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Hepatic Disposition and Biliary Excretion of the Organic Cations Thiazinamium and Thiazinamium Sulfoxide in Rats

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Received July 12, 1977, from the **Department of Pharmaceutical and Analytical Chemistry and the* ‡*Department of Pharmacology, State University of Groningen, Groningen, The Netherlands.* Accepted for publication December 6, 1977.

Abstract □ The disappearance from plasma and the excretion in bile of the monoquaternary thiazinamium (administered as the iodide) and one of its polar metabolites, thiazinamium sulfoxide (also administered as the iodide), were studied in the rat after intravenous injection to obtain more information on hepatic transport mechanisms for organic cations. Both compounds exhibited an extremely rapid plasma disappearance, partly due to a rapid liver uptake. After injection of thiazinamium iodide and thiazinamium sulfoxide iodide, 36 and 47%, respectively, of the administered dose were excreted in bile during 1 hr. TLC analysis of the bile showed at least two unidentified polar metabolites in addition to thiazinamium sulfoxide and only 3.8% unchanged thiazinamium after administration of thiazinamium iodide. The same metabolites were found after injection of thiazinamium sulfoxide iodide. Urinary excretion and intestinal secretion were 18 and 12%, respectively, for thiazinamium sulfoxide iodide and 27 and 9%, respectively, of the dose for thiazinamium iodide. It is concluded that, in spite of unequal physicochemical features, thiazinamium iodide and thiazinamium sulfoxide iodide differ only slightly in hepatic uptake and metabolism.

Keyphrases □ Thiazinamium—tissue distribution and biliary excretion in rats □ Distribution, tissue—thiazinamium in rats □ Excretion, biliary—thiazinamium in rats □ Phenothiazine derivatives—thiazinamium, tissue distribution and biliary excretion in rats

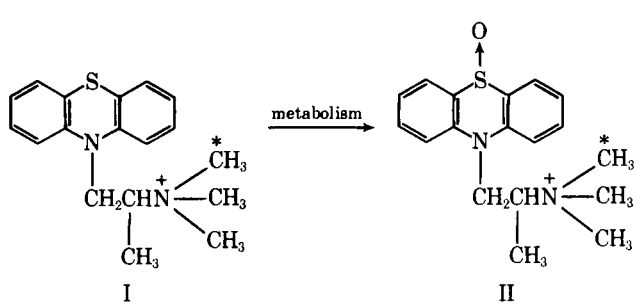
Factors such as molecular weight, polarity, molecular structure, lipophilicity, protein binding, and metabolism were reported (1–3) to influence biliary excretion of drugs. It was suggested (4) that biliary excretion of quaternary ammonium compounds requires a polar organic cation group and a relatively nonpolar ring structure in the molecule.

Thiazinamium (I) iodide is a phenothiazine derivative containing a quaternary ammonium group. No metabolites were detected in rats; no details were provided on biliary excretion of thiazinamium and its metabolites (5). In humans, dogs, and monkeys, one metabolite was detected (6) in bile and urine: thiazinamium sulfoxide (II) (Scheme I, asterisks indicate ^{14}C-label). Introduction of the oxygen in the phenothiazine nucleus results in a much more polar compound (Table I). Therefore, it was of interest to study whether this structural and physicochemical change would result in different hepatic disposition.

^{14}C-Thiazinamium iodide and ^{14}C-thiazinamium sulfoxide iodide were prepared and were used to investigate the distribution, biotransformation, and elimination in rats, with special reference to biliary excretion.

EXPERIMENTAL

Synthesis of ^{14}C-Thiazinamium Iodide—Synthesis of ^{14}C-thiazinamium iodide according to the reaction conditions described by Huang *et al.* (5) resulted in a radiochemical yield of only 8%. The conditions were modified, resulting in the following procedure. Promethazine, 500 mg, was allowed to react for 72 hr at 21° with 251 mg of ^{14}C-methyl iodide (100 μCi total activity) in 25 ml of acetone. After the acetone was removed, the substance was recrystallized (24 hr) in 20 ml of a hot mixture of dichloromethane–benzene (10:9). After drying, a chemically pure product (>99.5%) was obtained (95% yield). TLC analysis showed no radio-



Scheme I

chemical impurities. The specific activity was 0.13 μCi/mg (radiochemical yield of 86%, mp 217°).

Synthesis of ^{14}C-Thiazinamium Sulfoxide Iodide—Promethazine hydrochloride, 500 mg, was treated with 360 mg of 30% H_2O_2 in 1.25 g of 30% acetic acid at 40–60° for 1 hr. After extraction with dichloromethane and crystallization in carbon tetrachloride, promethazine sulfoxide was obtained. This product was used for quaternization with ^{14}C-methyl iodide as described for ^{14}C-thiazinamium iodide. No crystallization was necessary because thiazinamium sulfoxide iodide precipitates after its formation in acetone. Washing with acetone gave a chemically pure product (more than 99.5%). The yield was 700 mg (95%), with a specific activity of 0.12 μCi/mg (radiochemical yield of 84%). TLC analysis showed no radiochemical impurities.

Chemical Analysis—The authenticity of the compounds was checked by UV, IR, mass spectrometric, and TLC (methanol–water–ammonia–ammonium acetate, 200:42:6:8) analyses. Purity control was also performed by TLC.

Distribution–Metabolism Studies—Male Wister rats, 250–300 g, were anesthetized with pentobarbital sodium (0.35 ml ip of a 60-mg/ml solution). The animals were intubated and artificially respired. The abdomen was opened, and the bile duct was cannulated with a polyethylene cannula. A similar cannula was placed in the carotid artery for rapid blood sampling. During the experiment, a heating lamp was placed above the animal to maintain normal body temperature (37.8°). Drugs were injected intravenously during 10 sec (vena femoralis).

Immediately after injection, bile samples were collected at 5-min intervals. Blood samples (100 μl) were taken 30, 60, 90, 120, 150, 180, 210, and 240 sec after thiazinamium iodide injection and 30, 60, 90, 120, 180, 240, and 300 sec after thiazinamium sulfoxide iodide injection. After 1 hr, the liver, gut, and kidneys were rapidly removed and stored on ice. Urine was collected from the bladder with a syringe. The administered dose of thiazinamium iodide was 840 nmoles in a 1-ml solution by bolus injection. Thiazinamium sulfoxide iodide was administered in two doses of 2285 and 840 nmoles in separate groups of rats, also by bolus injection. Plasma disappearance rates at two doses of thiazinamium sulfoxide iodide, expressed as the percentage of the dose, were nearly identical. In another group of rats, liver and kidneys were removed 5 min after thiazinamium iodide (dose 840 nmoles) and thiazinamium sulfoxide iodide injection, and total activity in these organs was measured.

In some control experiments, carbon dioxide expired by the rats was trapped in a benzethonium chloride[1]–ethanol mixture to which thymolphthalein was added as indicator. Total absence of radioactivity in this solution indicated that no demethylation of ^{14}C-thiazinamium occurred.

Radiochemical Analysis—Thiazinamium and carbon-14-containing metabolites were determined by liquid scintillation counting (total radioactivity) in plasma, liver, gut, kidneys, bile, and urine. All samples were counted for 10 min, and counting was corrected for quenching by external standardization.

Plasma—Blood samples were heparinized and centrifuged. Then 25 μl of the supernate was mixed with 10 ml of premixed scintillator[2].

Bile—Aliquots of 25 μl were taken for TLC analysis. The remaining part of the samples was mixed with 10 ml of scintillator for counting.

Tissues—Liver, gut, and kidneys were dissected out, weighed, and cut into pieces. The tissues were homogenized with 40 ml of saline, and 0.5 ml of the homogenate was mixed with scintillator for radiochemical analysis.

Urine—An aliquot of 50 μl of urine was mixed with 10 ml of scintillator and counted.

TLC Analysis—Two systems were used to separate and detect the substances under study and their metabolites in bile. System I was the same as mentioned under *Chemical Analysis*, and System II was methanol–chloroform–sodium iodide (20:80:0.5 *M*). Bile samples of 25 μl were applied to silica gel 60 F_{254} alumina plates[3]. The position of the compounds was detected by cutting the plate in 0.5-cm pieces and liquid scintillation counting.

To remove the substances from the silica gel, 0.6 ml of a 10% NaI solution was added to the scintillator. Some plates were scanned[4] for radioactivity. Addition of labeled thiazinamium iodide and thiazinamium sulfoxide iodide to bile and subsequent TLC resulted in only one radioactive spot for each substance.

[1] Hyamine.
[2] Aquasol, New England Nuclear Chemicals.
[3] Merck.
[4] Dünnschicht-Scanner II, Berthold (nr. LB 2723).

Table I—Physicochemical Properties of Thiazinamium and Thiazinamium Sulfoxide

	Thiazinamium	Thiazinamium Sulfoxide
R_f value, TLC (silica gel, chloroform–methanol—0.5 *M* NaI)	0.64	0.50
Protein binding[a] (dialysis), % bound, 60–120 nmoles/ml of plasma	65	20
Lipid solubility[b], % in organic phase, 100 nmoles/ml of aqueous phase	75	3

[a] Protein binding was measured at two concentrations (the initial concentration in plasma is indicated). Percent binding was found to be similar at these concentrations, so values were averaged (n = 6). [b] Mean values of three experiments.

Determination of Lipid Solubility and Protein Binding—Lipid solubility was estimated by partition of the compounds between equal volumes of 1-octanol and a modified Krebs–Ringer solution from which carbon dioxide and sodium bicarbonate were omitted, and the pH was adjusted to 7.4 with sodium hydroxide (7). Recovery of the agents after equilibration was more than 95%. Protein binding was determined by equilibrium dialysis at 37° of 1 ml of defibrinated rat blood plasma and 2 ml of Krebs bicarbonate solution (pH 7.4). Virtually no binding occurred to the dialysis sacs.

RESULTS AND DISCUSSION

Introduction of oxygen by oxidation of the sulfur atom in the phenothiazine nucleus of thiazinamium resulted in a substance with quite different physicochemical features. Table I shows that R_f values with TLC, protein binding, and especially extraction into octanol were considerably lower for the thiazinamium sulfoxide than for the parent compound. Thus, two closely related organic cations with varying polarity due to the ring structure were obtained.

Figure 1 shows that the plasma concentration for both compounds after 4 min was less than 20% of the dose. After 5 min, about 40% of the dose of thiazinamium and thiazinamium sulfoxide was found in the liver and about 20% in the kidneys, expressed as percentage of radioactivity, indicating that the rapid elimination from the blood was predominantly due to uptake in these excretory organs. Despite unequal physicochemical properties, there was no major difference in the plasma elimination rates (Fig. 1), hepatic uptake, or biliary excretion patterns of thiazinamium and thiazinamium sulfoxide. Biliary concentrations were high compared with the plasma levels. This result may be due to both active secretion and diffusion followed by binding to micelles (4, 7, 8). After administration of thiazinamium iodide, TLC analysis of bile with Systems I and II showed at least two metabolites in addition to thiazinamium sulfoxide.

The same metabolites with identical R_f values in both TLC systems were found after injection of thiazinamium sulfoxide iodide. Figure 2 shows that the amount of polar metabolites relative to thiazinamium sulfoxide increased with time. The biliary excretion patterns of thia-

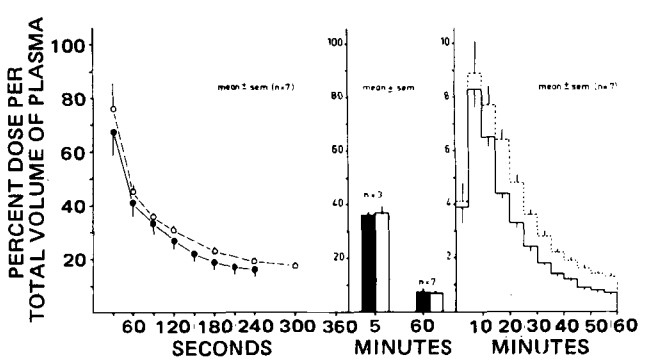


Figure 1—*Plasma disappearance (percent of dose in plasma) (left), hepatic content (percent of dose) 5 and 60 min after injection (middle), and biliary excretion (percent of dose per 5-min fraction, noncumulative) (right) after intravenous administration of thiazinamium iodide (●, ■, —) and thiazinamium sulfoxide iodide (○, □, - - -) (dose = 840 and 2285 nmoles, respectively). Total radioactivity in plasma, liver, and bile is indicated. The substances after injection were assumed to be distributed over the total plasma volume (8% of the body weight), in accordance with the observed plasma concentration at t = 0 from semilogarithmic plots of the plasma curve.*

Table II—Biliary Excretion [a] of Thiazinamium Cation, Thiazinamium Sulfoxide Cation, and Metabolites

Compound	Dose: Thiazinamium Iodide, 840 nmoles	Dose: Thiazinamium Sulfoxide Iodide, 2285 nmoles	Dose: Thiazinamium Sulfoxide Iodide, 840 nmoles
Thiazinamium cation	3.8	—	—
Thiazinamium sulfoxide cation	16.1	30.5	31.8
Metabolite 2	13.1	12.7	20.8
Metabolite 3	2.7	3.6	8.6

[a] Mean values of at least three experiments. Amounts in bile are indicated as percent of the dose excreted in 1 hr (total radioactivity).

zinamium sulfoxide and the two unidentified metabolites (Fig. 3) were very similar after injection of thiazinamium iodide and thiazinamium sulfoxide iodide. Only very small amounts (3.8% of the dose) of unchanged thiazinamium were excreted in bile following administration of thiazinamium iodide (Table II). These results indicate that thiazinamium, after being taken up in the liver, was rapidly oxidized to the sulfoxide and that the additional unknown metabolites may have arisen from biotransformation of this compound.

The present data on the nature of excreted material in bile after administration of thiazinamium iodide contrast with results of Huang *et al.* (5), who suggested that only unchanged thiazinamium is excreted in feces and urine. The distribution of radioactivity after injection of thiazinamium iodide and thiazinamium sulfoxide iodide in some excretory organs, bile, and urine is pictured in Fig. 4. Total recovery in bile, urine, and these organs amounted to 72 and 88% of the dose, respectively. Considerable amounts were detected in kidneys and gut 1 hr after injection. Although the nature of the radioactive material in the kidneys and gut was not studied, its presence probably was related to secretion processes for organic cations in the renal tubuli and in the intestinal mucosa (9).

Schanker (4) proposed that a nonpolar ring system and a polar cationic group would be required for hepatic transport of organic cations from plasma into bile. The present results support this hypothesis concerning the biliary excretion of thiazinamium. The presence of abundant amounts of the more polar thiazinamium sulfoxide in bile indicates that, in spite

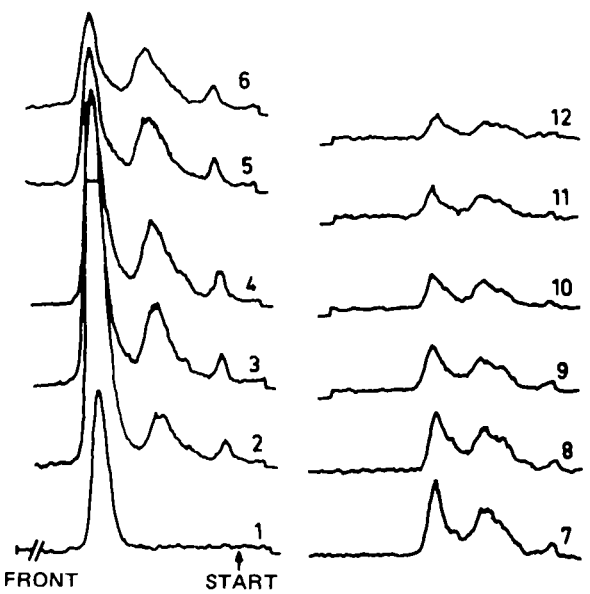


Figure 2—*Example of TLC analysis of 12 5-min bile samples collected after injection of thiazinamium sulfoxide iodide (numbers indicate first to 12th sample). TLC plates were developed with System I and scanned for radioactivity. The major peak (1–6) had an R_f value identical to thiazinamium sulfoxide. The two radioactive spots with lower R_f values probably represent more polar metabolites (Metabolites 2 and 3, Fig. 4). They were not detected when reference compounds thiazinamium iodide and thiazinamium sulfoxide iodide were added to rat bile and chromatographed using the same TLC system.*

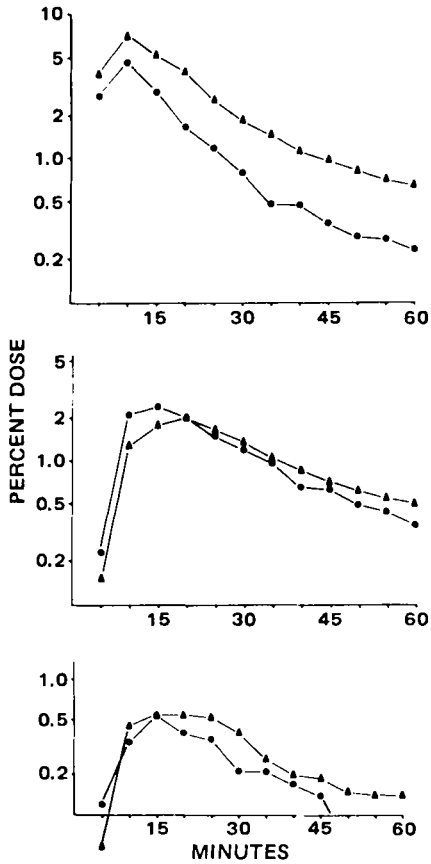


Figure 3—*Biliary excretion patterns (percent of dose per 5-min fraction, noncumulative) of thiazinamium sulfoxide (Metabolite 1) (top), Metabolite 2 (middle), and Metabolite 3 (bottom) after intravenous injection of thiazinamium sulfoxide iodide (▲) (2285 nmoles) and thiazinamium iodide (●) (840 nmoles). The compounds were separated by TLC and quantified by removal from the plates and scintillation counting. The values represent means of at least three experiments.*

of the change in polarity of the ring structure, enough hydrophobic sites remain to guarantee efficient biliary output. The data in Fig. 3 and Table II even suggest a more rapid biliary excretion of material after administration of thiazinamium sulfoxide than with thiazinamium iodide.

Furthermore, the uptake, as indicated by hepatic levels 5 min after injection of the compounds, was very similar, suggesting that the polarity of the ring structure in itself is not a critical factor in hepatic transport of these cations. Although oxidation of the phenothiazine nucleus of thiazinamium does not result in a major change in the hepatic disposition, previous studies with other anticholinergic agents (2), in which the ring structure of the agents was completely removed from the quaternary

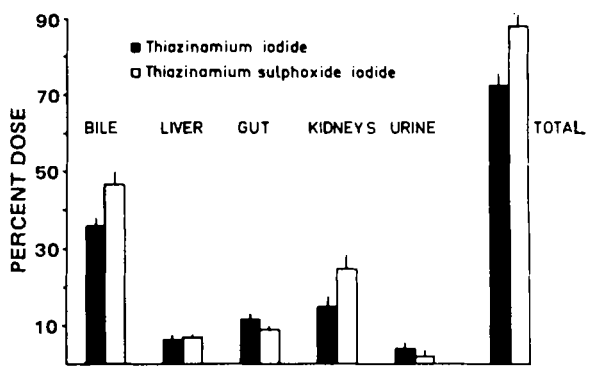


Figure 4—*Distribution of radioactivity 1 hr after intravenous injection of thiazinamium iodide (840 nmoles) and thiazinamium sulfoxide iodide (2285 nmoles). The amounts in the liver, kidneys, and gut and material cumulatively excreted in bile and urine are indicated as percent of administered dose (mean ± SEM, n = 7).*

ammonium part of the molecules by acid hydrolysis, indicate that the presence of a relatively nonpolar lipophilic part in the molecule of organic cations is a prerequisite for efficient biliary excretion.

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Interference in Assays for Hydralazine in Humans by a Major Plasma Metabolite, Hydralazine Pyruvic Acid Hydrazone

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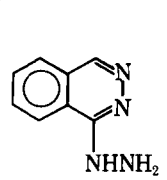
Received July 6, 1977, from the *Department of Clinical Pharmacology, The Queen Elizabeth Hospital, Woodville, 5011, South Australia.* Accepted for publication December 9, 1977.

Abstract □ The present study showed that published spectrophotometric and GLC methods for hydralazine in plasma do not distinguish between the drug and a major plasma metabolite, hydralazine pyruvic acid hydrazone. These methods involve the acidic treatment of the sample, which hydrolyzes the hydrazone back to hydralazine. A specific GLC assay for the hydrazone was developed and involves its selective extraction from plasma and transformation to 3-trifluoromethyl-*s*-triazolo[3,4-*a*]phthalazine. This derivative could be sensitively measured by GLC using an electron-capture detector. With this procedure, it was shown that most "apparent hydralazine" in plasma is the hydrazone, which forms rapidly from hydralazine and endogenous pyruvic acid. Previous work indicated that the hydrazone was inactive when administered intravenously to rabbits.

Keyphrases □ Hydralazine—interference in published GLC and spectrophotometric analyses in plasma by major metabolite □ Metabolites—hydralazine pyruvic acid hydrazone, GLC analysis in plasma □ GLC—analysis, hydralazine pyruvic acid hydrazone in plasma □ Antihypertensives—hydralazine, interference in published GLC and spectrophotometric analyses in plasma by major metabolite

The vasodilator hydralazine, 1-hydrazinophthalazine (I), is an effective agent for the treatment of arterial hypertension (1). It undergoes extensive metabolism in humans and animals, and Metabolites II–VIII were identified (1–11). Acetylation was proposed as the major pathway for hydralazine clearance (2–8), where the acetylated drug, 3-methyl-*s*-triazolo[3,4-*a*]phthalazine (III), may be further metabolized to 3-hydroxymethyl-*s*-triazolo[3,4-*a*]-phthalazine (IV) and its glucuronide conjugate (2, 11).

Although hydralazine can form hydrazones readily with compounds having an active carbonyl group, hydralazine pyruvic acid hydrazone, 1-hydrazinophthalazine pyruvic acid hydrazone (II), was identified only as a minor metabolite in rat urine following oral administration of hydralazine (2, 4). Fresh human blood contains pyruvic acid in a concentration of approximately 100 μmoles/liter, which is a considerable excess to the commonly found therapeutic levels of "apparent hydralazine" and appears to favor formation of II. Preliminary findings indicated (12) that plasma apparent hydralazine, or that measured



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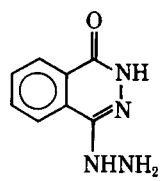

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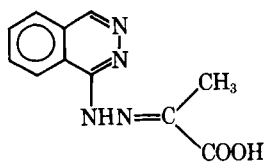

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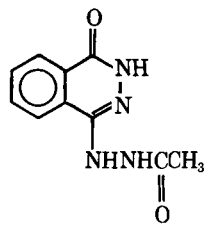

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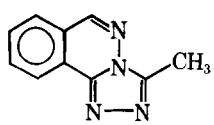

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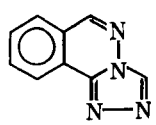

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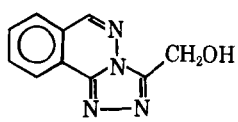

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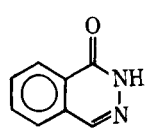

VIII

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