from EtOH, 1 g of **60** was obtained, mp 170–171°. The product obtained by method B was identical with that obtained by method A as shown by mixture melting point, TLC, and ir.

Determination of Apparent pK_a. The half-neutralization points or pK_a's were determined by potentiometric titration. The sample (0.05 mmol) was dissolved in 30% EtOH–H_2O (30 ml) containing NaOH (0.15 mmol) and back titrated with 0.5 *N* HCl using a glass calomel electrode system on an expanded pH scale. The apparent pK_a's were taken from the neutralization curve as the pH at the one-half neutralization point.

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3,5-Disubstituted 1,2,4-Triazoles, a New Class of Xanthine Oxidase Inhibitor

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3,5-Bis(4-pyridyl)-1,2,4-triazole (PPT), 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole (PMPT), and 3-(4-pyridazinyl)-5-(4-pyridyl)-1,2,4-triazole (PZPT) are among the most active competitive inhibitors of xanthine oxidase among a series of 3,5-disubstituted triazoles synthesized for this purpose, inhibition constants being less than 1×10^{-7} *M* for each. ED_{50} values in squirrel monkeys derived from first-order rate constants for the first and rate-limiting step of the sequence, xanthine → uric acid → allantoin + CO_2, range from 0.04 to 0.08 mg kg^{-1} orally, with unusually long durations of action attributable to asymmetric distribution of inhibitor within liver and gut as a consequence of enterohepatic recirculation. Sensitivity of rats, dogs, and anthropoid species to these, as to other xanthine oxidase inhibitors, is markedly less than that of the squirrel monkey, but the triazoles are at least an order of magnitude more active than the representative purine analogs tested.

Inhibition of uric acid biosynthesis as an alternative to uricosuria in the therapy of gout has become firmly established with the introduction of the xanthine oxidase inhibitor 4-hydroxypyrazolo[3,4-*d*]pyrimidine or allopurinol into clinical use.[1]

Virtually all xanthine oxidase inhibitors which are structural analogs of xanthine or hypoxanthine are substrates for phosphoribosyl transferases, forming ribonucleotides which possess various spectra of antimetabolite activities, e.g., 2,6-diaminopurine, 6-thioguanine, 8-azaguanine, 6-mercaptopurine,[2] 6-chloropurine,[3] 4-hydroxypyrazolopyrimidine,[4] and 4-mercaptopyrazolopyrimidine.[5] Thus, in addition to possibly augmenting normal autoregulatory mechanisms involving soluble nucleotide effects upon de novo purine biosynthesis,[6] a variety of inhibitions of interconversions and utilization of polynucleotide precursors occurs.

Because of this common inability of purine isosteres to inhibit xanthine oxidase in a highly selective manner, testing for xanthine oxidase inhibitory activity among novel structural types, unrelated to the purine nucleus, was undertaken in these laboratories.[7] The biological properties of three of the more active members of one such series are the subject of the present report.

Experimental Section

Materials and Methods. The syntheses of 3,5-bis(4-pyridyl)-1,2,4-triazole (PPT), 3-(4-pyrimidinyl)-5-(4-pyridyl)-1,2,4-triazole (PMPT), and 3-(4-pyridazinyl)-5-(4-pyridyl)-1,2,4-triazole (PZPT) are described elsewhere.[7] Bovine milk xanthine oxidase, uricase, and xanthine were purchased from Nutritional Biochemical Corp. and uric acid-2-^{14}C from Amersham Searle. Xanthine-6-^{14}C was prepared from guanine-6-^{14}C as previously described.[8]

Enzyme Inhibition in Vitro. For aerobic oxidation of xanthine, reactions were started by addition of enzyme (typically 1.0 ml of 1:300 dilution of Worthington Grade XOP enzyme to produce a ΔOD_{290} of 0.09–0.12 min^{-1}) to 2.0 ml of 0.05 *M* phosphate buffer, pH 7.5, containing xanthine and inhibitor. Initial reaction rates were determined directly from continuous recordings of OD_{290} vs.

Table I. Respective Inhibition of Oxygen and Ferritin Reduction by Xanthine Oxidase Inhibitors[a]

	Aerobic			
	I_{50}	K_1	Anaerobic I_{50}	Fe^{3+}/O_2
PPT	6.5×10^{-8}	9.6×10^{-8} (comp)	Inact	0
PMPT	4×10^{-8}	1.4×10^{-8} (comp)	Inact	0
PZPT	8×10^{-8}	6×10^{-8} (comp)	4×10^{-6}	0.02
4-Hydroxypyrazolopyrimidine	3×10^{-6}	2.1×10^{-7} (comp)	2.1×10^{-6}	1.40
4,6-Dihydroxypyrazolopyrimidine	6×10^{-6}	8.0×10^{-6} (uncomp)	1.2×10^{-6}	5.0
8-Azaadenine	1.8×10^{-7}	3×10^{-7} (noncomp)	1.6×10^{-4}	0.0011
8-Azaguanine	9.5×10^{-6}		6.9×10^{-4}	0.014
6-Mercaptopurine	3.9×10^{-5}		Inact	0
6-Chloropurine	6.7×10^{-4}		Inact	0

[a] For experimental conditions, see "Materials and Methods".

time. Ferritin reduction measurements were made as previously described.[9]

Enzyme Inhibition in Vivo. In rats, dogs, and squirrel monkeys, the latter anesthetized with pentobarbital (20 mg/kg iv), measurements of turnover of whole-body urate pools are based upon recovery of the 6-carbon label from xanthine in expired CO_2, i.e., xanthine-6-^{14}C → uric acid-6-^{14}C → $^{14}CO_2$ + allantoin, in which the first step is rate limiting. From a "deficit plot" of total ^{14}C recovery [1.00 − $^{14}CO_2/(^{14}C$, injected) vs. time], a first-order rate constant for urate turnover is obtained. Control values for rat, dog, and squirrel monkey averaged 0.0172 ± 0.0018, 0.0097 ± 0.0012, and 0.0165 ± 0.001 min^{-1}, respectively. Labeled xanthine (0.5 μCi/μmol) was injected intravenously admixed with inhibitor for iv inhibition testing or at intervals after oral administration of inhibitors. ED_{50} values for inhibitors were calculated from log dose vs. percent inhibition lines based upon a minimum of three dosage levels.

Since the spider monkey and gibbon lack uricase, measurement of urate pool turnover based upon the more convenient xanthine-6-^{14}C method is precluded. Accordingly, determinations of biosynthetic rates and pool sizes were based upon rate of decay of specific activity of the miscible urate pool labeled with uric acid-2-^{14}C by a modification of the method described by Sorensen.[10] Animals were anesthetized with Sernylan (1.0 mg/kg im) and pentobarbital (1 mg/kg iv as required) and infused with 5% glucose at ca. 0.3 ml min^{-1} kg^{-1}. Inhibitors were administered for subacute studies daily in diet or in acute inhibition experiments by gavage 60 min prior to labeled uric acid. This was administered via the infusion system over a 5-min interval at the level of ca. 5 μCi/kg. Urines were collected via catheterization of the ureter at 30-min intervals through 6 or 8 hr. After determination of total uric acid concentration by the enzymatic method,[11] 19-fold excess of unlabeled uric acid was added to an appropriate aliquot (typically 3.0 ml) of urine and uric acid twice recrystallized by dissolution in $LiHCO_3$ and subsequent acidification with acetic acid. Specific activities were based upon direct counting of recrystallized material after quantitation by absorption at 292 mμ in 0.1 N $LiHCO_3$. After chronic administration of inhibitors, urate levels in urine were, in some instances, below the level of precise determination by the enzymatic method. In these instances, uric acid was measured by isotope dilution using excess uric acid-3,5-$^{15}N_2$. Calculations were based upon isotope intensity ratios for molecular ions m/e 456/458 of trimethylsilyl derivatives of endogenous and heavy urate by GC–mass fragmentography.[12]

Results

I. In Vitro Inhibition. All three triazoles were found to be potent competitive inhibitors of the aerobic oxidation of xanthine by bovine milk xanthine oxidase (Table I). Unlike the representative purine, azapurine, and pyrazolopyrimidine structures tested, examination of absorption and fluorescence spectra of the triazoles after 60-min incubation with enzyme in the absence of xanthine revealed no suggestion that any was oxidized by xanthine oxidase.

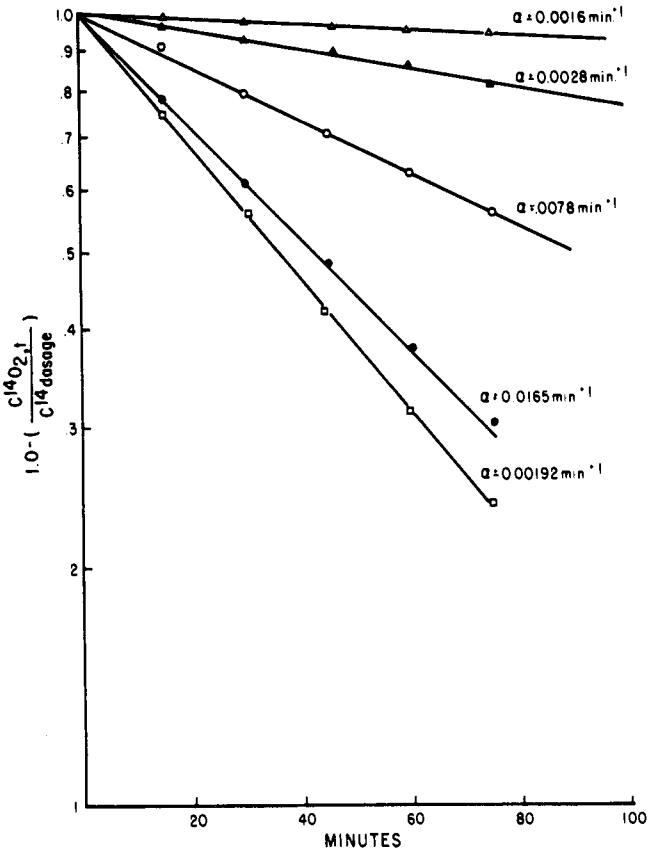

Figure 1. Turnover of oxypurine pool in the squirrel monkey and inhibition by 3,5-PPT. Male squirrel monkeys received 0.1 mg/kg of xanthine-6-^{14}C (50,000 dpm) at zero time, alone (●), and after 3,5-PPT by gavage at −60 min at 0.1 (○), 0.33 (▲), and 1.0 mg/kg (△); uric acid-6-^{14}C alone (□).

With ferritin as electron acceptor, PPT and PMPT were without inhibitory effect upon the anaerobic oxidation of hypoxanthine at concentrations 1000 times their I_{50} values with respect to aerobic oxidation of xanthine. PZPT was weakly inhibitory, ca. 100 times less active than with respect to the aerobic reaction (Table I). By contrast, the pyrazolopyrimidines were more active as inhibitors of ferritin reduction than of aerobic oxidation, azapurines resembled PZPT, and purines resembled PPT and PMPT in their respective inhibitory effects upon the two systems.

II. In Vivo Inhibitions. (a) Squirrel Monkey. The

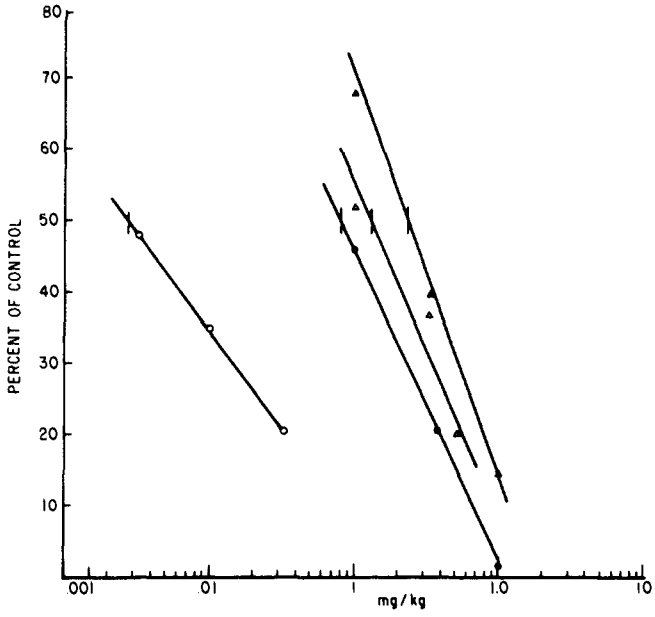

Figure 2. Dosage vs. inhibition curve for 3,5-PPT in the squirrel monkey: 3,5-PPT intravenously at zero time (○) or orally at 1 (●), 6 (△), and 16 hr (▲) before xanthine-6-^{14}C.

Table II. Duration of Effect of Xanthine Oxidase Inhibitors in the Squirrel Monkey

	ED_{50} (mg/kg) at				
	0 hr[a]	−1 hr	−3 hr	−6 hr	−16 hr
PPT	0.027	0.080	0.080	0.103	0.23
PMPT	0.055	0.080	0.13	0.19	0.80
PZPT	0.020	0.04	0.21	0.40	1.7
4-Hydroxypyrazolopyrimidine	0.85	0.94	3.3	3.5	5.0

[a] Inhibitor at zero time admixed with xanthine-6-^{14}C and administered iv; all other values for oral dosage at indicated intervals prior to xanthine-6-^{14}C.

derivation of the first-order rate constant (α) for turnover of the whole body oxypurine pool in the untreated monkey and dose-related reductions thereof by PPT are illustrated in Figure 1. Kinetics of the recovery of uric acid-6-^{14}C label and lack of effect of inhibitor thereupon demonstrate that (1) xanthine oxidase is rate limiting in the overall conversion xanthine-6-^{14}C → urate-6-^{14}C → $^{14}CO_2$, and (2) inhibition by PPT is specifically of xanthine oxidase and not of uricase.

From individual rate constants obtained as above, log dose vs. percent inhibition curves were constructed for each inhibitor as administered intravenously at zero time and orally at intervals preceding measurement of turnover of the oxypurine pool. A representative series, for PPT in the monkey, presented in Figure 2, demonstrates an excellent duration of action, ED_{50} at 6 hr being only 28% higher than at 1 hr, and at 16 hr, ca. threefold higher. Corresponding values for the three triazoles and for 4-hydroxypyrazolopyrimidine in the monkey are summarized in Table II. PMPT is comparable to 3,5-PPT through 3 hr, but its activity decreases at a significantly greater rate thereafter. PZPT, which is the most active through 1 hr, has only one-eighth the activity of PPT at 16 hr. 4-Hydroxypyrazolopyrimidine is at least an order of magnitude less active at all time points.

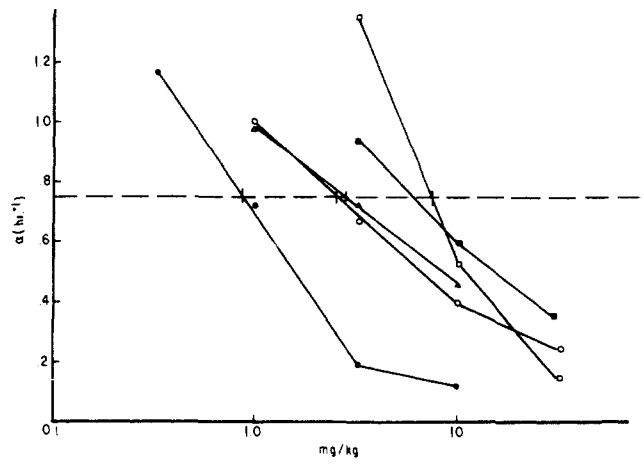

Figure 3. Dose-response curves for xanthine oxidase inhibitors in the rat. Inhibitors at dosages indicated by gavage 60 min prior to xanthine-6-^{14}C: PMPT (○), PZPT (●), PPT (△), 8-azaguanine (■), 4-hydroxypyrazolopyrimidine (□).

Table III. Inhibition of Xanthine Oxidase in the Rat[a]

Inhibitor	Xan-6-^{14}C → → $^{14}CO_2$ ED_{50}, mg/kg	Hypoxan-8-^{14}C incorpn, dpm per mg of RNA	
		Liver	Gut
None		113 ± 26	345 ± 63
PPT	2.4	2,077 ± 101	24,300 ± 3,430
PMPT	2.2	4,160 ± 610	28,500 ± 4,700
PZPT	0.85	3,500 ± 320	18,600 ± 1,250
4-HPP	7.5	1,270 ± 240	4,050 ± 550
8-AG	7.0	N.A.	N.A.

[a] Male Sprague-Dawley (200-230 g) rats received inhibitors by gavage 60 min prior to xanthine-6-^{14}C (4×10^5 dpm) or hypoxanthine-8-^{14}C. Estimates of ED_{50} for inhibition of $^{14}CO_2$ recovery based upon log dose vs. inhibition curves constructed from a minimum of three dosage levels. Values for polynucleotide labeling are means for three animals sacrificed 2 hr post hypoxanthine-8-^{14}C.

(b) Rat. Control rate constants for recovery of xanthine-6-^{14}C and uric acid-6-^{14}C labels in $^{14}CO_2$ were virtually identical with those in squirrel monkeys, i.e., 0.0172 and 0.0198 min^{-1}, respectively. Sensitivity to all inhibitors tested in this species, however, was markedly less, oral ED_{50} values for each inhibitor (Figure 3) ranging from 9- to 30-fold higher than in the monkey, but the relative order of activities was the same (Table III).

As an additional measure of acute inhibition of xanthine oxidase in rats, effects of inhibitors upon incorporation of hypoxanthine-8-^{14}C label into polynucleotide purines were determined. At 100 mg/kg, each inhibitor effected greater than tenfold increases in labeling of RNA in liver and gut (Table III).

As an index of effects of subacute treatment with inhibitors upon purine catabolism in rats, daily urinary excretions of allantoin were measured before, during, and after four daily treatments with inhibitor in rats in which the experimental hyperuricemic agent, 2-aminothiadiazole, was implanted intraperitoneally in a silastic depot formulation designed to deliver 10–15 mg/kg/day. In these hyperallantoinuric animals, each triazole at 3.3 mg/kg once daily reduced control allantoin excretion by ca. 50% (Table IV).

(c) Dogs. Control rate constants for turnover of oxypurine pools in dogs (0.0086 ± 0.0012 min^{-1}) were smaller than

Table IV. Inhibition of Allantoin Production in Aminothiadiazole-Induced Rats[a]

Treatment	mg/kg	Urinary allantoin, mg/rat/day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
None		29.0	43.5	50.5	63.0	60.0	74.0	60.0	62.5	50.5	46.0	48.5	50.5	50.5	46.0
PPT	3.3	31.5	42.5	49.0	59.5	56.0	28.5	29.0	27.5	18.5	19.5	42.0	44.5	43.0	42.5
PMPT	3.3	30.0	43.5	48.5	57.0	59.5	27.5	26.0	19.0	22.5	30.3	44.0	47.5	43.5	47.0
PZPT	3.3	28.5	39.0	47.5	54.0	51.0	37.5	39.0	23.0	22.5	22.5	25.0	37.0	44.5	43.5
	10	34.0	41.5	51.0	58.5	53.0	9.5	19.5	14.0	24.0	17.5	44.0	43.5	50.0	47.0

[a]Values are means for two pairs of 200-220-g rats implanted with depot formulation of 2-amino-1,3,4-thiadiazole on day 1 and treated once daily with inhibitors at dosages indicated on days 5-9. Urinary allantoin measurements as per Young and Conway.[22]

Table V. Inhibition of Xanthine Oxidase in the Dog[a]

Inhibitor	Dosage, mg/kg po	Rate constant (hr^{-1})			% inhibn	
		Control	1 hr	6 hr	1 hr	6 hr
PPT	1.0	0.50	0.083	0.174	83	65
PMPT	3.3	0.56	0.28	0.60	50	0
PMPT	10	0.53	0.17	0.50	68	6
PZPT	1.0	0.56	0.055	0.21	90	63
PZPT	3.3	0.62	0.030	0.17	95	73
4-Hydroxypyrazolopyrimidine	10	0.70	0.44	0.60	37	15
4-Hydroxypyrazolopyrimidine	10	0.59	0.34	0.50	43	15
8-Azaguanine	10	0.66	0.40	0.55	39	14
8-Azaguanine	10	0.63	0.44	0.55	30	12

[a]For experimental details, see text, section IIc.

for monkey and rat and more subject to interindividual variation (Table V). Therefore, each animal was made to serve as its own control as follows. After ca. 1×10^5 dpm of xanthine-6-^{14}C iv, $^{14}CO_2$ was collected for 15-min intervals through 75 min (control phase); inhibitor was administered at 90 min and ca. 3×10^5 dpm of xanthine-6-^{14}C at 150 min, after which an additional five collections of $^{14}CO_2$ were made ("1-hr post drug"); at 450 min, an additional 5×10^5 dpm of xanthine-6-^{14}C was injected and $^{14}CO_2$ collected incrementally for 75 min ("6-hr post drug"); recoveries for the second and third rate determinations were based upon the sum of count injected plus residual amounts calculated by extrapolation of deficit plots for previous injection(s).

PPT and PZPT were significantly more active than PMPT both with respect to initial intensity and duration of inhibition, ED_{50}'s being clearly less than 1 mg/kg. In each of two animals, 4-hydroxypyrazolopyrimidine and 8-azaguanine at 10 mg/kg effected a transient inhibition of less than 50%.

(d) Gibbon and Spider Monkey. The gibbon, being a true anthropoid, lacks uricase; thus, uric acid is the end product of purine catabolism in this species and attains plasma and urine levels of an order providing a direct parameter for estimation of biosynthetic rates. In the spider monkey (Ateles, sp.), recently found to be devoid of uricase,[13] control values for urate pool size and biosynthetic rate fall within the same ranges as in gibbons. The two species have therefore been used interchangeably. A typical control experiment is illustrated in Figure 4.

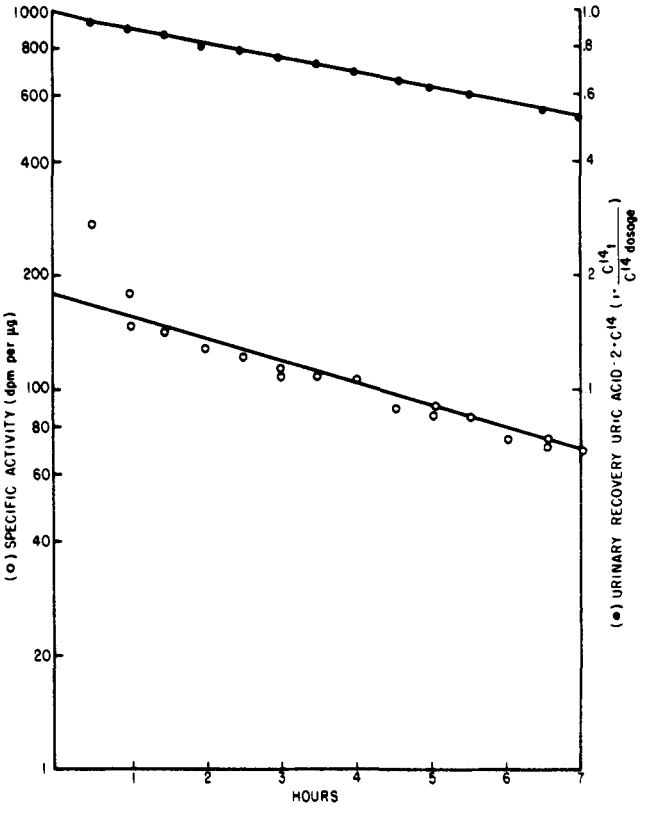

Figure 4. Turnover of uric acid pool in the gibbon. A 6.5-kg gibbon received a total of 650 μg of uric acid-2-^{14}C (11×10^6 dpm) intravenously; calculated pool size, 58 mg; biosynthetic rate 0.138 hr^{-1}.

In each of six animals, pool size and biosynthetic rate were determined three times at biweekly intervals: (1) before drug (control phase); (2) after a single iv dosage of inhibitor, and (3) following daily oral dosage of inhibitor for 7 days. The results of representative experiments with each of the three triazoles and 4-hydroxypyrazolopyrimidine are summarized in Table VI. PPT was clearly the most active, a single dosage reducing biosynthetic rate by 60%, and chronic dosage resulting in a threefold reduction in the miscible urate pool and ca. 90% inhibition in biosynthetic rate.

Discussion

The intrinsic inhibitory activities of PPT, PMPT, and PZPT are of an order second only to those of 2-amino-4-

Table VI. Inhibition of Uric Acid Biosynthesis in Spider Monkeys[a]

Animal	Treatment	Plasma urate, mg %	Miscible pool, mg	β, hr^{-1}	Urate synthesis, mg/hr
SM No. 8 (6.3 kg)	None	3.1	83.3	0.197	16.3
	PPT, 5 mg/kg	3.1	65.0	0.098	6.4
	PPT, 5 mg/kg/day	1.9	25.9	0.039	1.0
SM No. 331 (6.6 kg)	None	3.0	67	0.145	9.8
	PZPT, 5 mg/kg	2.9	75	0.102	7.1
	PZPT, 5 mg/kg/day	2.2	52	0.032	1.7
SM No. 300 (5.4 kg)	None	4.5	78	0.165	12.9
	PMPT, 5 mg/kg	3.6	73	0.120	8.8
	PMPT, 5 mg/kg/day	2.8	51	0.106	5.4
SM No. 2 (6.8 kg)	None	4.8	78	0.128	11.2
	4-HPP,[b] 10 mg/kg	4.4	91	0.087	7.9
	4-HPP, 10 mg/kg b.i.d.	2.9	28	0.087	2.5

[a] For details of treatment schedules and of pool size and biosynthetic rate, see text and Figure 4. [b] 4-Hydroxypyrazolopyrimidine.

hydroxy-6-formylpteridine[14] and certain imidazole-4,5-dicarboxylic acids (Baldwin, unpublished results). Although competitive with respect to oxypurines, the triazoles, unlike various classes of purine and pteridine isosteres, do not appear to be oxidized by the purified enzyme.

The ratios of oral to parenteral activities of the triazoles suggest that they are well absorbed, but bioavailability studies are limited by the relative insensitivity of fluorescent methods presently available and by their prompt clearance from peripheral plasma, terminal elimination rates ranging from 0.28 hr^{-1} for PPT to 1.2 hr^{-1} for PMPT (unpublished results). The relative durations of action of the triazoles in vivo (Table II), while qualitatively related to their respective half-lives, are, in absolute terms, unexpectedly long. A given dosage of PPT, for example, after 16 hr (6.4 half-lives) still effects an inhibition of ca. half that at 1 hr (Figure 2); PMPT after 30 half-lives still exerts one-tenth its initial inhibition. These observations would suggest either biotransformation to active metabolite(s) of longer biological half-life or an asymmetric distribution of the parent inhibitors providing disproportionate concentrations at the major sites of enzyme activity.

The major metabolites of the triazoles are their *N*-glucosides, which are extensively secreted in bile.[15] In the case of PMPT, substantial portal–peripheral plasma gradients of aglycone are apparent by 4 hr, reaching values of >10:1 at 8 hr. This delayed enteropheptatic reentry of active drug is presumably mediated via hydrolysis of the glucoside conjugates in the distal gut. PPT, in addition to conjugation and excretion as the glucoside, also undergoes oxidation of one pyridyl nitrogen, the resulting *N*-oxide secreted in bile, then reduced anaerobically by enteric bacteria (unpublished results), providing an additional mechanism for enterohepatic recirculation. In any event, a disproportionate fraction of dosage remains confined to the liver and gut, a fortuitous disposition for a xanthine oxidase inhibitor, which provides a basis for the unusual duration of effect of these inhibitors.

In the dog, PZPT, which had the least impressive duration of action in the spider monkey, is most active with respect to both initial intensity and duration of effect, while PMPT is least active, only ca. three times more so than azaguanine and 4-hydroxypyrazolopyrimidine.

In gibbon and spider monkeys which probably represent the most definitive experimental model in terms of their phylogenetic proximity to man, PPT and PZPT are clearly more active. Significant reductions in plasma level and pool size, and drastic reduction in biosynthetic rate on a once daily dosage schedule in species in which urate pool turnover rate is ca. five times that in man, is the most convincing evidence for an excellent duration of inhibitory effect.

In addition to inhibitory potency, biochemical rationale for preventing formation of excess uric acid, whether from exogenous purine bases or from those synthesized de novo, should include the following considerations.

(1) Inhibition should be specifically of xanthine oxidase and/or other enzymes concerned exclusively with the catabolism of purine bases, e.g., guanase. The consequent accumulation of soluble nucleotides of natural purines would be expected to provide autoregulation of de novo purine biosynthesis[16] precluding the need for nucleotide analogs, formed from the inhibitor, for this purpose.

(2) Since xanthine oxidase is multifunctional, inhibition should, ideally, be specifically of that function concerned with uric acid formation.

With respect to the first and more important of these criteria, it is apparent that all inhibitors of xanthine oxidase which are structural analogs of xanthine or hypoxanthine are substrates for phosphoribosyl transferases, forming nucleotide analogs. While these may exert a pseudo-feedback inhibition of de novo synthesis, a desirable attribute in primary hyperuricemia, they also function as antimetabolites at various stages of interconversion and utilization of polynucleotide precursors, e.g., 8-azaadenine, 2,6-diaminopurine, 6-mercaptopurine, 6-thioguanine,[2] 6-chloropurine,[3] and 4-hydroxypyrazolopyrimidine.[4]

The triazole inhibitors, while not isosteric with the purine nucleus, share one functional element with purines, viz. a weakly acidic imino group. By analogy to the *N*-ribonucleosides of imidazoleacetic acid[17] and 2-hydroxynicotinic acid,[18] presumably formed via hydrolysis of their ribonucleotides, the triazoles might be expected to form ribonucleotides. No evidence for this has been found; rather their *N*-glucosides are the major, and probably only, conjugates found in vivo.[15] Furthermore, utilization of adenine, guanine, and orotic acid for polynucleotide synthesis is not affected by them (unpublished results), suggesting no significant locus of action in vivo other than xanthine oxidase.

Regarding the second criterion stated above, it might be expected that xanthine oxidase inhibitors which are structural analogs of xanthine would have greater specificity with respect to that function of the enzyme concerned with purine oxidation. Dissociation of the respective inhibitions of purine and of aldehyde oxidations does not appear to occur.[19] On the other hand, selective inhibitions with respect to the nature of the electron acceptor are common.[20] One of the more important physiological roles of xanthine oxidase involving reduction of acceptors other than oxygen is in the mobilization of storage iron from ferritin.[21] The triazole inhibitors, like purine and azapurines, but unlike pyrazolopyrimidines, are virtually devoid of inhibitory activity with respect to this system.

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Chemical Differentiation of Histamine H_1- and H_2-Receptor Agonists[1]

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Histamine exists predominantly as the N^{τ}-H tautomer of the monocation (IIa) at a physiological pH of 7.4 and structure–activity studies indicate that this tautomer is likely to be the pharmacologically active species for both H_1 and H_2 receptors. Effective H_2-receptor agonists appear to require a prototropic tautomeric system whereas H_1-receptor agonists do not need to be tautomeric. This identifies a chemical difference in the receptor requirements which provides the basis for obtaining selective histamine H_1-receptor agonists. Thus 2-(2-aminoethyl)thiazole and 2-(2-aminoethyl)pyridine are nontautomeric and are highly selective agonists for histamine H_1 receptors (H_1:H_2 ca. 90:1 and 30:1, respectively). In conjunction with the selective H_2-receptor agonist, 4-methylhistamine, they are of great value for studying the pharmacology of histamine receptors.

The pharmacological actions of histamine are mediated by at least two distinct classes of receptor. One receptor type, designated H_1 by Ash and Schild,[2] mediates histamine-induced contraction of smooth muscle of the small intestine and bronchi. A second receptor type, designated H_2 and characterized by Black and coworkers,[3] mediates the action of histamine in vivo in stimulating gastric acid secretion and in vitro in inhibiting contractions of the rat uterus and in increasing the rate of beating of the guinea-pig atrium. The receptors are defined pharmacologically from the antagonists which selectively block the responses of these tissues to histamine stimulation.[2,3] The differentiation of these two receptor types is also reflected in the relative agonist activities of certain histamine analogs.[2-4] Thus from an analysis of agonist activities in three H_2-receptor systems and two H_1-receptor systems it was shown that 4-methylhistamine (**1**, Table I) is highly selective in stimulating H_2 receptors and that 2-methylhistamine (**2**) is a relatively selective H_1-receptor stimulant[3] (for histamine nomenclature[5] see Scheme I, I). In the work presented herein, H_2-receptor agonist activity is determined by stimulation of rat gastric acid secretion, and H_1-receptor agonist activity is measured on the isolated guinea-pig ileum. In these assays, the relative activities (H_1:H_2) are in the ratios of approximately 1:200 for 4-methylhistamine and approximately 8:1 for 2-methylhistamine. Activity in the H_1- and H_2-receptor systems is highly sensitive to apparently minor structural changes in the agonist molecule. This suggests

Scheme I. Ionic and Tautomeric Equilibria between Histamine Species