

EXAMPLE V
Preparation of 6-Nitroimidazo [1,2a] pyrimidine-5,7-diol.

A solution of sodium ethoxide was prepared by dissolving sodium [4.6 g (0.2 formula weights)] in 250 ml of absolute ethanol. Diethyl nitromalonate [20.5 g (0.1 mole)] and 2-aminomimidazole, hemisulfate [13.2 g (0.1 mole)] were added to the sodium ethoxide solution. The mixture was refluxed with stirring for 9 hours, and then evaporated to dryness at reduced pressure. The residue was dissolved in 250 ml of water, and the pH adjusted to 1-2 by the addition of 6N hydrochloric acid. The product was separated by filtration, washed thoroughly with water and dried. Purification was afforded by recrystallizing the product from dilute sodium hydrosulfide solution by the addition of 6N hydrochloric acid to afford 5.9 g (30%) of analytically pure product. Mp, 305°-37°C (dec); λ max (pH 1) 207 nm (ε, 23,300) and 320 nm (ε, 6,300); λ max (pH 11) 226 nm (ε, 13,300) and 342 nm (ε, 4,900).

Anal. calc'd. for C17H14N3O4: C, 36.7; H, 2.04; N, 28.6.
Found: C, 36.8; H, 2.16; N, 28.4.

EXAMPLE VI
Preparations of 6-Phenylazoimidazo [1,2a] pyrimidine-5,7-diol.

A. A solution of sodium ethoxide was prepared by dissolving sodium [1.98 g (0.086 formula weights)] in 150 ml of absolute ethanol. Diethylphenylazomalonate [11.35 g, 43 m moles] and 2-aminomimidazole, hemisulfate [5.68 g, 43 m moles] were added to the sodium ethoxide solution. The mixture was refluxed with stirring for 8 hours, and then evaporated to dryness at reduced pressure. The residue was dissolved in 300 ml of water and the pH adjusted to 5-6 by the addition of a glacial acetic acid. The product was separated by filtration, washed with water, and purified by recrystallizing from ethanol to afford 3.40 g (31%) of analytically pure product that had a melting point of 277°-29°C (dec); λ max (pH 1) 205 nm (ε, 23,000) and 408 nm (ε, 27,800); λ max (pH 11) 217 nm (ε, 19,400) and 384 nm (ε, 20,900).

Anal. calc'd. for C21H16N2O4: C, 56.5; H, 3.53; N, 27.4.
Found: C, 56.7; H, 3.62; N, 26.6.

B. A solution of benzaldehydrazon chloride was prepared by treating a solution of aniline [0.31 g, 3.3 m moles] in 7 ml of 1.7N hydrochloric acid with a solution of sodium nitrite [0.25 g, 0.0036 formula weights] in 5 ml of water at 5°C. The benzene diazoniun chloride solution was added dropwise to a stirred solution of imidazo [1,2a] pyrimidine-5,7-diol [0.58 g, 3.3 m moles] in 10 ml of 1.25 N sodium hydrosulfide solution at 10°C. After the addition was complete, the mixture was stirred at 10°C for 30 minutes and then at room temperature for 30 minutes. The solid was separated by filtration, and purified by recrystallizing from a dilute sodium hydrosulfide solution by the addition of acetic acid.
A final purification by recrystallizing from absolute ethanol afforded 0.41 g (40%) of analytically pure product that had a melting point of 277°-8° (dec). The ultraviolet absorption spectra of this product is identical to the spectra of the compound prepared by Method A.

EXAMPLE VII
Preparation of Pyrazolo [1,5a] pyrimidine-5,7-diol.

This compound was prepared by the procedure of Y. Makukumi as described in the Chemical and Pharmaceutical Bulletin (Tokyo), 10, 612 (1962). This procedure is as follows:

A solution of sodium ethoxide was prepared by dissolving sodium [4.6g (0.2 formula weights)] in 300 ml of absolute ethanol. Diethylmalonate [16.0g, 0.1 mole] and 3-aminopyrazole [8.3g, 0.1 mole] were added to the sodium ethoxide solution. The solution was stirred and slowly warmed to reflux at which time a sodium salt began to precipitate. The mixture was refluxed for 6 hours and then allowed to cool to room temperature. The sodium salt was separated by filtration, washed with absolute ethanol, and dried. The sale was dissolved in 250 ml of water and the product precipitated by the addition of 6 N hydrochloric acid until a pH of 1~2 was obtained. The product was separated by filtration, washed with the water, and recrystallized from water to afford 7.4g (49%) of analytically pure product; mp 247°-8° (dec); λ max (pH 1) 218 nm (ε, 14,700) and 276 nm (ε, 7,300); 229 nm (ε, 22,200) and 270 nm (ε, 10,900).

Anal. calcd. for C_{12} H_{10} N_{2} O_{2}: C, 47.7; H, 3.31; N, 27.8. Found: C, 47.6; H, 3.42; N, 27.7.

EXAMPLE VIII
Preparation of 6-Methylpyrazolo [1,5a] pyrimidine-5,7-diol.

A solution of sodium ethoxide was prepared by dissolving sodium [2.3g (0.1 formula weights)] in 150 ml of absolute ethanol. Diethylmalonate [8.7g, 50 m moles] and 3-aminopyrazole [4.15g, 50 m moles] were added to the sodium ethoxide solution. The mixture was refluxed and stirred for 3 hours, and then evaporated to dryness at reduced pressure. The residue was dissolved in 125 ml of water and the pH adjusted to 1~2 by the addition of 6 N hydrochloric acid. The product was separated by filtration, washed with water, and dried at 100°. Purification was achieved by reprecipitating the product from dilute sodium hydroxide solution by adding the solution of 6 N hydrochloric acid to afford 4.37g (54%) on analytically pure product; mp, 358°-60° dec; λ max (pH 1) 209 nm (ε, 21,300) and 275 nm (ε, 10,700); λ max (pH 11) 226 nm (ε, 22,700) and 280 nm (ε, 13400).

Anal. calcd. for C_{12} H_{10} N_{2} O_{2}: C, 50.9; H, 4.24; N, 25.4. Found: C, 50.9; H, 4.36; N, 25.1.

EXAMPLE IX
Preparation of 6-Phenylazopyrazolo [1,5a] pyrimidine-5,7-diol.

A solution of sodium ethoxide was prepared by dissolving sodium [1.52g (0.066 formula weights)] in 150 ml of absolute ethanol. Diethylphenylazomalonate [8.71g, 33 m moles] and 3-aminopyrazole [2.74g, 33 m moles] were added to the sodium ethoxide solution. The mixture was stirred and heated at reflux for 6 hours, and then evaporated to dryness at reduced pressure. The residue was dissolved in 300 ml of water and the pH adjusted to 2 by the addition of 6 N hydrochloric acid. The solid was separated by filtration, washed with water and ethanol, and air dried. Purification was achieved by reprecipitating the product from dilute sodium hydroxide solution by the addition of 6 N hydrochloric acid to afford 2.54g (28%) of analytically pure product; mp, 300°-2° (dec); λ max (pH 1) 202 nm (ε, 25,200) and 246 nm (ε, 9,700), and 406 nm (ε, 30,100); λ max (pH 11) 210 nm (ε, 19,900) and 386 nm (ε, 20,000).

Anal. calcd. for C_{12} H_{10} N_{2} O_{2}: C, 56.5; H, 3.53; N, 27.5. Found: C, 57.0; H, 3.84; N, 27.9.

EXAMPLE X
Preparation of 3-Chloropyrazolo [1,5a] pyrimidine-5,7-diol.

A solution of sodium ethoxide was prepared by dissolving sodium [0.98g (0.0428 formula weights)] in 50 ml of absolute ethanol. Diethylmalonate [3.50g, 21.9 m moles] and 3-amino-4-chloropyrazole [2.30g, 2.14 m moles] were added to the sodium ethoxide solution. The mixture was stirred and slowly warmed to reflux. After refluxing for 8 hours the mixture was allowed to cool to room temperature and the sodium salt separated by filtration. The sodium salt was dissolved in 100 ml of water and the pH adjusted to 1~2 by the addition of 6 N hydrochloric acid. The product was separated by filtration, washed with ice water, and dried. Recrystallization from water afforded 1.48g (37%) of analytically pure product that had a melting point of 270°-2° (dec); λ max (pH 1) 209 nm (ε, 32,140) and 286 nm (ε, 16,680); λ max (pH 11) 231 nm (ε, 47,000) and 275 nm (ε, 18,520).

Anal. calcd. for C_{12} H_{10} Cl N_{2} O_{2}: C, 38.8; H, 2.16; N, 22.6. Found: C, 38.6; H, 2.46; N, 22.9.

EXAMPLE XI
Preparation of 3-Nitropyrazolo [1,5a] pyrimidine-5,7-diol, hemihydrate.

A solution of sodium ethoxide was prepared by dissolving sodium [0.92g (0.04 formula weights)] in 50 ml of absolute ethanol. Diethylmalonate [3.2g, 20 m moles] and 3-amino-4-nitropyrazole [2.56g, 20 m moles] were added to the sodium ethoxide solution. The mixture was stirred and refluxed for 16 hours and then allowed to cool to room temperature. The sodium salt was separated by filtration, washed with absolute ethanol and air dried. The sodium salt was dissolved in 100 ml of water and the pH of this solution adjusted to 1~2 by the addition of 6 N hydrochloric acid. The product was separated by filtration, washed with cold water, and recrystallized with water to afford 3.0g (77%) of analytically pure product; Mp, 226°-8°; λ max (pH 1) 218 nm (ε, 17,600) and 319 nm (ε, 8,700); λ max (pH 11) 227 nm (ε, 12,700) and 332 nm (ε, 13,700).

Anal. calcd. for C_{12} H_{10} N_{2} O_{1}: C, 35.1; H, 2.45; N, 27.3. Found: C, 35.1; H, 2.55; N, 27.6.

EXAMPLE XII
Preparation of 3-Bromopyrazolo [1,5a]-pyrimidine-5,7-diol.

A solution of sodium ethoxide was prepared by dissolving sodium [2.76g (0.120 formula weights)] in 50 ml of absolute ethanol. Diethylmalonate [9.6g, 60.2 m moles] and 3-aminopyrazole [4.15g, 50 m moles] were added to the sodium ethoxide solution. The mixture was stirred and heated at reflux for 6 hours, and then evaporated to dryness at reduced pressure. The residue was dissolved in 300 ml of water and the pH adjusted to 2 by the addition of 6 N hydrochloric acid. The solid was separated by filtration, washed with water and ethanol, and air dried. Purification was achieved by reprecipitating the product from a dilute sodium hydroxide solution by the addition of 6 N hydrochloric acid to afford 2.54g (28%) of analytically pure product; mp, 300°-2° (dec); λ max (pH 1) 202 nm (ε, 25,200) and 246 nm (ε, 9,700), and 406 nm (ε, 30,100); λ max (pH 11) 210 nm (ε, 19,900) and 386 nm (ε, 20,000).

Anal. calcd. for C_{12} H_{10} N_{2} O_{2}: C, 56.5; H, 3.53; N, 27.5. Found: C, 57.0; H, 3.84; N, 27.9.
mole) were added to the sodium ethoxide solution. The mixture was stirred at room temperature for 3 days, and the sodium salt separated by filtration. The sodium salt was dissolved in 100 ml of water and the pH of this solution adjusted to 1-2 by the addition of 6 N hydrochloric acid. The product was separated by filtration, washed with cold water, and dried. Purification was accomplished by reprecipitating the product from a dilute sodium hydroxide solution by the addition of 6 N hydrochloric acid. The analytically pure material weighed 4.3 g (31%) and decomposed without melting at 265°-7° C max (pH 1) 211 nm (ε, 20,300) and 283 nm (ε, 9,870); λ max (pH 1) 233 nm (ε, 32,800) and 273 nm (ε, 12,850).

Anal. calc. for C₉H₆BrN₂O₂: C, 31.3; H, 1.72; N, 18.2. Found: C, 31.2; H, 1.84; N, 18.3.

**EXAMPE XIII**

Preparation of 5-Chloropyrazolo [1,5a] pyrimidine-7-ol

A. 5,7-Dichloropyrazolo [1,5a] pyrimidine

A mixture of pyrazolo [1,5a]-pyrimidine-5,7-diol (32 g) and phosphorus oxychloride (160 ml) was stirred and heated at 110° C for 5 h. At the end of 1 h a complete solution was obtained, and this solution was heated for an additional 5 h. The excess phosphorus oxychloride was removed at reduced pressure using the steam bath as the source of heat. The red syrup was added slowly to 400 g of crushed ice with good stirring. This mixture was stirred at 5° C for 30 minutes, and then extracted with anhydrous ether (400 ml). The ethereal extract was washed with water, washed with dilute sodium bicarbonate solution, and dried over anhydrous sodium sulfate. Evaporation of the ethereal extract afforded 23 g of slightly yellow product, which was sublimed at reduced pressure (1 mm) and elevated temperature (70°-100° C) to afford 11.9 g (29%) of analytically pure product that had a melting point of 72°-74°C. λ max (CH₃OH) 235 nm (ε, 32,000) and 290 nm (ε, 2,200).

Anal. calc. for C₈H₇Cl₂N₂: C, 38.3; H, 1.59; N, 22.3. Found: C, 38.2; H, 1.78; N, 22.4.

B. 5-Chloropyrazolo [1,5a] pyrimidine-7-ol

A solution of 5,7-dichloropyrazolo [1,5a] pyrimidine [6.0 g 31.9 m moles] in 1.25N sodium hydroxide solution (120 ml) was heated at 95° C for 1 h. The hot solution was treated with decolorizing carbon and filtered. Acrification of the filtrate with 6 N hydrochloric acid until a pH of 2 was obtained, afforded the desired product. Recrystallization from a mixture of water and ethyl alcohol (1:1) gave 4.83 g (89%) of analytically pure product that had a melting point of 295°-8°C (dec); λ max (pH 1) 223 nm (ε, 34,000) 263 nm (ε, 4,900) and 305 nm (ε, 5,100); λ max (pH 11) 227 nm (ε, 18,200), 281 nm (ε, 8,000) and 303 nm (ε, 7,300).

Anal. calc. for C₉H₈ClN₂O Cl: C, 42.5; H, 2.35; N, 24.8. Found: C, 42.3; H, 2.76; N, 24.7.

**IV**

Preparations of Pyrazolo [1,5a]-pyrimidine-7-ol

A. A mixture of 5-chloropyrazolo [1,5a]-2-pyrimidine-7-ol [8.50 g 50 m moles]; 150 ml of methanol, 10 ml of 1.25 N sodium hydroxide solution, and 10% palladium or charcoal catalyst was placed in a Paro hydrogenation apparatus and hydrogenated at a pressure of 42 lbs/in². Within 5 hours, the calculated quantity of hydrogen (Ca. 4.5 lbs/in²) had been absorbed. The mixture was filtered and the filtrate acidified with 6 N hydrochloric acid until a pH of 2 was obtained. The acidified solution was evaporated to dryness and the residue washed with cold water. The white crystalline product was recrystallized from a mixture of methanol and water (2:5:1) to afford 3.13 g (46%) of analytically pure product that had a melting point of 327°-9°C (dec); λ max (pH 1) 216 nm (ε, 10,600), 260 nm (ε, 6,900) and 295 nm (ε, 4,900); λ max (pH 11) 226 nm (ε, 4,900), 280 nm (ε, 8,600) and 308 nm (ε, 7,700).

Anal. calc. for C₈H₆ClN₂O: C, 53.3; H, 3.70; N, 31.1. Found: C, 53.3; H, 3.97; N, 30.9.

B. A mixture of ethylacetate [17.8 g, 0.2 mole] and ethylformate [14.8 g (0.2 mole) was added dropwise to a suspension of finely cut sodium [4.6 g (0.2 formula weights)] in 300 ml of absolute ether with vigorous stirring. After the addition was complete the mixture was stirred vigorously for 20 hours, during which time the sodium salt of malonaldehydic ester precipitated. A solution of 3-amino-pyrazol [16.6 g, 0.2 mole] in 250 ml of absolute ethanol was added to the suspension, and the resulting mixture heated at 70° until the ether had evaporated. The resulting mixture was refluxed for 8 hours and then evaporated to dryness at reduced pressure. The residue was dissolved in 200 ml of water and this solution treated with decolorizing carbon and filtered. The filtrate was acidified to pH 2 by the addition of 6 N hydrochloric acid, and the precipitated product separated by filtration. Recrystallization from a methanol and water mixture afforded 3.1 g (11%) of analytically pure product that had a melting point of 325°-8°C (dec). The ultra-violet absorption spectra of this compound is identical to the spectra of the compound prepared by Method A.

**EXAMPLE XV**

In this example, the inhibitory activity of the compounds of this invention was determined by recording spectral changes at a constant wavelength of 290 nm, using a Cary Model 15 recording spectrophotometer equipped with an 0-0.1 O.D. slidewire. To facilitate dissolution in the incubation mixture, the compounds of this invention were dissolved in DMSO (Pure spectral grade), which is a good solvent and also one which does not inhibit the enzyme at the indicated concentrations. After dissolving in DMSO, the compounds were introduced into the incubation mixture containing 150 micromoles of tris HCl buffer (pH 7.83), 0.6 micromoles EDTA, 0.013 micromoles xanthine, and 20 to 40 micrograms of xanthine oxidase in a final volume of 3.0 milliliters. The xanthine oxidase was purified milk xanthine oxidase from Worthington Biochemical Corporation and had a specific activity of 0.29 U/mg of protein. The assay was carried out by mixing all the components in a cuvette at 25°C. The reaction was started by the addition of the enzyme with the aid of a stirrer-adder. Changes in O.D. were measured for several minutes, and the rate (Δ O.D. per minute) was calculated from the spectral change over the first 15 seconds.

In order to fully evaluate the inhibitory activity and to compare the relative inhibitory abilities of the compounds of this invention with allopurinol, experiments were carried out in which the concentration of inhibi-
tor was varied over a range of approximately $10^{-4}$ to about $10^{-7}$ molar and the initial velocity of xanthine oxidation was measured. From a plot of the log of inhibitor concentration against the percent inhibition, the concentration of inhibitor giving 50% inhibition, ($I_{50}$), was calculated. This value was obtained from a linear regression analysis of the straight lines obtained in this graph. The data is presented in Tables I and II which follow as (I/S)$_{50}$ values. The lower the (I/S)$_{50}$ value, the more potent the inhibitory capability of the compound.

### TABLE I

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>R</th>
<th>(I/S)$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>3.57</td>
</tr>
<tr>
<td>(2)</td>
<td>NH$_4$</td>
<td>H</td>
<td>CH$_3$</td>
<td>OH</td>
<td>100.1</td>
</tr>
<tr>
<td>(3)</td>
<td>H</td>
<td>CH$_3$</td>
<td>OH</td>
<td>H</td>
<td>1.54</td>
</tr>
<tr>
<td>(4)</td>
<td>H</td>
<td>Br</td>
<td>OH</td>
<td>H</td>
<td>0.92</td>
</tr>
<tr>
<td>(5)</td>
<td>H</td>
<td>phenyl-</td>
<td>OH</td>
<td>H</td>
<td>0.17</td>
</tr>
<tr>
<td>(6)</td>
<td>NH$_4$</td>
<td>H</td>
<td>phenyl-</td>
<td>H</td>
<td>0.55</td>
</tr>
<tr>
<td>(7)</td>
<td>NH$_4$</td>
<td>H</td>
<td>Br</td>
<td>OH</td>
<td>7.65</td>
</tr>
<tr>
<td>(8)</td>
<td>H</td>
<td>NO$_2$</td>
<td>OH</td>
<td>H</td>
<td>2.80</td>
</tr>
<tr>
<td>(9)</td>
<td>Allopurinol</td>
<td></td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
</tbody>
</table>

* Included for comparison only; outside scope of invention.

### TABLE II

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$R_4$</th>
<th>(I/S)$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>0.447</td>
</tr>
<tr>
<td>(2)</td>
<td>H</td>
<td>CH$_3$</td>
<td>OH</td>
<td>H</td>
<td>5.37</td>
</tr>
<tr>
<td>(3)</td>
<td>H</td>
<td>phenyl-</td>
<td>OH</td>
<td>H</td>
<td>3.22</td>
</tr>
<tr>
<td>(4)</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>1.08</td>
</tr>
<tr>
<td>(5)</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>2.42</td>
</tr>
<tr>
<td>(6)</td>
<td>NH$_4$</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>32</td>
</tr>
<tr>
<td>(7)</td>
<td>NH$_4$</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>33.4</td>
</tr>
<tr>
<td>(8)</td>
<td>Allopurinol</td>
<td></td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
</tbody>
</table>

* Included for comparison only; outside scope of invention.

Referring now more particularly to Table I, it will be observed that Compounds (1), (3), (4), (5), and (8) demonstrated significant inhibitory activity. Compound (5), the 5,7-dihydroxyimidazoo compound in which $R^2$ is phenylazo, demonstrated exceptional activity, being approximately 3½ times superior to allopurinol, which is also included in Table I for comparison. It will also be observed that Table I includes compounds in which an amino group is present at the 7 position. A comparison of the activity results between Compounds (1) and (2), (5) and (6), and (4) and (7), shows that the inhibitory activity is decreased significantly. A comparison of Compounds (1) and (7) and (5) and (6) in Table II shows similar results.

It should also be noted, with respect to Table II, that Compounds (1), (2), (3), (4), and (5) demonstrated significant ability to inhibit the enzyme xanthine oxidase. Compound (1) was especially good, being superior to allopurinol, which has an (I/S)$_{50}$ of 0.58.

**EXAMPLE XVI**

Oxidation tests were also conducted on the compounds of the present invention to determine their stability against enzyme oxidation. The compounds were tested for their ability to serve as alternate substrate in two ways: (1) by substituting the test compound for xanthine in the basic incubation mixture described with respect to Example XV above and measuring the change in O.D. from 220 to 380 nm over a 60-minute period, using the repetitive scan accessory of the Cary spectrophotometer, and (2) using the method of Johns, Spector, and Robins, Bioch. P. Col., 18, p. 2371–83 (1969) in which phenozone methosulfate and cytochrome C were substituted for oxygen as the electron carrier. Changes in O.D. of the test compound (Method 1) or of the phenozone methosulfate (Method 2) were observed for 30 minutes at the appropriate wavelength values (550 nm for phenozone methosulfate).

As noted previously, it is known that allopurinol is oxidized to 4,6-dihydroxypyrazolo[3,4,d]pyrimidine by xanthine oxidase. Because of this, the ability of the compounds of this invention to be oxidized by xanthine oxidase were determined. The reactions were carried out in vitro using the methods described above. Under the same conditions, allopurinol was slowly oxidized to 4,6-dihydroxypyrazolo [3,4,d]pyrimidine when oxygen was used to accept electrons and was completely oxidized to such compound in the presence of phenozone methosulfate in less than 5 minutes incubation at 25°C. In contrast, the compounds of this invention shown in Tables I and II above were completely stable to enzymatic oxidation under either condition.

**EXAMPLE XVII**

The compounds of this invention shown in Tables I and II were next subjected to kinetic analysis using the methods of Lineweaver and Burke, or Hanes *Enzymes*, M. Dixon and E. C. Webb, 1964, Acad. Press, New York, N.Y., to determine the type of inhibition they were exhibiting. Allopurinol has been shown to be a competitive inhibitor of xanthine oxidase. As can be seen from Tables III and IV which follow, the 5,7-dihydroxy compounds having the indicated $R_2$ substituents were non-competitive inhibitors. The $K_i$ values shown in Tables III and IV were calculated from a Dixon-plot (1) against 1/v at the two indicated substrate concentrations.

### TABLE III

<table>
<thead>
<tr>
<th>Imidazo[1,2,a]pyrimidines</th>
<th>Imidazo[1,2,a]pyrimidines</th>
<th>Type of Inhibition</th>
<th>Non-Inhibited Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>R$_1$ R$_2$ R$_3$ R$_4$</td>
<td>Km x 10$^4$</td>
<td>Vmax x 10$^4$</td>
<td>K$_i$ x 10$^4$</td>
</tr>
<tr>
<td>H Br OH H</td>
<td>2.83</td>
<td>13.2</td>
<td>11.9, 17.1</td>
</tr>
<tr>
<td>H phenyl- OH OH H</td>
<td>3.98</td>
<td>13.2</td>
<td>2.75, 3.12</td>
</tr>
<tr>
<td>H NO$_2$ OH H</td>
<td>5.51</td>
<td>16.7</td>
<td>29.8, 38.3</td>
</tr>
<tr>
<td>H CH$_3$ OH H</td>
<td>2.68</td>
<td>14.8</td>
<td>23.6, 26.2</td>
</tr>
<tr>
<td>H H OH H</td>
<td>2.68</td>
<td>14.8</td>
<td>34.0, 51.8</td>
</tr>
</tbody>
</table>
TABLE IV

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Type of Inhibition</th>
<th>Km × 10⁻⁴</th>
<th>Vₘ⁺ × 10⁶</th>
<th>Kᵣ × 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>Non-Comp.</td>
<td>1.99</td>
<td>11.3</td>
<td>6.08, 6.95</td>
</tr>
<tr>
<td>H</td>
<td>phenyl-</td>
<td>OH</td>
<td>H</td>
<td>&quot;</td>
<td>1.40</td>
<td>10.8</td>
<td>101, 116</td>
</tr>
<tr>
<td>H</td>
<td>CH₃</td>
<td>OH</td>
<td>H</td>
<td>&quot;</td>
<td>3.23</td>
<td>13.3</td>
<td>75.9, 90.8</td>
</tr>
</tbody>
</table>

EXAMPLE XVIII

Using the general procedure and conditions described with respect to Example XV, the inhibitory activity of the compounds set forth in Table V was determined. The incubation mixture again had a total reaction volume of 3.0 milliliters and the xanthine concentrations was 1.32 × 10⁻⁶ M. It will be seen from Table V that the compounds in which R₄ is NO₂, Cl or Br, demonstrated high inhibitory activity. Also shown in Table V for comparison is Compound (1) of Table II. It will be appreciated that the three other compounds shown in Table V exhibited very good inhibition capability with the halogenated compounds showing significantly better activity, for example less than 5% by weight preferably less than about 1%, magnesium stearate or other flowing agent, such as “Avicel” (carboxymethylcellulose).

We claim:

1. A compound of the structure

```
```

wherein Y₁ and Y₂ are C or N;
R₁ is H or an alkali metal or ammonium;
R₂ is H, CH₃, halogen, or NO₂;
R₃ is OR₁, H, or halogen;
and R₄ is H, NO₂ or halogen; provided that when one of Y₁ or Y₂ is C, the other is N;
that when Y₁ is C, Y₂ is N and R₂ is H, R₃ is H, halogen or OR₁, where R₁ is an alkali metal or ammonium and R₂ is H, CH₃, NO₂ or halogen; and that when Y₁ is N and Y₂ is C, R₄ is H.

2. The compound of claim 1 in which Y₁ is C and Y₂ is N, and R₁, R₂ and R₃ are H and R₄ is OH.

3. The compound of claim 1 in which Y₁ is C and Y₂ is N, and R₁, R₂ and R₄ are H and R₃ is Cl.

4. The compound of claim 1 in which Y₁ is C and Y₂ is N, and R₁ and R₂ are H, R₃ is OH and X is NO₂.

5. The compound of claim 1 in which Y₁ is C and Y₂ is N, and R₁ and R₂ are H, R₃ is OH and R₄ is Cl.

6. The compound of claim 1 in which Y₁ is C and Y₂ is N, and R₁ and R₃ are H, R₄ is OH and X is Br.

7. The compound of claim 1 in which Y₁ is N and Y₂ is C, R₁, R₂, and R₄ are H, and R₃ is OH.

8. The compound of claim 1 in which Y₁ is N and Y₂ is C, R₁, R₃, and R₄ are H, and R₂ is OH.

9. The compound of claim 1 in which Y₁ is N and Y₂ is C, R₁, and R₄ are H, R₃ is Br and R₂ is OH.

10. The compound of claim 1 in which Y₁ is N and Y₂ is C, and R₁, and R₄ are H, R₃ is NO₂ and R₂ is OH.

* * * * *