ORAL AND TOPICAL ABSORPTION, DISPOSITION KINETICS, AND THE METABOLIC FATE OF TRANS-METHYL STYRYL KETONE IN THE MALE FISCHER 344 RAT

JOHN-MICHAEL SAUER, RICHARD L. SMITH, JINGQI BAO, MARGARET J. KATTNIG, ROBERT K. KUESTER, THOMAS D. MCCLURE, MICHAEL MAYERSON, AND I. GLENN SIPES

Department of Pharmacology and Toxicology and Center for Toxicology, The University of Arizona

(Received December 16, 1996; accepted March 10, 1997)

ABSTRACT:

trans-Methyl styryl ketone (MSK; trans-4-phenyl-3-buten-2-one) is a β-unsaturated ketone that has a wide range of uses in industry and is present in numerous consumer products. Although MSK has been shown to be positive in several in vitro mutagenic assays, it does not seem to be overly toxic in animal models. This lack of toxicity may relate to its poor absorption and/or rapid elimination. However, little is known about the fate of MSK in the body. Studies were conducted to characterize the absorption, and disposition kinetics of MSK after intravenous, oral, and topical administration to male Fischer 344 rats. After intravenous administration of [14C]MSK (20 mg/kg, 120 μCi/kg), blood concentration-time data could be characterized with a biexponential equation and apparent first-order elimination kinetics. The following pharmacokinetic parameter values were obtained (mean ± SD): terminal disposition half-life, 17.7 ± 0.08 min; apparent steady-state volume of distribution, 0.89 ± 0.14 liters/kg; systemic body clearance, 68.9 ± 10.0 ml/kg * min; and mean residence time, 13.1 ± 2.2 min. Within 48 hr, 95.5% of the dose was excreted in the urine and 2.7% in the feces. The major blood metabolite after intravenous administration was identified by GC/MS as the 4-phenyl-3-buten-2-ol (methyl styryl carbinol). After oral administration of [14C]MSK (200 mg/kg, 100 μCi/kg), ~96.6% of the dosed radioactivity was recovered in the urine and 4.8% in the feces within 48 hr. Major urinary metabolites identified by LC-MS/MS and quantified by HPLC radioassay were N-phenylethyl-L-glutamic acid (64.9% of dose) and N-benzyl-L-glutamic acid (9.9% of dose). Parent compound could not be detected in the blood after oral administration, and 14C-equivalents in the blood never exceeded 1.3% of the dose. Results suggest near-total pre-systemic elimination of the oral dose. After topical application of [14C]MSK (250 mg/kg, 50 μCi/kg), >60% of the dose was absorbed, and the majority of the dose was excreted into the urine (55% of dose) in the form of metabolites. Urinary metabolites were similar to those described after oral administration. 14C-equivalents were not detected in the blood at any time after topical administration. These results indicate that MSK is almost totally metabolized before systemic distribution after oral or topical administration. The systemic exposure dose of MSK seems to be exceedingly low at the doses studied herein.

MSK1 [3-buten-2-one, 4-phenyl-8CI, 9CI]; CAS registry no. 1896-62-4] has a wide range of industrial and commercial uses. It is a reactive carbonyl compound used in many types of organic synthesis reactions, electroplating, as well as serving as a pharmaceutical intermediate in preparation of drugs. Furthermore, it has recently been identified as a tobacco flavoring additive in cigarettes (1). MSK is known to occur naturally, but its extensive use as a flavoring and fragrance agent in consumer products suggests potential for widespread human exposure. MSK has been used in foods such as baked goods, gelatins, puddings, candies, and beverages. MSK is also used as a fragrance additive in soaps, detergents, lotions, creams, and perfumes (2).

MSK is relatively nontoxic when administered to rats (oral LD50 2031–3536 mg/kg). However, a large oral dose of MSK (5000 mg/kg) has been reported to cause necrosis and hemorrhage in the glandular gastric mucosa, as well as enlargement of the kidneys and liver (3). Histological changes in the kidney were suggestive of epithelial regeneration, whereas liver enlargement was thought to be an adaptive response related to xenobiotic metabolism and not a toxic response. MSK is an electrophilic substrate and a Michael reaction acceptor that can cause the induction of glutathione-S-transferase and quinone reductase in humans and animals (4), which might be responsible for the changes in the liver and kidney. The electrophilic nature of MSK could also lead to the loss of vital cofactors and represents a potential mechanism for the precipitation of toxic or neoplastic events (5). However, there is no information regarding the chronic effects of MSK in the literature.

MSK shares structural similarities with cinnamic acid, cinnamaldehyde, and cinnamyl alcohol. The disposition and metabolism of these compounds have been reported (6, 7). The metabolites of these compounds include: hippuric acid, benzyl glucuronide, benzoic acid, acetophenone, 3-hydroxy-3-phenylpropionic acid, and cinnamoylglycine. Although MSK is structurally similar to several well-characterized chemical compounds, little is known about its fate in the body. The objectives of this study are to determine the oral and topical

1 Abbreviations used are: MSK, trans-methyl styryl ketone; JVC, jugular vein cannulation; CLsys, systemic clearance; VSSp, steady-state volume of distribution; t1/2T, terminal half-life; MRT, mean residence time; AUC, area under the plasma concentration-time curve; AUMC, area under the first moment of the plasma concentration-time curve; DCM, dichloromethane; CID, collision-induced dissociation; EI, electron impact; NIEHS, National Institute of Environmental Health Sciences.

Send reprint requests to: Dr. I. Glenn Sipes, Department of Pharmacology and Toxicology, College of Pharmacy, P.O. Box 210207, The University of Arizona, Tucson, AZ 85721-0207.
absorption of MSK, and to characterize its disposition kinetics and metabolic fate in the rat.

Materials and Methods

Chemicals. Radiolabeled [14C]-MSK (benzalacetone; trans-4-phenyl-3-buten-2-one) was obtained from Chemsyn Science Laboratories (Lenexa, KS) and was >98% pure, as determined by both normal and reversed-phase HPLC radiochemical analysis. The specific activity was reported to be 56.4 mCi/ mmol by Chemsyn Science Laboratories. Unlabeled MSK was obtained from Acros Organics (Pittsburgh, PA). Its chemical purity was >99%. All other reagents used in these experiments were either of analytical or HPLC grade.

Animal Studies. Animals. Male Fischer 344 rats (175–250 g) with and without JVC were obtained from Hilltop Lab Animals, Inc. (Scottsdale, PA). The animals were acclimated for 5–7 days in a temperature-controlled (25°C) environment.

Preliminary Studies. For each route of administration, rats were housed in air-tight glass metabolism cages and radiolabel collected for at least 48 hr. [14C]-MSK equivalents associated with exhaled organs were trapped in 2-methoxyethyl ether (Malinnckrodt Chemical; Paris, KY), and [14C]CO2 was trapped in CarboSorb (Packard; Meriden, CT)/ethyleneglycol (Malinnckrodt Chemical) (2:1, v/v). Trapping solvents were changed and measured for total radioactivity by scintillation counting at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 48 hr after administration of [14C]-MSK.

Intravenous Administration. [14C]-MSK (20 mg/kg, 120 μCi/kg) in emulphor/ethanol/saline (3:4:12, v/v/v) was administered via a jugular vein cannula to male Fischer 344 rats (over 5 sec). The injection (2 ml/kg) was followed by an equal volume of normal saline to flush the cannula. The animals were then placed in Nalgene metabolism cages to allow collection of urine (6, 12, 24, and 48 hr) and feces (24 and 48 hr) throughout the 48-hr experiment. Blood samples (250 μl) were collected via the jugular cannula at 0, 0.017, 0.035, 0.117, 0.17, 0.20, 0.25, 0.33, 0.5, 0.75, 1, 6, 12, 24, and 48 hr and analyzed immediately by normal phase HPLC. At the end of 48 hr, the animals were euthanized by carbon dioxide inhalation. Blood was collected immediately from the posterior vena cava into a heparinized syringe, and major tissues were harvested as described. The skin at the treatment site was washed with 50 ml of methanol, and aliquots of the wash were analyzed using liquid scintillation counting. Furthermore, the excised skin site was analyzed for radioactivity by tissue oxidation. Radiolabeled MSK was also analyzed by exhaustive extraction with methanol and dichloromethane. Aluminum traps were washed in acetone (3 × 100 ml) and analyzed for total radioactivity. Aliquots of the desorbing solvent, as well as trap washings, were analyzed using liquid scintillation counting.

Data Analysis. The plasma concentration-time data after intravenous bolus dosing were analyzed by both compartmental and noncompartmental methods. Disposition parameter values best describing a linear multicompartmental model, and assuming first-order kinetics for all processes, were determined from nonlinear regression analysis using a weighting scheme of $1/\lambda^2$ (12). The most appropriate model was determined with application of the F-test (13). The parameters of the model were used to calculate values for $CL_{tot}$, $V_{SS}$, $t_{1/2}$, and MRT. The average plasma concentration-time data were also fit to the appropriate model to provide a graphical display of the data.

The noncompartmental analysis involved determination of the terminal disposition rate constant ($k_t$) from a log–linear regression of the data in the terminal phase and from which $t_{1/2}$ was estimated (0.693/$k_t$). The AUC was estimated with the use of the linear trapezoidal rule up to the last measured concentration. The terminal area, extrapolated to time infinity, was estimated by dividing the last measured concentration (on the regression line) by $k_t$. The total AUC was determined as the sum of the two former areas. $CL_{tot}$ was calculated from dose/AUC. The AUMC ($C' × t vs. t$) was determined with the trapezoidal rule along with extrapolation to time infinity. MRT and $V_{SS}$ were determined from: $MRT = AUMC/AUC$; $V_{SS} = MRT × CL_{tot}$. Individual data sets were analyzed as described. Average parameter values ($\pm$SD) are expressed as the arithmetic average, with the exception of $t_{1/2}$ and MRT, which are expressed as the harmonic mean and “pseudo” standard deviation (14).

Analytical Methods. HPLC Analysis of MSK and Its Metabolites. Blood samples were measured directly for total radioactivity by scintillation counting (2 × 25 μl of whole blood). MSK was extracted from 150 μl blood with an equal volume of 0.1 N HCl and 300 μl of DCM. The samples were vortexed, centrifuged, and organic extracts removed. The organic portion of the extraction procedure was repeated two more times and the extracts pooled. Samples were dried by vacuum centrifugation and reconstituted with 150 μl hexane/ethyl acetate (90:10, v/v). The recomposed samples (100 μl) were injected onto a 250 × 4.6 mm silica (10 μm) column (Phenomenex, Torrance, CA) and eluted with a mobile phase of hexane/ethyl acetate (90:10, v/v) at a flow rate of 1 ml/min, with a run time of 50 min. The HPLC (Spectra Physics, SP 8800, San Jose, CA) was equipped with an autosampler (Thermo Separation Products-Spectra Systems, AS 3000, Fremont, CA). The column effluent was monitored in tandem with a UV/visible detector (Spectra Physics, Spectra 100) at a wavelength of 280 nm and radiochemically with a β-RAM flow-through monitor (IN/US Systems, Inc., model 2 with WinFlow, Tampa, FL).

For analysis of urinary metabolites, urine samples were diluted 1:1 (v/v) with water and centrifuged to remove any precipitate. Prepared urine samples (50 μl) were injected onto a 250 × 4.6 mm Whatman Partisil ODS-2 (10 μm) column (Whatman Lab Sales, Inc., Hillsboro, OR), and metabolites were eluted with a mobile phase of water and acetonitrile both containing 0.1% acetic acid at a flow rate of 1 ml/min, with a total run time of 80 min. The mobile phase gradient was run from 100% water for 5 min, then to 20% acetonitrile over 15 min, to 30% acetonitrile over 20 min, and to 100% acetonitrile over 10 min with the final conditions held for 10 min. The column was then brought back to initial conditions over 20 min. The HPLC (Spectra Physics, SP 8800, San Jose, CA) was equipped with an autosampler (Spectra Physics, SP 8775). The column effluent was monitored in tandem with a UV/visible detector (Spectra Physics, Spectra 8450) at a wavelength of 280 nm and radiochemically with a β-RAM flow-through monitor (IN/US Systems, Inc., model 2 with WinFlow). Urine samples were also incubated with β-glucuronidase (2000 units/ml) or sulfatase (100 units/ml) according to the method of Peters and Caldwell (7). Samples were incubated for at least 24 hr and then analyzed by HPLC.
Metabolite Identification Using MS and MS/MS Analysis. For metabolite isolation and identification, pooled urine samples (6, 12, and 24 hr collection) from each route of administration were diluted with an equal volume of water and centrifuged to remove any precipitate. HPLC peaks containing radioactivity were collected and concentrated by lyophilization. The concentrated fractions were diluted with equal volumes of 10 mM ammonium acetate and acetonitrile (1:1, pH 9) for negative electrospray analysis, and water (1:1) for atmospheric pressure chemical ionization analysis. Samples were analyzed on a Finnigan TSQ 7000 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an atmospheric pressure source. Samples were introduced into the mass spectrometer ion source by flow injection at 0.5 ml/min. Ions with $m/z$ values corresponding to putative metabolites were subjected to collision-induced dissociation (CID) with argon gas and the subsequent product ion signal masses analyzed to produce a product ion mass spectrum. Logical fragmentation patterns observed in the resulting MS/MS spectrum provided further evidence as to metabolite identity.

Collected urine samples (0–24 hr), as well as isolated HPLC peaks were also extracted with ethyl acetate, and extracted metabolites were separated using a Fisons GC-8000 gas chromatograph coupled to a Fisons MD800 quadrupole mass spectrometer (Fisons Instruments, Beverly, MA). The samples (1 μl) were injected onto a DB-SMS capillary column (0.25-μm film thickness, 0.25 mm diameter, 15 m; J & W Scientific, Folsom, CA). The oven temperature was initially maintained at 50°C for 5 min, then increased at 10°C/min for the next 25 min to a final temperature of 300°C and maintained at 300°C for 5 min. The injector, source, and interface temperatures were 250°C, 250°C, and 275°C, respectively. Mass spectra were scanned from $m/z$ 50 to 650 in 1 sec. The retention times and mass spectra of putative metabolites in the samples were compared with the mass spectra of authentic standards to verify compound identity.

For isolation and identification of metabolites from blood after intravenous administration, blood samples (6 ml) were extracted with DCM as described. Extracts were dried by vacuum centrifugation and reconstituted with hexane/ethyl acetate (90:10, v/v). Samples were subjected to normal phase HPLC. Peaks containing radioactivity were collected and concentrated by vacuum centrifugation. Concentrated samples were analyzed by GC/MS as described.

Results

Intravenous Administration. The blood concentrations of MSK declined rapidly after intravenous administration, and values were below the limit of detection within 60 min (fig. 1). The concentration-time profile could be adequately described by a biexponential equation, consistent with a linear two-compartmental model. The average $(±SD)$ $t_{1/2}$, apparent $V_{SS}$ and $CL_S$ values were, respectively: 17.7 ± 0.08 min, 0.89 ± 0.14 liters/kg, and 69.8 ± 10.0 ml/kg * min. The MRT was 13.1 ± 2.2 min.

Within 6 hr of intravenous administration, >55% of the dose of [14C]MSK had been excreted into the urine (fig. 2A). Recovery of total radioactivity (at 48 hr) in the urine, feces, and tissues is shown in table 1. Only a small percentage of the dose appeared in the feces
A blood/ated peaks is shown in table 2. The mass spectrum for metabolite
the samples were compared with the mass spectra of authentic stan-
matrix. The retention times and mass spectra of putative metabolites in
of the analytes, these analytes were separated and analyzed by GC/
ion corresponding to $m/z$ 108. The EI spectra of the

Radioactivity represented only 1.3% of the total dose. After oral administration of $[^{14}C]$MSK, the
majority of the radiolabel was excreted into the urine (table 1); >70% within 6 hr. Cumulative excretion of radioactivity in the urine and feces is shown in fig. 2A. Tissue oxidation indicated that little radioactivity was retained in tissues 48 hr after treatment (0.4%). Essentially, the entire dose was recovered in urine and feces.

**Oral Administration.** After oral administration of $[^{14}C]$MSK, the
majority of the radiolabel was excreted into the urine (table 1); >70% within 6 hr. Cumulative excretion of radioactivity in the urine and feces is shown in fig. 2B. The recovery pattern was virtually identical to that following intravenous administration (table 1). The radioactivity associated with the exhaled carbon dioxide and organic vapors were at or below background levels (0.1%). Tissues retained little radioactivity 48 hr after treatment (0.12%). Whole blood samples after oral $[^{14}C]$MSK dosing were analyzed for parent compound and metabolites. Importantly, no parent MSK could be detected in the blood after oral exposure; however, some metabolites were present in the blood after oral exposure. At the time of peak blood concentration (1 hr), radioactivity represented only 1.3% of the total dose.

**Topical Administration.** After topical application of $[^{14}C]$MSK (250 mg/kg), 39.8% of the radioactive dose was associated with the activated charcoal, skin trap, and skin at the site of application, whereas the remainder of the dose was absorbed. Elimination of total radioactivity after topical exposure is shown in table 1. The majority of the radiolabel was eliminated in the urine (55% of dose at 48 hr). However, <8% of the dose was excreted by 6 hr. Cumulative excretion of radioactivity in the urine and feces is shown in fig. 2C. The excretion pattern was similar to those after intravenous and oral administration in that 91.0% of the dose absorbed was recovered in urine and 2.0% was recovered in feces. Tissue oxidation showed that little radioactivity was retained in the tissue 120 hr after treatment (0.3%). Although >60% of the dose was absorbed, at no time could $^{14}$C-equivalents be detected in the blood.

**Determination of Blood and Urinary Metabolites.** Blood Metab-
ollies. After intravenous administration of $[^{14}C]$MSK (20 mg/kg), normal phase HPLC analysis of blood revealed four major radioactive peaks ($R_p$: 10, 12, 31, and 39 min) (fig. 3). Because of the volatility of the analytes, these analytes were separated and analyzed by GC/MS. The retention times and mass spectra of putative metabolites in the samples were compared with the mass spectra of authentic standards to verify compound identity. The GC/MS analysis of the isolated peaks is shown in table 2. The mass spectrum for metabolite $A_{blood}$ indicated a molecular ion corresponding to $m/z$ 148 (table 3). The EI spectra of the $m/z$ 148 molecular ion were characteristic of 4-phenyl-3-buten-2-ol (methyl styryl carbinol) (II; fig. 4). The mass spectrum for metabolite $B_{blood}$ contained an analyte with a molecular ion corresponding to $m/z$ 146. The EI spectra of the $m/z$ 146 molecular ion corresponded to the parent molecule, MSK (Ia). The mass spectrum for metabolite $C_{blood}$ yielded a molecular ion corresponding to $m/z$ 108. The EI spectra of the $m/z$ 108 molecular ion were charac-

$^{a}$ Mean % of dose $\pm$ SD, $N = 3$; oral and intravenous studies were conducted for 48 hr, whereas the topical study was conducted for 120 hr.

$^{b}$ Includes percutaneous skin trap, activated charcoal, and skin at the site of MSK application.

$^{c}$ Exhaled radioactive CO$_2$ and organic accounted for <0.6% of the total dose; data not included in total recovery.

### TABLE 1

<table>
<thead>
<tr>
<th>Route</th>
<th>% of Radioactive Dose Recovered$^a$</th>
<th>Total Recovery$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td>Intravenous (20 mg/kg)</td>
<td>95.5 ± 1.5</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Oral (200 mg/kg)</td>
<td>96.6 ± 0.6</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>Topical (250 mg/kg)</td>
<td>55.0 ± 11.5</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

Peaks are identified by letters that correspond to those listed in table 3.
metabolite I contained a signal corresponding to an [M+H]^+ ion at m/z 310. The major product ion from the [M + H]^+ ion was at m/z 147, which represents a 4-phenyl-2-butan-one fragment. Another product ion was at m/z 162, which is characteristic of an N-acetyl-l-cysteine fragment. These products are indicative of metabolite I being N-acetyl-S-(4-phenyl-2-butan-one)-l-cysteine (VI). The mass spectrum for metabolite J contained a signal corresponding to the [M−H]^− ion at m/z 310. The major product ion from the [M−H]^− ion was at m/z 181, which represents an addition of a sulfur to methyl styril carbinol. Another product ion was at m/z 162, which is characteristic of an N-acetyl-l-cysteine fragment. These products are indicative of N-acetyl-S-(4-phenyl-2-butan-ol)-l-cysteine (VII).

Because of the volatility of the analytes, metabolite peak K was separated and analyzed using a GC/MS. Metabolite K had two major GC peaks containing analytes with molecular ions corresponding to m/z 146 and 148 (table 2). The EI spectra of the m/z 146 molecular ion was characteristic of 4-phenyl-3-buten-2-one (MSK) (Ia), whereas the EI spectra of the m/z 148 molecular ion corresponded to 4-phenyl-3-buten-2-ol (methyl styril carbinol) (II). After extraction of undiluted urine with ethyl acetate, acetophenone and benzoic acid were also found in the urine by GC/MS analysis, but these were not associated with any isolated HPLC peak and may not be derived from MSK.

Six minor metabolites were not identified by either LC/MS or GC/MS, because of the low amount of material associated with the collected peaks. Taken together, these unidentified metabolites represent between 5.4–7.2% of the dose, depending on the route of administration.

**Discussion**

trans-MSK is a reactive carbonyl compound used in organic synthesis, as well as an intermediate in preparation of pharmaceutical agents. It has been shown to be mutagenic in several short-term assays. For example, in the Ames *Salmonella typhimurium* assay, MSK produced a positive mutagenic response in strain TA100 with S9 activation, but not in strain TA98 regardless of activation (15). In the mouse lymphoma L5178Y TK^+/− assay, MSK was positive but only in the presence of the S9 fraction (16). Thus, it is apparent that MSK requires metabolic activation to exert its mutagenic effects. However, MSK does not seem to be overtly toxic in animal models (3). This lack of toxicity may relate to its rapid metabolism and/or excretion. Because of the extensive use of MSK as a flavoring and fragrance agent in consumer products, widespread human exposure occurs. Thus, it is critical to know more about the fate of this compound in the body. The work described herein represents a comprehensive study of the absorption, disposition, and metabolism of MSK in the male Fischer 344 rat.

It is important to note that, at the doses of MSK used in these experiments, no signs of toxicity were observed. After MSK administration, the majority of the dose was readily absorbed, metabolically cleared, and excreted into the urine regardless of route of administration. Tissue oxidation indicated that very little radioactivity was retained in the tissues. Although there was a distinct distributive phase, the Vss (0.89 liters/kg) is not especially large, suggesting that only a limited amount of the dose distributes to the tissues. Assuming that MSK is totally metabolized by the liver, CL_S will be approximately equivalent to hepatic clearance. The value for CL_S (69.8 ±
10.0 ml/kg * min) is about equal to estimates of liver blood flow (17). This would suggest that MSK is nonrestrictively cleared and that it should have a high hepatic intrinsic clearance (and high hepatic extraction ratio). The latter is consistent with the oral dosing results as noted herein. As a consequence of this high clearance and small to intermediate apparent volume of distribution, MSK has a short disposition half-life (17.7 ± 0.08 min).

We were unable to detect, at our limit of quantitation, intact MSK in the blood at any time after oral or topical administration. On the basis of this discussion, the anticipated high hepatic intrinsic clearance of MSK would suggest that the compound undergoes substantial presystemic or first-pass hepatic clearance after oral dosing. This would likely explain the immeasurable low blood concentrations. The most reasonable explanation for the undetectable blood concentrations after topical dosing is that absorption following topical exposure occurs so slowly, and MSK is so rapidly cleared that measurable concentrations are not seen. For both routes of administration, however, small quantities of intact compound (0.8 – 1.6%) were detected in the urine, suggesting that at least a small percentage of the compound is absorbed intact and escapes metabolism. This issue was further clarified by examining the in vitro transport of MSK across excised percutaneous skin and everted intestinal tissue. MSK was readily transported intact across those isolated preparations, with only a small percentage being converted to the methyl styryl carbinol metabolite (unpublished data). However, at the doses used herein, it is likely that a very low systemic exposure to MSK occurs after oral or topical administration.

A schematic representation for the proposed metabolic fate of MSK consistent with the data reported herein is shown in fig. 4. The metabolism of MSK is similar to that reported for cinnamic acid and cinnamaldehyde (6, 7). The urinary metabolites of cinnamic acid and cinnamaldehyde have been shown to include: hippuric acid, benzoyl glucuronide, 3-hydroxy-3-phenylpropionic acid, and cinnamoyl glycine. These compounds have also been shown to react with glutathione (18, 19). Analysis of urinary samples from MSK-treated rats revealed metabolites that included phenaceturic acid, hippuric acid, and two mercapturate conjugates. However, unlike either cinnamic acid or cinnamaldehyde, benzoyl glucuronide was not detected as a urinary metabolite. In fact no glucuronide or sulfate conjugates were observed after administration of MSK. This difference in metabolic products (i.e. hippuric acid vs. benzoyl glucuronide) may be due to the limited formation of benzoic acid (7–10% of the dose) available for glucuronidation.

The principle metabolite of MSK detected in the blood was methyl styryl carbinol, which represented 4.4% of the dose at peak blood concentration after intravenous administration. The formation of methyl styryl carbinol suggests that the keto group is more readily reduced than the unsaturated bond of MSK. Interestingly, whole blood has the ability to reduce MSK to methyl styryl carbinol. However, this conversion accounts for only a minor amount of the total dose converted to methyl styryl carbinol in the whole animal (unpublished data). The reduction of MSK in the blood is likely mediated by ketone

**Fig. 4.** Metabolic scheme proposed for MSK in the male Fischer 344 rat.

(I) trans-4-phenyl-3-buten-2-one (MSK); (II) 4-phenyl-3-buten-2-ol (methyl styryl carbinol); (III) benzyl alcohol; (IV) N-benzyl-L-glycine (hippuric acid); (V) N-phenylacetyl-L-glycine (phenaceturic acid); (VI) N-acetyl-S-(4-phenyl-2-butan-one)-L-cysteine; and (VII) N-acetyl-S-(4-phenyl-2-butan-ol)-L-cysteine. Note: S-Cys-N-acetyl is an N-acetyl-S-cysteine conjugate; GS is a glutathione conjugate.

**Fig. 5.** HPLC radiochromatograms of pooled (6-, 12-, and 24-hr collection) urine samples from male JVC Fischer 344 rats after (A) intravenous (20 mg/kg, 120 μCi/kg), (B) oral (200 mg/kg, 100 μCi/kg), or (C) topical (250 mg/kg, 50 μCi/kg) administration of [14C]MSK. Peaks are identified by letters that correspond to those listed in table 5.
reductase, an enzyme found in erythrocytes of several species (20). Although methyl styryl carbinol was the major blood metabolite after intravenous administration, it comprised only a minor amount of the dose (0.1%) excreted in the urine. Thus, it seems that the formation of methyl styryl carbinol is essential for the subsequent steps in the metabolic conversion of MSK to its oxidized metabolites (i.e., phenylacetic acid and benzoic acid). It has been shown that MSK can be metabolized to the completely reduced carbinol (4-phenyl-2-butanol) (21). Because 4-phenyl-2-butanol was not detected in either the blood or urine of MSK-treated rats, it is unclear whether this possible metabolite plays a major role in the metabolic pathway described herein.

Phenylacetic acid and benzoic acid are apparently the major phase I metabolites of MSK in the rat. β-Upsaturated ketones are commonly metabolized to either benzoic acid or phenylacetic acid according to the number of carbon atoms associated with their alkene side chain (21). If the side chain contains an even number of carbon atoms, phenylacetic acid is formed, whereas an odd number of carbon atoms yield benzoic acid. However, both phenaceturic acid and hippuric acid were present in the urine of rats administered MSK, in ratios of 6:1 to 4:1 depending on the route of administration. This metabolic pathway seems to be analogous to the oxidative metabolism of several foreign ω-phenyl unsaturated fatty acids, such as cinnamic acid and phenylisocrotoic acid (6, 21). Xenobiotic carboxylic acids that enter the cell are readily converted to their acyl-coenzyme A thioesters and undergo the β-oxidation pathway of fatty acid catabolism resulting in the formation of benzoic acid and/or phenylacetic acid (22). However, MSK lacks a carboxylic acid required for metabolism via B-oxidation.

In addition to the oxidation of the alkene side chain, it is apparent that MSK can undergo nucleophilic attack by glutathione resulting in the formation of N-β-acetyl-L-glutathione. This metabolite is further metabolized, resulting in the urinary excretion of N-β-acetyl-L-glutathione and N-β-acetyl-L-γ-glutathione. These mercapturates accounted for 4.2–18.7% of the dose, depending on the route of administration. It is likely that glutathione conjugation occurs in the liver, because oral administration of MSK results in the depletion of hepatic glutathione (~34%) within 12 hr (unpublished data). MSK did not affect pulmonary or renal glutathione levels. Depletion of hepatic glutathione could represent an important mechanism by which large doses of MSK could participate in chemical–chemical interactions.

### Table 5

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>HPLC RT</th>
<th>Peak Identification</th>
<th>% of Total Dose in Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td></td>
<td>Oral (200 mg/kg)</td>
</tr>
<tr>
<td>Undefined</td>
<td>2</td>
<td>A</td>
<td>—</td>
</tr>
<tr>
<td>Undefined</td>
<td>10</td>
<td>B</td>
<td>1.8</td>
</tr>
<tr>
<td>Undefined</td>
<td>16</td>
<td>C</td>
<td>—</td>
</tr>
<tr>
<td>Undefined</td>
<td>21</td>
<td>D</td>
<td>3.6</td>
</tr>
<tr>
<td>Undefined</td>
<td>23</td>
<td>E</td>
<td>—</td>
</tr>
<tr>
<td>N-benzyl-l-glycine</td>
<td>24</td>
<td>F</td>
<td>9.9</td>
</tr>
<tr>
<td>Undefined</td>
<td>25</td>
<td>G</td>
<td>—</td>
</tr>
<tr>
<td>N-phenylacetyl-l-glycine</td>
<td>26</td>
<td>H</td>
<td>64.9</td>
</tr>
<tr>
<td>N-acetyl-S-(4-phenyl-2-butano-l)-l-cysteine</td>
<td>33</td>
<td>I</td>
<td>5.6</td>
</tr>
<tr>
<td>N-acetyl-S-(4-phenyl-2 butanol)-l-cysteine</td>
<td>36</td>
<td>J</td>
<td>2.2</td>
</tr>
<tr>
<td>MSK</td>
<td>51</td>
<td>K_1</td>
<td>1.6</td>
</tr>
<tr>
<td>Methyl styryl carbinol</td>
<td>51</td>
<td>K_2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Total dose in urine (0–24 hr)</td>
<td></td>
<td></td>
<td>89.6%</td>
</tr>
</tbody>
</table>

*a* Reverse phase HPLC retention time.

*b* See fig. 5.

![Representative LC-MS/MS spectra of the urinary metabolite N-phenylacetyl-l-glycine (phenaceturic acid) and N-benzyl-l-glycine (hippuric acid) obtained from male JVC Fischer 344 rats after administration of [14C]MSK.](https://dmd.aspetjournals.org/doi/10.1093/dmd/738/4)

Fig. 6. Representative LC-MS/MS spectra of the urinary metabolite N-phenylacetyl-l-glycine (phenaceturic acid) and N-benzyl-l-glycine (hippuric acid) obtained from male JVC Fischer 344 rats after administration of [14C]MSK. Spectra are from a pooled urine sample from an orally dosed rat. (A) Product ion spectra of [M-H] m/z 192. (B) Product ion spectra of [M+H]^+ m/z 180.
DISPOSITION AND ABSORPTION OF trans-METHYL STYRYL KETONE

The other routes of metabolism for MSK, such as alkene side-chain oxidation, also likely occur primarily in hepatic tissue. This has been determined using liver, lung, and kidney precision-cut tissue slices. Although the lung and kidney have the ability to metabolize MSK, the liver possessed the greatest ability to reduce MSK to methyl styryl carbinoil, as well as to form benzyl alcohol (unpublished data).

In summary, results of this study indicate that the majority of the dose is readily absorbed after oral or topical administration of MSK. Although absorption through the skin is significantly slower than from the gastrointestinal tract, in both cases it is likely that MSK is absorbed intact. However after absorption, on the basis of blood measurement of intact compound, MSK is rapidly metabolized. This is a result of the high hepatic extraction ratio of MSK. As a consequence, systemic exposure to MSK after oral or topical dosing is expected to be exceedingly low. There may be a similar minimum exposure of tissues to metabolites, because most are readily eliminated. The increased water solubility of these metabolites tends to restrict them from being distributed into tissues. Future studies should, therefore, include experiments to determine the tissue disposition of MSK at early time points, as well as the organ(s) responsible for its metabolism.

Acknowledgments. We thank the Synthetic Core and Analytical Core of the Southwest Environmental Health Science Center. Special thanks to Mary Lou Eckerson, as well as Drs. Eugene A. Mash and Yushun Li, for providing assistance with this project. Furthermore, the author would like to thank Drs. H. B. Matthews and M. L. Cunningham of the National Toxicology Program (National Institute of Environmental Health Sciences) for their advice and support.

References

16. NCI: “NCI Short-Term Test Results.” National Cancer Institute, Division of Cancer Etiology Short-Term Test Program, Bethesda, MD, 1994.