Porfiromycin (PM, Fig. 1) is the aziridine N-methyl analog of mitomycin C (MC), a bioreductive alkylating agent that has been shown to be preferentially cytotoxic to hypoxic tumor cells in vitro and in vivo (Iyer and Szybalski, 1964; Keyes et al., 1985; Fracasso and Sartorelli, 1986; Marshall and Rauth, 1988; Rockwell et al., 1993). Interestingly, PM generates a greater differential toxicity to hypoxic EMT6 and Chinese hamster ovary tumor cells versus their aerobic counterparts than that of MC (Rockwell et al., 1988; Belcourt et al., 1996). Furthermore, human phase I and phase II clinical trials have demonstrated that PM is tolerated at a dose 3-fold higher than that of MC (Foley et al., 1967; Loo et al., 1967; Izbicki et al., 1972; Grage et al., 1975; Baker et al., 1976). The findings indicate that PM is potentially superior to MC in clinical applications for the treatment of solid tumors. Currently, PM is under development for the treatment of head and neck cancers as an adjunct to radiation therapy in phase III clinical trials.

In vitro metabolism and bioreductive activation of MC under hypoxic conditions have been extensively studied (Schwartz, 1962; Tomasz and Lipman, 1981; Kennedy et al., 1982; Pan et al., 1984). The enzymes responsible for the reductive metabolism of MC are NADPH:cytochrome c (P-450) reductase (Keyes et al., 1984; Pan et al., 1986), xanthine oxidase (Pan et al., 1984, 1986), xanthine dehydrogenase (Gustafson and Prisots, 1992), DT-diaphorase (Siegel et al., 1990; Pan et al., 1992, 1995; Robertson et al., 1992; Beall et al., 1994), and NADH:cytochrome b_5 reductase (Hodnick and Sartorelli, 1993). In contrast, the information on the metabolism and disposition of PM is limited. To date, three metabolites of PM resulting from biotransformation at the C-1 position in the presence of purified NADPH:cytochrome c reductase and xanthine oxidase have been identified (Pan and Iracki, 1988; Pan, 1990). Recently, we reported the identification of several C-1 and C-10 metabolites of PM arising from a rat liver preparation under aerobic conditions (Lang et al., 2000). However, the in vivo metabolism and disposition of PM under physiological conditions are still unclear.

To understand the metabolic fate of PM, we conducted the studies on the metabolism and disposition of PM in dogs and humans. In this article, we present: 1) urinary and fecal excretion of radioactivity derived from [methyl-^3H]PM in dogs; 2) isolation and identification of urinary metabolites of PM in dogs and humans; 3) comparison of urinary metabolite profiles between the two species; 4) chemical syntheses of the major urinary metabolites; and 5) postulation of metabolic pathways of PM on the basis of the metabolite profiles.

**Experimental Procedures**

**Materials.** Porfiromycin injection, 15 mg, (Promycin) was manufactured at Chesapeake Biological Lab., Inc. (Owings Mills, MD). [Methyl-$^3$H]PM (1.4 mg/ml of ethanol), specific activity 235 mCi/mmol, was purchased from Moravek Biochemicals, Inc. (Brea, CA). Methanol and potassium phosphate monobasic were of HPLC grade, and were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Fisher Scientific Co. (Pittsburgh, PA), respectively.
Ammonium acetate, potassium hydroxide, and 37% hydrochloric acid were used from American Chemical Society reagents and were purchased from J. T. Baker, Inc. (Phillipsburg, NJ). Tris(hydroxymethyl)-aminomethane, l-cysteine, and N-acetyl-l-cysteine were purchased from Aldrich Chemical Co. Milli-Q Plus ultra pure water was used throughout the study.

Animals, Dosing, and Sample Collection. Three adult male Beagle dogs (Marshall Laboratories of North Rose, NY), weighing from 8.4 to 9.2 kg, were used in this study. A dosing solution was prepared by dissolving six vials of porfiromicron injection (15 mg) and [methyl-\textsuperscript{3}H]PM (0.7 mg) into a final volume of 45 ml of water to produce a 2 mg/ml [methyl-\textsuperscript{3}H]PM solution (specific activity 5.46 μCi/mg). The dogs were given a single i.v. dose of PM dosing solution at 2 mg/kg (∼40 mg/m\textsuperscript{2}) and housed individually in metabolism cages with access to water and food. Urine and feces of the test animals were collected at 0 to 4, 4 to 8, and 8 to 24 h, and at 24-h intervals thereafter for 7 days. The samples were stored immediately at −20°C.

Human Urine Sample. A human urine sample obtained from a patient who received a PM/radiation therapy trial at Yale New Haven Hospital is presented in this article. The patient was given a single i.v. dose of PM at 40 mg/m\textsuperscript{2}. The urine sample was collected at 0 to 2.5 h after the dose and stored at 80°C until used.

Urine Sample Preparation. After thawing, each 10-ml urine sample was filtered through a 0.45-μm nylon filter. The filtrate was loaded onto two Waters Sep-Pak Plus C18 cartridges connected in tandem (sorbent weight of 360 mg each). The cartridge set, preconditioned with 5 ml of methanol and 10 ml of water sequentially, was washed with 10 ml of water, and then eluted with 5 ml of methanol/water (2:1, v/v). The methanolic effluent was collected and concentrated under a stream of nitrogen. Aliquots of 100 μl of the resulting solution were repeatedly injected onto HPLC for isolation.

HPLC. A Hewlett-Packard 1100 liquid chromatography system (Wilmington, DE) was used for isolation of urinary metabolites and the determination of PM in dog urine. The LC system consisted of a quaternary pump G1311A, an autosampler G1313A, a diode array detector G1315A, and a column thermostat G1316A. Hewlett-Packard LC3D ChemStation software was used for autosampler G1313A, a diode array detector G1315A, and a column thermostat G1316A. Tris(hydroxymethyl)-aminomethane, L-cysteine, and N-acetyl-L-cysteine-S-yl)-7-aminomitosene (M1). l-Cysteine (17.8 mg) and PM (5.1 mg) were dissolved in 2.0 ml of an aqueous 0.1 M Tris/160 HCl buffer, pH 7.0. The solution was deaerated by bubbling He gas for 5 min, then 2.1 mg of platinum oxide was added. The reaction was carried out by bubbling H\textsubscript{2} gas at 60–65°C for 2 h. The reaction mixture was then exposed to air, filtered, and concentrated in vacuo.

The product was purified by reversed-phase high performance liquid chromatography (RP-HPLC). Electrospray ionization-mass spectrometry (ESI-MS): m/z (intensity %) 379 (MH, 100), 258 (56); \textsuperscript{1}H NMR (methyl-d\textsubscript{4}): \textit{δ} 1.78 (s, C(6)CH\textsubscript{3}), 2.42 (s, NCH\textsubscript{3}), 2.72 (dd, J = 16.4, 4.8 Hz, C(1)H\textsubscript{2}), 2.77 (dd, J = 14.4, 9.9 Hz, C(1)H), 3.14 (dd, J = 14.5, 3.7 Hz, C(1)'H), 3.18 (dd, J = 16.5, 7.3 Hz, C(1)H\textsubscript{2}), 3.75 (dd, J = 9.7, 3.7 Hz, C(2)H\textsubscript{2}), 3.83 (1/2ABq, J = 13.3, C(10)H\textsubscript{2}), 3.94 (m, C(2)H\textsubscript{2}), 3.96 (1/2ABq, J = 13.4, C(10)H), 4.02 (dd, J = 13.1, 4.6 Hz, C(3)H\textsubscript{2}), and 4.37 (dd, J = 12.1, 6.7 Hz, C(3)H\textsubscript{2}).

1.2-trans and 1.2-cis-1-Hydroxy-2-methylamino-7-aminomitosenes (M2 and M4). PM (14.0 mg) was dissolved into 10 ml of an aqueous 0.05 N HCl solution and stirred at room temperature for 3 h. The reaction solution was neutralized with a saturated aqueous NaHCO\textsubscript{3} solution and concentrated in vacuo. The crude diastereomers (M2 and M4) were separated and purified by semipreparative HPLC. The purification was performed on a Ultracearb ODS (30) column, 5 μm, 250 × 10 mm, at 30°C. The column was eluted with methanol/50 mM KHP\textsubscript{4}, pH 6.0, (3:7, v/v) at a flow rate of 2.0 ml/min. The effluent was collected with a Gilson fraction collector. Fractions containing the same product were pooled and concentrated in vacuo. The final solution of each product was purified using Sep-Pak Plus C18 cartridges to remove salts. The yield was 18% for M2 and 50% for M4. M2—ESI-MS: m/z (intensity %) 335 (MH, 80), 317 (7) and 274 (100); \textsuperscript{1}H NMR (methyl-d\textsubscript{4}): \textit{δ} 1.80 (s, C(6)CH\textsubscript{3}), 2.45 (s, NCH\textsubscript{3}), 3.62 (m, C(2)H), 4.01 (dd, J = 13.4, 3.2 Hz, C(3)H\textsubscript{2}), 4.47 (dd, J = 13.3, 6.3 Hz, C(3)H\textsubscript{2}), 4.96 (d, J = 2.3 Hz, C(1)H\textsubscript{2}), 5.16 (1/2ABq, J = 12.6 Hz, C(10)H\textsubscript{2}), 5.24 (1/2ABq, J = 12.6 Hz, C(10)H), 5.41 (1/2ABq, J = 12.9 Hz, C(9)H\textsubscript{2}), 5.25 (1/2ABq, J = 12.9 Hz, C(10)H).

10-Decarbamoyl-2-methylamino-10-(N-acetyl-L-cysteine-S-yl)-7-aminomitosenes (M5). N-Acetyl-l-cysteine (39.6 mg) and Tris base (30.2 mg) were dissolved in 2.0 ml of water to form a solution (pH 6.5), then 11.7 mg of PM was added. The solution was deaerated by bubbling He gas for 5 min, and then 2.1 mg of platinum oxide was added. Hydrogenation proceeded by bubbling H\textsubscript{2} gas at 50–55°C, and was terminated at 2 h. The reaction mixture was exposed to air and filtered through a 0.45-μm nylon filter. The filtrate was concentrated in vacuo, and the residue was further purified by RP-HPLC. ESI-MS: m/z (intensity %) 421 (MH, 100); \textsuperscript{1}H NMR (methyl-d\textsubscript{4}): \textit{δ} 1.79 (s, C(6)CH\textsubscript{3}), 1.94 (s, C(O)CH\textsubscript{3}), 2.72 (s, NCH\textsubscript{3}), 2.67 (dd, J = 13.2, 7.0 Hz, C(1)'H), 3.09 (dd, J = 13.2, 4.4 Hz, C(1)H), 3.15 (dd, J = 18.1, 2.9 Hz, C(1)H\textsubscript{2}), 3.21 (dd, J = 17.7, 7.0 Hz, C(1)H\textsubscript{2}), 3.62 (1/2ABq, J = 14.2, C(10)H\textsubscript{2}), 4.07 (d, J = 14.2, C(10)H), 4.23 (dd, J = 6.0, 4.4 Hz, C(2)H\textsubscript{2}), 4.25 (m, 1H, C(2)H\textsubscript{2}), 4.33 (dd, J = 14.2, 2.9 Hz, C(3)H\textsubscript{2}), 4.46 (dd, J = 14.2, 6.1 Hz, C(3)H\textsubscript{2}).
and the residue was purified by RP-HPLC. The purification was performed on a Ultrasphere ODS (30 cm, 5 μm, 250 × 10 mm) at 50°C. The column was eluted with MeOH/50 mM KH₂PO₄ buffer, pH 7.30 (45:55, v/v) at a flow rate of 2.0 ml/min. ESI-MS: m/z (intensity %) 319 (MH², 100), 258 (9). ¹H NMR (methanol-d₄): δ 1.78 (s, C(6)CH₃), 2.40 (s, NCH₃), 2.71 (dd, J₁ = 4.7 Hz, C(3)H), 3.17 (dd, J = 16.5, 7.3 Hz, C(1)H), 3.90 (m, C(2)H₃), 3.98 (dd, J = 12.9, 4.7 Hz, C(3)H₃), 4.37 (dd, J = 12.8, 6.7 Hz, C(5)H₅), 5.14 (s, C(10)H₂).

2-Methylamino-7-aminomitosene (M6). PM (24.7 mg) was dissolved in 10 ml of an aqueous 0.1 M KH₂PO₄ buffer, pH 4.55, and then 5.7 mg of platinum oxide was added. The mixture was deaerated by bubbling He gas for 5 min and followed by bubbling H₂ gas at room temperature for 1 h. The reaction mixture was exposed to air, and then filtered. The filtrate was concentrated in vacuo and the residue was purified by RP-HPLC. The purification was performed on a Ultrasphere ODS (30 cm, 5 μm, 250 × 10 mm) at 50°C. The column was eluted with MeOH/50 mM KH₂PO₄ buffer, pH 7.30 (45:55, v/v) at a flow rate of 2.0 ml/min. ESI-MS: m/z (intensity %) 319 (MH², 100), 258 (9). ¹H NMR (methanol-d₄): δ 1.78 (s, C(6)CH₃), 2.40 (s, NCH₃), 2.71 (dd, J₁ = 4.7 Hz, C(3)H), 3.17 (dd, J = 16.5, 7.3 Hz, C(1)H), 3.90 (m, C(2)H₃), 3.98 (dd, J = 12.9, 4.7 Hz, C(3)H₃), 4.37 (dd, J = 12.8, 6.7 Hz, C(5)H₅), 5.14 (s, C(10)H₂).

Results

Chemical Syntheses. Urinary metabolites M1, M2, M4, M5, and M6 were synthesized in analytical or semipreparative scales using PM as a starting material. Of these compounds, M1, M5, and M6 were prepared under a reductive condition using catalytic hydrogenation by palladium on charcoal. M1 and M4 were prepared in analytical or semipreparative scales using PM as a starting material. Of these compounds, M1, M5, and M6 were prepared under a reductive condition using catalytic hydrogenation by palladium on charcoal. M1 and M4 were prepared in analytical or semipreparative scales using PM as a starting material.

Urinary Excretion in Dogs. Cumulative urinary excretions of radioactivity derived from [methyl-³H]PM and the recoveries of PM in three Beagle dogs after a single i.v. dose over 7 days are given in Table 1. An average of 48.0% of total radioactivity given to the dogs was cumulatively excreted in urine over a period of 7 days after the dose. In the first 24 h, about 43.6% of the administered dose was excreted in urine; this accounted for approximately 92% of total radioactivity excreted in urine over the entire period studied. Unchanged parent drug excreted in urine accounted for 10.8% of the administered dose over 7 days. The findings indicated that the majority of excreted dose in dog urine was in the form of metabolites, and urinary excretion of the metabolites was rapid and intensive in dogs.

Identification of Urinary Metabolites. Representative HPLC profiles showing human and dog urinary metabolites are given in Fig. 2. PM and its metabolites were selectively detected at 550 nm due to their characteristic indigoquinone chromophores. Therefore, PM metabolites could be readily differentiated from other complex components present in urine matrices. A total of seven major metabolites M1 to M7 were observed in human urine (Fig. 2A), and a similar HPLC pattern was obtained for dog urine (Fig. 2B). A major peak M1 at Rₜ 12.70 min observed in both human and dog urine had a typical mitosone UV absorption pattern (λmax, 250, 310, and 550 nm). The APC1 mass spectrum of M1 showed a cluster of molecular ions at m/z 379 (M+H)⁺, 401 (M+Na)⁺, and 417 (M+K)⁺ in Fig. 3A. The observation of two fragment ions at m/z 335 (MH⁻ CO₂)⁺ and 317 (MH⁻ COOH⁻ NH₃)⁺ suggested the existence of carboxylic acid and amino groups in the molecule. A base peak at m/z 292 (MH⁻ CH₅ C(NH₂)COOH⁻) and two related fragment ions at m/z 260 (MH⁻ HISC=CH(NH₂)COOH⁻) and 258 (MH⁻ cysteine⁻) indicated that M1 was a cysteine conjugate. Further confirmation of the structure of M1 was obtained by comparison with our synthetic standards. It was found that the HPLC Rₜ and MS fragmentation pattern of M1 matched those of 10-decarbamoyl-2-methylamino-10-(L-cysteine-S-yl)-7-aminomitosene. The findings supported our structural assignment of M1.

Metabolites M2 and M4 appeared at Rₜ 13.28 and 14.65 min, respectively, by HPLC analysis. Identical UV spectra and similar mass spectra were obtained for the two compounds, indicating that the two metabolites are isomers. The APC1 mass spectra showed a cluster of molecular ions at m/z 335 (M+H)⁺, 357 (M+Na)⁺, and 373 (M+K)⁺ in Fig. 4. A series of fragment ions at m/z 288 (MH⁻ HOOC(OH)NH₂ H₂O+ MeOH⁻), 274 (MH⁻ HOOC(O)NH₂⁻), 258 (MH⁻ HOOC(O)NH₂⁻ NH₂), and 256 (MH⁻ HOOC(O)NH₂ H₂O⁻) were also observed. The MS fragmentation pattern indicated that the two metabolites contained the C-10 carbamate group and a hydroxyl group. HPLC Rₜ and MS fragmentation patterns of M2 and M4 matched those of the reference standards of 1,2-trans and 1,2-cis-1-hydroxy-2-methylamino-7-aminomitosene, respectively. Thus, M2 was assigned as 1,2-trans-hydroxy-2-methylamino-7-aminomitosene and M4 as 1,2-cis-hydroxy-2-methylamino-7-aminomitosene.

### TABLE 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Percentage of Total Radioactivity in Urine</th>
<th>Percentage of Total Radioactivity in Feces</th>
<th>Percentage of PM Recovered in Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>10.7 ± NA*</td>
<td>12.9 ± 12.1†</td>
<td>6.2 ± NA*</td>
</tr>
<tr>
<td>4–8</td>
<td>21.7 ± 18.0</td>
<td>NA</td>
<td>3.4 ± 1.8</td>
</tr>
<tr>
<td>8–24</td>
<td>11.2 ± 15.4</td>
<td>NA</td>
<td>1.2 ± 1.9</td>
</tr>
<tr>
<td>24–48</td>
<td>1.5 ± 0.3</td>
<td>16.1 ± 13.2</td>
<td>ND†</td>
</tr>
<tr>
<td>48–72</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>ND</td>
</tr>
<tr>
<td>72–96</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>96–120</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>120–144</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>144–168</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± NA</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>48.0 ± 2.2</td>
<td>31.8 ± 2.0</td>
<td>10.8 ± 8.6</td>
</tr>
</tbody>
</table>

*NA, not applicable because no sample was collected for one dog during the period (n = 2).
†The sample was collected at 0 to 24 h.

ND, not detectable.

### FIG. 2

Representative chromatograms (550 nm) of human urine (0–2.5 h) after administration of a single i.v. dose of 40 mg/m² of PM (A) and dog urine (0–8 h) after administration of a single i.v. dose of 2 mg/kg of [³H]PM (B).

HPLC conditions are described in Experimental Procedures.
The UV spectrum of M3 showed typical mitosene absorption peaks at 250, 310, and 550 nm. The APCI-mass spectrum of M3 showed a protonated molecular ion at \(m/z\) 379 and a related potassium adduct ion at \(m/z\) 417 in Fig. 3B. A predominant fragment ion at \(m/z\) 292 (\(\text{MH}\cdot \text{CH}_3=\text{C(NH}_2\text{)COOH}^+\)) was also observed, indicating that M3 may be an isomer of M1. The major difference of M3 from M1 in MS fragmentation was the absence of the fragment ion at \(m/z\) 319 was not observed (data not shown). Two fragment ions at \(m/z\) 275 (\(\text{MH}\cdot \text{CO}_2\text{H}^+\)) and 258 (\(\text{MH}\cdot \text{OOC(O)NH}_3^+\)) corresponded to the losses of carbon dioxide and carbamic acid, respectively. The MS fragmentation pattern, HPLC Rt, and UV spectrum of M6 were consistent with those of the reference standard of 2-methylamino-7-aminomitosene.

The mass spectrum of M7 showed a cluster of molecular ions at \(m/z\) 421 (\(\text{M}^+\)), 443 (\(\text{M}^+\cdot \text{Na}^+\)), and 459 (\(\text{M}^+\cdot \text{K}^+\)). The major fragment ions appeared at \(m/z\) 292, 258, and 229 (Fig. 6), which were comparable with those of M5. Thus, M7 is believed to be an isomer of M5. The absence of the thermolabile fragment ion at \(m/z\) 260 for M7 indicated that the N-acetylcysteine moiety could be attached to the C-1 position. According to the mechanism of the formation of M1 and M3, M7 could be assigned as 10-decarbamoyl-2-methylamino-1-(N-acetyl-L-cysteine-S-yl)-7-aminomitosene (Fig. 5).

**Discussion**

Recently, we reported the identification of metabolites of PM formed in the presence of a rat liver preparation (Lang et al., 2000). Several C-1 and C-10 metabolites of PM were characterized as mitosene analogs in the in vitro system. The findings suggest that the formation of the metabolites is initiated by enzymatic activation of PM to a reactive intermediate with two reactive centers, which subsequently link with environmental nucleophiles in the aqueous medium. The current in vivo studies were our continuing effort to explore the metabolic fate of PM.

Tritium labeled to the N-methyl group of PM was used in the dog study. The isotope is not exchangeable with mobile protons in an aqueous medium. The potential loss of the label by demethylation catalyzed by demethylases has been previously investigated in P388 cell homogenates and liver microsomes (Pan, 1990). The results have demonstrated that PM is a poor substrate for the enzymes. So far, no demethyl metabolites of PM have been found. During the dog study, the effluents from HPLC column and Sep-Pak Plus C18 cartridges were collected, and were subjected to liquid scintillation counting for evaluation of the recoveries of radioactivity in each step. The radiochromatograms of the dog urine samples (data not shown) matched the metabolite profiles of HPLC monitored at 550 nm.

Three phase I metabolites (M2, M4, and M6) were identified in both human and dog urine. The findings are consistent with those previously obtained in vitro (Pan and Iaracci, 1988; Lang et al., 2000). Most importantly, we found that M6 is a reactive metabolite that can be directly converted to cysteinyl S-conjugates (M1 and M5) via 13.60 min in dog urine was not observed in human urine. This peak, showing typical UV absorption of mitosene, is believed to be a metabolite of PM, but has not been identified.

Metabolite M5 appeared at \(R_t\) 14.85 min in human urine (Fig. 2A) and gave a typical mitosene UV spectrum (\(\lambda_{\text{max}}\) at 250, 310, and 550 nm). The mass spectrum of M5 showed a cluster of molecular ions at \(m/z\) 421 (\(\text{M}^+\)), 443 (\(\text{M}^+\cdot \text{Na}^+\)), and 459 (\(\text{M}^+\cdot \text{K}^+\)) (data not shown). The major fragment ions of M5 at \(m/z\) 292 (\(\text{MH}\cdot \text{CH}_3=\text{C(NH}_2\text{)COOH}^+\)), 260 (\(\text{MH}\cdot \text{SCH}^+=\text{C(NH}_2\text{)COOH}^+\)), and 258 (\(\text{MH}\cdot \text{CH}_3=\text{C(NH}_2\text{)COOH}^+\cdot \text{H}_2\text{S}^+\)) were similar to those of M1 (Fig. 3A). On the basis of the MS data, we assigned M5 as a mercapturic acid derivative, in which an N-acetyl-cysteine residue conjugated at the C-10 position. The HPLC Rt and MS fragmentation of M5 matched those of the synthetic compound of 10-decarbamoyl-2-methylamino-10-(N-acetyl-L-cysteine-S-yl)-7-aminomitosene (see structure in Fig. 5).

Metabolite M6 was observed at \(R_t\) 15.84 min in both human and dog urine. The mass spectrum of M6 showed a strong potassium adduct ion at \(m/z\) 357 and a sodium adduct peak at \(m/z\) 341 although the protonated molecular ion at \(m/z\) 319 was not observed (data not shown). Two fragment ions at \(m/z\) 275 (\(\text{MH}\cdot \text{CO}_2\text{H}^+\)) and 258 (\(\text{MH}\cdot \text{OOC(O)NH}_3^+\)) corresponded to the losses of carbon dioxide and carbamic acid, respectively. The MS fragmentation pattern, HPLC Rt, and UV spectrum of M6 were consistent with those of the reference standard of 2-methylamino-7-aminomitosene.

The mass spectrum of M7 showed a cluster of molecular ions at \(m/z\) 421 (\(\text{M}^+\)), 443 (\(\text{M}^+\cdot \text{Na}^+\)), and 459 (\(\text{M}^+\cdot \text{K}^+\)). The major fragment ions appeared at \(m/z\) 292, 258, and 229 (Fig. 6), which were comparable with those of M5. Thus, M7 is believed to be an isomer of M5. The absence of the thermolabile fragment ion at \(m/z\) 260 for M7 indicated that the N-acetylcysteine moiety could be attached to the C-1 position. According to the mechanism of the formation of M1 and M3, M7 could be assigned as 10-decarbamoyl-2-methylamino-1-(N-acetyl-L-cysteine-S-yl)-7-aminomitosene (Fig. 5).
further biotransformation at the C-10 position (Fig. 5). The further metabolism of M6 is believed to be through the loss of the C-10 carbamate group to generate a positive charge, which is stabilized by the indole nitrogen (Iyer and Szybalski, 1964). The confirmation of the existence of such a metabolic pathway of M6 was conducted in vitro by direct incubation of M6 with L-cysteine to produce M1 under a reductive condition via catalytic hydrogenation (data not shown). No corresponding C-10 cysteine S-conjugates for 1-hydroxy-mitosenes (M2 and M4) were identified under the same conditions.

A pair of isomeric phase II metabolites (M1 and M3) as cysteine S-conjugates of mitosene analogs at C-1 and C-10 were identified in both human and dog urine, whereas the pair of metabolites (M5 and M7) as N-acetylcysteine S-conjugates (mercapturic acids) were only observed in human urine (Fig. 2). The formation of mercapturic acids in general is believed to be through N-acetylation of cysteine conjugates in the kidney. The reaction may be catalyzed by the cysteine conjugate N-acetyl-S-transferase (Jakoby, 1978; Elfarra and Anders, 1984). The observation of the differences in the formation of mercapturic acids between humans and dogs indicates that the enzymatic activity present in humans may be much higher than that in dogs. In addition, the identification of the phase II metabolites in urine suggests that GSH conjugation may be involved in the biotransformation of PM. As expected, the significant amount of total radioactivity (31.8%) was found in feces after i.v. administration of [methyl-H3]PM to three dogs (Table 1) because GSH conjugates are commonly excreted bile by a carrier-mediated transport system (Ketterer et al., 1983). Two cysteine S-conjugates of PM identified in both dog and human urine may be the products of subsequent metabolism of the GSH conjugates (Igwe, 1986). In summary, the identification of the urinary metabolites of PM linked to the thiol species in dog and human urine at the C-1 and C-10 positions is a novel finding. On the basis of the metabolite profiles, we propose the in vivo metabolic pathways of PM in Fig. 5. The identification of the urinary metabolites of PM is important to understand its in vivo metabolic fate and disposition of PM in dogs and humans.

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References


