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Metabolism of 5-(*p*-Hydroxyanilino)-1,2,3,4-thiatriazole† in Rats

George J. Ikeda

Drug Metabolism Department, Abbott Laboratories, North Chicago, Illinois 60064. *Received March 30, 1973*

Plasma level, excretion, and metabolite identification studies were performed after oral administration of tritiated 5-(*p*-hydroxyanilino)-1,2,3,4-thiatriazole to male Sprague–Dawley rats. At a dose of 250 mg/kg, an average of 74.8 and 19.8% of the administered radioactivity was excreted in the urines and feces, respectively; at a dose of 1 g/kg, the values averaged 44.1 (urines) and 49.7% (feces). The major metabolic pathway for the disposition of the title compound in these rats was ethereal sulfate conjugation. The glucuronide conjugate and products resulting from fragmentation of the thiatriazole moiety were found in smaller amounts.

The compound, 5-(*p*-hydroxyanilino)-1,2-3,4-thiatriazole (1), has been under investigation at Abbott Laboratories as a possible antihypertensive agent. In order to obtain some idea of its metabolism in animal systems, studies using tritium-labeled drug were performed in rats. These include studies on blood levels of radioactivity, excretion, and metabolite identification.

Results and Discussion

Results of the blood level studies appear in Table I and Figure 1. It appears that more than one molecular species are present in the plasma. If the initial decline can be attributed to the parent drug, the half-life of this portion is 4.5-5.5 hr. The rise in plasma levels of radioactivity at about 6 hr may be due to the formation of metabolite(s) which have different volumes of distribution from that of 1.

If rats are sacrificed at 4 hr after an oral dose (250 mg/kg) and the tissues are analyzed for drug radioactivity, a large quantity of radioactivity (60%) was found in the gastrointestinal tract.‡ This may indicate either (1) a slow absorption or (2) biliary excretion of drug. The results obtained from bile-duct cannulated rats (Table II) seem to favor the idea of slow absorption of drug, since apparently no great amount of drug is routed *via* the bile. It also ap-

Table I. Blood Levels of Radioactivity after Administration of Abbott-31699-^{3}H to Male Sprague–Dawley Rats[a]

Time after administration, hr	Plasma levels of radioactivity, μg of drug/ml of plasma	
	Rat α	Rat β
0.25	12.8	17.0
0.50	21.5	26.9
1.0	56.6	24.7
2.0	23.3	20.5
3.0	21.3	17.6
4.0	19.4	16.4
5.0	15.3	19.7
6.0	15.6	19.9
7.5	16.3	20.2
12	12.0	17.4
24	5.88	11.3
30	5.03	10.1
48	3.71	7.92

[a] Dose = 250 mg/kg orally in tragacanth suspension.

† Abbott-31699.

‡ G. J. Ikeda, unpublished observations, Abbott Laboratories, North Chicago, Ill.

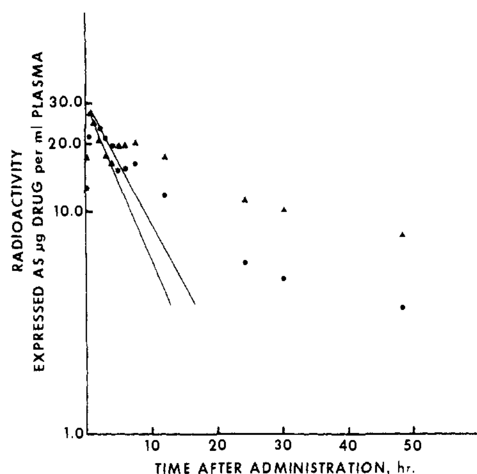

Figure 1. Semilogarithmic plot of plasma levels of radioactivity after administration of Abbott-31699-^{3}H to rats: dose, 250 mg/kg po.

pears that once the drug is absorbed, it is not distributed to any single target organ in any great degree but is probably conjugated by the liver and excreted *via* the kidneys.

Excretion of radioactivity after oral administration of high and low levels of drug is summarized in Tables III and IV. A considerably greater per cent of drug is adsorbed when 250 mg/kg is given compared to 1000 mg/kg, as evidenced by the greater percentages excreted in the urine in the former case. This is reasonable if a constant amount of drug is absorbed during a given experiment, and a saturation phenomenon occurs. The bile-duct cannulation study showed that only a few per cent of the drug were excreted *via* the bile (Table II); hence, most of the radioactivity which appears in the feces following an oral dose is due to unabsorbed drug. Thin-layer chromatography of methanolic fecal extract in several solvent systems (1, 2, 5, and 8) confirmed that unchanged 1 was the only major radioactive component in feces.

The excretion of radioactivity as tritiated water was low initially (Tables V and VI). This indicates that there was little or no labile tritium in the labeled drug. As the molecule remains in the animal for a longer period of time, there would be metabolism of the phenyl moiety with resultant removal of tritium from the phenyl ring. This tritium is then excreted in the urine as tritiated water.

Enzymic hydrolysis of the 0-48-hr rat urines and solvent extraction revealed that there was extensive conjugation of this drug. Glusulase, containing aryl sulfatase activity in addition to β-glucuronidase, and Ketodase, a β-glucuronidase preparation, were used to treat urine and served to reveal the nature of the conjugates found in urine. Results of this study are summarized on Table VII. It can be seen from this table that sulfate conjugation appears to be the predominant mode of detoxification of the drug and its metabolites in the rat.

Thin-layer chromatographic examination of rat urines after hydrolysis with Glusulase showed several radioactive regions on the chromatogram. Numerous solvent systems were experimented with, but the best resolution was obtained using solvent system 8. Quantum silica gel plates in this solvent system showed R_f's of 0–0.27 for *p*-aminophenol, 0.71 for *N*-acetyl-*p*-aminophenol (APAP), *p*-hydroxyphenylurea, *p*-hydroxyphenylthiourea, and an unknown metabolite, and 0.90 for 1. Sequential scraping of the thin-layer chromatograms and liquid-scintillation counting of the scrapings served to quantify the amount of radioactivity found in each region. Isotopic dilution using APAP and *p*-hydroxyphenylthiourea served to resolve and quantify the region of R_f 0.71.

Unchanged 1 accounted for 70% of the radioactivity in urine by isotopic dilution. *p*-Hydroxyphenylthiourea was found by isotopic dilution to account for 2.9%; APAP was found by isotopic dilution to account for 2.3%; *p*-hydroxyphenylurea was estimated to account for 3% by thin-layer chromatographic analysis of the pH 8 extract; and *p*-aminophenol and metabolites of similar polarity accounted for 6% of the radioactivity in urine.

Attempts at isolation of compounds by extraction and column chromatography have yielded the parent compound, *p*-hydroxyphenylurea, a compound described below, and elemental sulfur. Whether the elemental sulfur is derived from the parent compound or from *p*-hydroxyphenylthiourea which is then converted *in vitro* to *p*-hydroxyphenylurea is not known.

A compound was isolated from the acidic extract which the author has not been able to identify. High-resolution mass spectrometry indicated a parent ion of mass 194.0267, corresponding to an empirical formula of $C_7H_6N_4OS$, identical with the parent. Yet, the fragmen-

Table II. Excretion of Radioactivity in Bile after Administration of Abbott-31699-^{3}H to Bile-Duct Cannulated Male Sprague–Dawley Rats[a]

Time after dosing, hr	% of administered dose excreted in the bile	
	Rat 1	Rat II
24	1.96	0.10
48	2.29	1.37
72	0.51	3.03
96	0.03	0.62
120	0.01	0.04
Totals	4.80	5.16

[a] Dose = 250 mg/kg orally in tragacanth suspension.

Table III. Excretion of Radioactivity after Administration of Abbott-31699-^{3}H to Male Sprague–Dawley Rats[a]

Rat	% found in			
	Urine + cage wash	Feces	Carcass	Total
1	70.0	14.4	7.49	91.8
2	52.4	40.1	3.33	95.8
3	35.3	54.4	2.98	92.7
4	48.6	60.6	*b*	109.3
5	42.7	65.7	*b*	108.4
6	35.3	53.6	*b*	88.9
7	36.7	62.5	1.79	101.0
8	40.5	51.9	2.18	94.6
9	30.4	50.3	2.09	82.8
10	48.7	43.2	1.83	93.7
Mean ± S.E.M.	44.06 ± 3.64	49.67 ± 4.67	3.10 ± 0.76	95.90 ± 2.62

[a] Dose = 1 g/kg orally in tragacanth suspension; duration of experiment = 6 days. [b] Not determined.

Table IV. Excretion of Radioactivity after Administration of Abbott-31699-^{3}H to Male Sprague–Dawley Rats[a]

Rat	% found in		
	Urine + cage wash	Feces	Total
A	66.1	23.5	89.6
B	90.0	9.12	99.1
C	85.8	16.2	102.0
D	69.7	15.4	85.1
E	66.0	31.7	97.7
F	71.2	22.9	94.1
Mean ± S,E,M,	74.80 ± 4.26	19.80 ± 3.22	94.60 ± 2.58

[a] Dose = 250 mg/kg orally in tragacanth suspension; duration of experiment = 5 days.

Table V. Excretion of Tritiated Water in Urine of Rats after Administration of Abbott-31699-^{3}H[a]

Time interval after dosing, hr	% of urinary radioactivity attributable to tritiated water					
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean ± S.E.M,
0–24	0.54	0.52	0.59	0.09	0.61	0.47 ± 0.10
24–48	1.73	12.1	2.52	2.32	3.49	4.43 ± 1.94
48–72	5.31	38.1	8.05	7.14	10.3	13.78 ± 6.13
72–96	21.5	36.0	30.0	26.9	34.1	29.70 ± 2.59
96–120	39.1	48.8	41.4	40.0	39.4	41.74 ± 1.81

[a] Dose = 1 g/kg orally in 0,3% tragacanth suspension,

Table VI. Excretion of Tritiated Water in Urine of Rats after Administration of Abbott-31699-^{3}H[a]

Time interval after dosing, hr	% of urinary radioactivity attributable to tritiated water						
	Rat A	Rat B	Rat C	Rat D	Rat E	Rat F	Mean ± S.E.M.
0–24	0.33	0.34	0.24	0.27	0.19	0.20	0.26 ± 0.03
24–48	2.69	1.37	8.86	6.23	0.51	2.62	3.71 ± 1.30
48–72	23.2	19.4	44.3	29.0	12.3	12.4	23.43 ± 4.93
72–96	46.6	38.8	54.3	46.8	14.2	12.7	35.57 ± 7.28
96–120	57.1	36.8	60.5	46.8	26.4	25.7	42.22 ± 6.13

[a] Dose = 250 mg/kg orally in 0.3% tragacanth suspension,

tation pattern of this compound in the mass spectrometer indicated a compound different from the parent drug when the mass spectra were compared. Nmr spectrometry indicated a para-substituted phenol. The ir spectrum was inconclusive, There was no evidence of azide, which would be indicative of an opened thiatriazole ring. Whether a tetrazole ring is formed by rearrangement of the thiatriazole moiety is not clear. Attempts to synthesize the 1-*p*-hydroxyphenyltetrazoline-5-thione have failed. However, an unsubstituted phenyltetrazole (1-phenyltetrazoline-5-thione) was used for comparison purposes. Comparison of the ir spectrum of the authentic 1-phenyltetrazoline-5-thione with the spectrum obtained from the unknown indicated markedly different absorption bands. Yet, mass spectrometry seemed to indicate sufficiently similar fragmentation patterns for the unknown and the tetrazole analog, so it is possible that the unknown is a tetrazole. Because the parent drug 1 does not undergo a similar change in structure under the extraction conditions (pH 8 or 2), it seems unlikely that this unknown compound is an artifact arising from alteration of the parent drug under extraction conditions. It appears, rather, to be a metabolite which was formed *in vivo*.§

The major metabolic pathway for the disposition of 1 in rats is ethereal sulfate conjugation of the phenol; sulfate conjugates account for some 66% of the radioactivity in the urine, Glucuronide conjugation appears to play a relatively minor role at the dosage employed (250 mg/kg); glucuronide conjugates account for some 6–7% of the urinary radioactivity. There were found some minor metabolites indicating some fragmentation of the thiatriazole moiety (at least 14%). The unknown metabolite described above accounts for some 7% of the urinary radioactivity. A summary of the metabolic fate of 1 is shown as Figure 2.

§ See paragraph at end of paper regarding supplementary material.

Table VII. Extraction of 0–48 hr Rat 31699-^{3}H Urines after Treatment with Enzymes

Extract	% of radioactivity extracted after	
	Ketodase treatment	Glusulase treatment
pH 8 EtOEt extract	6.65[a]	72.3[a]
pH 2 EtOEt extract	0.90	7.45
Aqueous residue	92.4	20.6

[a] Results are average of duplicate determinations.

It appears reasonable to postulate that the minor metabolites arise from a ring-opened azide intermediate as shown in Figure 2. This azide would be unstable and would quickly decompose to give the *p*-hydroxyphenylthiourea and possibly the tetrazole or *p*-aminophenol. The *p*-hydroxyphenylthiourea could further be metabolized or may degrade during work-up, yielding *p*-hydroxyphenylurea and elemental sulfur. The *p*-aminophenol would be acetylated *in vivo* to form APAP. Since azides are known to have antihypertensive activity,[1] it is possible that the agent responsible for the antihypertensive activity of 1 is the proposed transient azide.

Experimental Section

Drug. Tritiated 1 was prepared from back-exchanged and purified Wilzbach-tritiated *p*-aminophenol by Mr. Arthur Alter of our laboratories. Chemical and radiochemical purity of the final preparation was established by thin-layer chromatography on

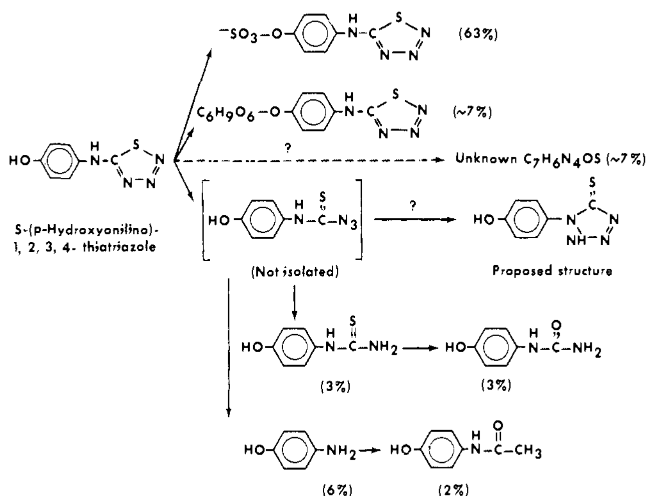

Figure 2. Metabolic fate of 5-(*p*-hydroxyanilino)-1,2,3,4-thiatriazole in the rat: dose, 250 mg/kg po.

silica gel plates in at least four different solvent systems (systems 1-4). Whenever unlabeled drug was required, lot no. 811-308 was used. The initial specific activity of the labeled drug was 1.0 μCi/mg.

Animals. The animals used were male rats of the Sprague-Dawley strain obtained from the Charles River Laboratories, North Wilmington, Mass., and weighed approximately 200 g each. The rats were fasted overnight prior to experimentation and, with the exception of the bile-duct cannulated rats, were allowed Purina laboratory chow and water *ad libitum* after dosing. The bile-duct cannulated rats were in restraining cages and were maintained on Aminosol during these experiments.

Radiochemical Analysis of Samples. Aliquants of urine and cage washes obtained from the animals as well as other liquid samples were counted directly in diotol scintillator solution (a mixture of 350 ml of toluene, 350 ml of dioxane, and 210 ml of methanol, containing 73 g of naphthalene, 4.6 g of 2,5-diphenyloxazole, and 80 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene). Tissue, bile, and fecal samples were assayed radiochemically either by a direct counting procedure described by Petroff and coworkers[2] or by the Schöniger oxygen flask combustion method as modified by Thomas.[‡] All samples were counted in a liquid-scintillation counter at conditions suitable for measurement of tritium and corrected for quenching by the internal standard technique.

Thin-Layer Chromatography. Thin-layer chromatographic studies were performed on commercially prepared plates coated with silica gel. These were either Brinkmann silica gel F-254, 250 μ, or Quantum Q1F, 250 μ. After development in solvent, radioactivity was measured either by scanning on a Packard radiochromatogram scanner or by scraping sequential 0.5-cm sections into scintillation vials containing liquid-scintillation phosphor solution and counting in the liquid-scintillation counter. The solvent systems used for thin-layer chromatography were (1) chloroform-methanol-NH_4OH (85:15:1, v/v), (2) chloroform-methanol (85:15, v/v), (3) 1-butanol-acetic acid-water (2:1:1, v/v), (4) chloroform-methanol (80:20, v/v), (5) chloroform-acetone-water (40:58:2, v/v), (6) chloroform-acetic acid (95:5, v/v), (7) chloroform-methanol-acetic acid (75:25:1, v/v), (8) chloroform-acetic acid-water (lower phase, 2:2:1, v/v), and (9) ethyl acetate-methanol-28% NH_4OH (80:20:1, v/v).

Blood Level Studies. Two rats were each administered a single oral dose (250 mg/kg) of tritiated 1 in tragacanth suspension *via* stomach tube. Blood samples were drawn from the tail vein at various times, and plasma samples were obtained by centrifugation. These plasma samples were assayed radiochemically by Schöniger combustion.

Absorption and Excretion Studies. Low Dose. Six rats were administered single oral doses (250 mg/kg) of tritiated 1 in 0.3% tragacanth suspension *via* stomach tube. Urines and feces were collected for 5 days after administration of the dose and assayed for radioactivity.

High Dose. Ten rats were administered single oral doses (1 g/kg) of tritiated 1 in 0.3% tragacanth suspension *via* stomach tube. Urine and feces were collected for 5 days after administration of the dose. At the end of 5 days, the animals were sacrificed and the carcasses and excreta were assayed for radioactivity.

Determination of Drug Excreted in Bile. Two rats were surgically fitted with bile-duct cannulae and placed in restraining cages. The rats were dosed orally with 250 mg/kg of tritiated 1, and the excreted bile was collected at 24-hr intervals. The volumes of bile samples were measured and appropriate aliquants were assayed for radioactivity by the modified Schöniger oxygen flask combustion method.

Determination of Tritiated Water in Rat Urines. A sample of the rat urine to be tested was placed in a vessel equipped with a cold finger trap. The urine was frozen, the system was placed under vacuum, and the water which was vaporized from the urine was trapped on the cold finger. This trapped water was then assayed for tritium, and the radioactivity attributable to tritiated water (THO) was calculated and expressed as per cent of the urinary radioactivity.

Isotopic Dilution. A measured portion of the urine obtained from the rats was placed in an erlenmeyer flask and treated with Glusulase to hydrolyze the glucuronide and ethereal sulfate conjugates. A solution of *N*-acetyl-*p*-aminophenol (APAP) in methanol (4 mmol) was added to the flask and the contents were mixed thoroughly. The solution was then evaporated to dryness on a rotary evaporator. The residue was dissolved in hot water and decolorized with carbon. A crop of APAP was obtained upon chilling for a few hours. After filtration and determination of its specific activity, the crop was dissolved in ethanol, decolorized with carbon, and recrystallized from ethanol-water. Recrystallizations from methanol-water and hot water were alternately repeated until there resulted a product with constant specific activity upon recrystallization. Five to seven recrystallizations were usually sufficient to achieve constant specific activity. This method was used to calculate the per cent of urinary radioactivity attributable to APAP.

Isotopic dilution studies using unlabeled *p*-hydroxyphenylthiourea and 1 were performed in a similar fashion, using appropriate solvents (ethanol-water, methanol-water, or acetone-water) for recrystallization of the compounds.

Enzymic Hydrolysis of Conjugates. Enzymic hydrolyses were performed on 0-48-hr urines from the rats. The urine was adjusted to pH 5 with glacial acetic acid; then a half-volume of pH 5.0, 0.1 *M*, acetate buffer was added to the urines. A few milligrams of EDTA (to chelate any metal ions) and a few drops of chloroform (preservative) were added to each flask containing the incubation mixture; then 10,000 Fishman units[3] of β-glucuronidase activity were added for each 5 ml of urine. The flasks containing the incubation mixture were loosely stoppered and placed in a 37° water bath and gently agitated for 72 hr. Equivalent amounts of both Glusulase and Ketodase were used in these studies.

Solvent Extraction of Urines. The enzyme-hydrolyzed urines were made alkaline (pH 8) with $NaHCO_3$ and extracted four times with equal volumes of ethyl ether. The aqueous phase was then acidified to pH 2 (6 *N* H_2SO_4) and extracted an additional four times with equal volumes of ethyl ether. Finally, the aqueous phase was saturated with $(NH_4)_2SO_4$ and extracted four times with ethyl ether. Each of the extracts and the aqueous residue were sampled for radioactivity to determine the distribution of radioactivity into the various phases. The pH 8 extraction removed 72% of the urinary radioactivity. The pH 2 ether extraction removed 7.5% more, and the ether extract of $(NH_4)_2SO_4$-saturated urine removed another 11% of the urinary radioactivity.

Work-Up of Extracts. Each extract was concentrated to a small volume and analyzed by thin-layer chromatography on commercial silica gel chromatoplates using several solvent systems. Authentic compounds, where available, were cochromatographed in order to identify the constituents of the extracts. Unchanged parent drug was recovered from the pH 8 extracts by concentration of the extracts and recrystallization of the crystalline crop.

The pH 2 extracts were concentrated and placed on a 2.5 × 60 cm column of Florisil packed in benzene. The column was eluted with benzene containing increasing concentrations of acetone, and the major radioactive peak was eluted with 5-10% acetone. The fractions comprising this peak were combined and concentrated, streaked onto preparative thin-layer chromatoplates (Quantum PQ1), and developed in solvent system 9. The single radioactive region was eluted with acetone and decolorized with carbon. The

‡ R. C. Thomas, The Upjohn Company, Kalamazoo, Mich., personal communication.

acetone solution, when taken to dryness, yielded a solid which was analyzed by nmr, ir, and mass spectrometry.

The major constituent of the ether extract of $(NH_4)_2SO_4$-saturated urine was purified by successive chromatography on three silica gel columns, using benzene or chloroform with increasing concentrations of methanol. The fine white needles which were obtained were recrystallized from a mixture of methanol and chloroform and then analyzed by nmr, ir, and mass spectrometry. It was found to be identical with a synthetic sample of *p*-hydroxyphenylurea prepared by the method of Kalckhoff.[4]

Spectral Analyses. The nmr spectra were obtained at 100 MHz on a Varian Associates HA-100 spectrometer, using acetone-d_6 as solvent. The ir spectra were obtained on a Perkin-Elmer Model 521 infrared spectrometer, using KBr pellets. The mass spectra were obtained on an AEI-MS-902 mass spectrometer at 70 eV, using direct probe for introduction of the sample.

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Supplementary Material Available. Supplementary material consisting of the mass spectrum of authentic 5-(*p*-hydroxyanilino)-1,2,3,4-thiatriazole, mass and ir spectra of the unknown metabolite of 5-(*p*-hydroxyanilino)-1,2,3,4-thiatriazole, and the mass and ir spectra of the synthetic analog, 1-phenyltetrazoline-5-thione, will appear following these pages in the microfilm edition of this volume of the journal, Photocopies of the supplementary material from this paper only on microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-73-1157,

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Synthesis of Antimicrobial Nitroimidazolyl 2-Sulfides, -Sulfoxides, and -Sulfones

Robert C. Tweit, E. M. Kreider,* and R. D. Muir

Departments of Chemical and Biological Research, Searle Laboratories, A Division of G. D. Searle & Co., Chicago, Illinois 60680.
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Imidazoles having a variety of alkyl and aralkyl sulfur substituents at the 2 position, and their 5- and 4-nitro analogs, were synthesized and tested for a broad spectrum of biological activities. Many of the nitroimidazoles were potent *in vitro* trichomonacides; other activities observed among the structural series prepared include antibacterial, antifungal, antinematode, and antiinflammatory.

The introduction of 1-(2-hydroxyethyl)-5-nitro-2-methylimidazole (Flagyl; metronidazole) as a highly effective agent for treatment of human trichomoniasis and of 1,2-dimethyl-5-nitroimidazole (Emtryl; dimetridazole) for turkey histomoniasis has stimulated a number of synthetic programs involving nitroimidazoles, This work has resulted in several compounds which are potential products in the human or animal fields: *e.g.*, 1-methyl-2-isopropyl-5-nitroimidazole (ipronidazole);[1] 1-(2-morpholinoethyl)-2-methyl-5-nitroimidazole (nitrimidazine);[2] 1-methyl-2-carbamoyloxymethyl-5-nitroimidazole (Ridzole; ronidazole);[3] 1-(2-hydroxyethyl)-2-(*p*-fluorophenyl)-5-nitroimidazole (flunidazole);[4] 1-methyl-2-(*p*-fluorophenyl)-5-nitroimidazole (MK-910);[5] 1-(2-ethylsulfonylethyl)-2-methyl-5-nitroimidazole (Fasigyn; tinidazole);[6] 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole (CL 64885);[7] and various 2-nitroimidazole derivatives,[8] This paper describes several series of 2-(substituted mercapto)imidazoles and their nitro derivatives which were made in a search for a potent antitrichomonal agent with a broader biological activity profile than metronidazole.

Chemistry. The 1-alkyl-2-imidazolyl sulfides (Tables I, II, and VI-IX) were prepared by alkylation of the corresponding 1-alkyl-2-mercaptoimidazole with the appropriate halides in dioxane or 2-propanol.

Nitration of the sulfides was carried out by heating at 100° for 0.5-1.5 hr in aqueous nitric acid (100 parts of 70% HNO_3 to 40 parts of H_2O), This procedure was found to be preferable to H_2SO_4-HNO_3 nitrations which frequently became violent, Longer heating was inadvisable for arylmethyl sulfides owing to oxidative cleavage at the *S*-methylene bond as shown by the isolation of the corresponding benzoic acid, This oxidation could usually be detected by the appearance of solid after 45 min of heat-