SULFOXIDES AS URINARY METABOLITES OF S-ALLYL-L-CYSTEINE IN RATS: EVIDENCE FOR THE INVOLVEMENT OF FLAVIN-CONTAINING MONOOXYGENASES

RENEE J. KRAUSE, STEVEN C. GLOCKE, AND ADNAN A. ELFARRA

Department of Comparative Biosciences and the Center for Molecular and Environmental Toxicology, University of Wisconsin–Madison, Madison, Wisconsin

(Received June 3, 2002; accepted July 12, 2002)

ABSTRACT:

S- Allyl-L-cysteine (SAC), a component of garlic and a metabolite of allyl halides, is a known substrate for multiple flavin-containing monooxygenases (FMOs). In the current study, we characterize the in vivo SAC metabolism by investigating the presence of SAC, N-acetyl-S-allyl-L-cysteine (NASAC), and their corresponding sulfoxides in the urine of rats given SAC (200 or 400 mg/kg i.p.). In some experiments, rats were given aminooxyacetic acid (AOAA), an inhibitor of cysteine conjugate β-lyase, or methimazole, an alternative FMO substrate, 30 min prior to treatment with 200 mg/kg SAC. Nearly 40 to 50% of the dose was recovered in the 24-h collection period. In all treatment groups, the majority of the metabolites were excreted within 8 h. The major metabolites detected were NASAC and NASAC sulfoxide (NASACS; nearly 30–40% and 5–10% of the dose, respectively). Only small amounts of the dose (approximately 1.5%) were recovered as SAC and SAC sulfoxide (SACS). Methimazole pretreatment significantly reduced amounts of both SACS and NASACS detected in the urine when compared with rats given SAC only, whereas AOAA pretreatment had no effect. In vitro assays using rat liver microsomes were also carried out to compare the sulfoxidation rates of SAC and NASAC. The results showed that SAC was much more readily oxidized than NASAC. Collectively, the results provide evidence for the involvement of FMOs in the in vivo metabolism of SAC and that SAC is a much better substrate for FMOs than its corresponding mercapturic acid.

S-Allyl-L-cysteine (SAC)\(^1\) is a significant water-soluble allyl sulfur component in garlic preparations (Weinberg et al., 1993), a component which has been shown to have antioxidant and anticancer properties in animals (Sumiyoshi and Wargovich, 1990; Hatano et al., 1996; Ho et al., 2001). SAC has been shown to have antiproliferative effects on neuroblastoma (Welch et al., 1992), melanoma (Takeyama et al., 1993), and prostate carcinoma cells (Pinto et al., 1997). However, the mechanisms of the anticancer properties of SAC are unclear.

SAC is also a known metabolite of allyl halides and allyl esters, including allyl chloride, allyl bromide, sodium allyl sulfate, and allyl nitrate (Kaye et al., 1972; Kaye, 1973). It results from the formation of the glutathione conjugate of the allyl halide or ester. The glutamate and glycine moieties of S-allyl-glutathione are then cleaved by γ-glutamyl transpeptidase and dipeptidases to yield SAC.

This research was supported by Grant DK44295 (A.A.E.) from the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institute of Health. A preliminary report of this data was presented at the Federation of the American Societies for Experimental Biology meeting held in New Orleans, LA on April 20–24, 2002.

\(^1\)Abbreviations used are: SAC, S-Allyl-L-cysteine; FMOs, flavin-containing monooxygenases; SACS, S-Allyl-L-cysteine sulfoxide; NASAC, N-acetyl-S-allyl-L-cysteine; NASACS, N-acetyl-S-allyl-L-cysteine sulfoxide; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; AOAA, aminooxyacetic acid; FAB-MS, fast atom bombardment mass spectroscopy; NASBC, N-Acetyl-S-benzyl-L-cysteine; ACN, acetonitrile; GC, gas chromatography; P450, cytochrome P450.

Address correspondence to: Adnan Elfarra, Ph.D., School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706. E-mail: elfarra@svm.vetmed.wisc.edu

Our laboratory has previously shown that SAC is a substrate for multiple rabbit and human flavin-containing monooxygenases (FMOs). FMOs are microsomal enzymes that catalyze the NADPH-dependent oxidation of many compounds that contain sulfur, nitrogen, selenium, and phosphorus (Ziegler, 1993). Previously, we have shown that SAC is metabolized to the greatest extent by cDNA-expressed rabbit FMO3 followed by FMO1 and then FMO2, but SAC showed no activity with rabbit FMO5 (Ripp et al., 1997). More recently and using cDNA-expressed human FMOs, SAC has been shown to be a good substrate for FMO1, FMO3, and FMO4, and although the activity is low, it is also a substrate for human FMO5 (Ripp et al., 1999a,b; Krause et al., 2002).

Since SAC is a good substrate for multiple FMOs in vitro, it is logical to expect formation of S-allyl-L-cysteine sulfoxide (SACS; Fig. 1) after SAC treatment in vivo. SAC and SACS may also be N-acetylated to form N-acetyl-S-allyl-L-cysteine (NASAC) and N-acetyl-S-allyl-L-cysteine sulfoxide (NASACS), respectively. NASAC is an expected metabolite because it has been detected in the urine of rats, mice, and dogs dosed with SAC (Kaye et al., 1972; Nagae et al., 1994) and in the urine of humans who consumed garlic (de Rooij et al., 1996). The studies by Kaye and coworkers (Kaye et al., 1972; Kaye, 1973) used paper chromatography to suggest the formation of NASACS in rats given allyl chloride, allyl bromide, allyl iodide, triallyl phosphate, sodium allyl sulfate, or allyl nitrate, but no quantitation of NASACS formation was provided and to date, no study has examined the role of FMOs in SAC metabolism in vivo. Thus, the purpose of the current study was to characterize and quantify the major metabolites formed from SAC in vivo, especially those that are due to sulfoxidation by the FMOs, and to determine whether addition...
of the N-acetyl moiety affects SAC's ability to be oxidized by rat liver microsomes.

Materials and Methods

Chemicals. SAC was a generous gift from Wakunaga Pharmaceutical of America (Mission Viejo, CA). SACS was synthesized as previously described (Ripp et al., 1997). HPLC-grade acetoniurite, ethyl acetate, tetrahydrofuran, methanol, and 30% hydrogen peroxide were obtained from EM Science (Gibbstown, NJ). Acetic anhydride, acetic acid, trifluoroacetic acid (TFA), 1-methyl-3-nitro-1-nitosoguanidine, methimazole, aminoxyacetic acid (AOAA), sodium iodide and NADPH were purchased from Aldrich Chemicals.

NASAC was synthesized as described by Stoll and Seebeck (1948). Briefly, SAC (0.7 mmol) was dissolved in 2.8-ml ice-cold 1N NaOH and placed on ice. Acetic anhydride (100 µl, 1 mmol) was added dropwise, followed by an additional 2.8 ml of NaOH and another 100 µl of acetic anhydride. The reaction was stirred for 20 min after which it was acidified to pH 1.2 with 6N HCl. The solution was concentrated to half its volume by rotary evaporation and extracted with ethyl acetate (2 x 2 ml). The ethyl acetate extracts were dried with sodium sulfate and evaporated to dryness under N₂. The residue was dissolved in a small volume of hot acetone and allowed to precipitate at 4°C. The resulting precipitate was collected by filtration. The reaction yield was 91% and was ≥95% pure as determined by HPLC with UV detection at 220 nm. Identity of NASBC was also confirmed by ¹H NMR, and the chemical shifts match well with what has been reported in the literature (Glass et al., 1989).

Animals. Male Sprague-Dawley rats (240-300 g) were purchased from Sasco Inc. (Omaha, NE). Rats were maintained on a 12-h light/dark cycle and allowed food and water ad libitum. Rats were housed individually in metabolic cages (Nalgene, Rochester, NY). Rats were injected with either 200 or 400 mg/kg (1242 or 2484 µmol/kg) SAC i.p. These doses were chosen because they were similar to what was used in the chemoprevention studies (Sumiyoshi and Wargovich, 1990; Hatono et al., 1996) and the dose that showed protection against acetaminophen hepatotoxicity in mice (Wang et al., 1996).

For the in vitro studies, rat liver microsomes containing 20% glycerol were prepared as previously described (Sausen and Elfarra, 1990) and stored at -80°C until use. In some experiments, microsomes were solubilized using a buffer comprised of 0.1M KH₂PO₄, 0.1 M KCl, 5 mM EDTA containing 1% Emulgen 911 at pH 7.4 by stirring on ice for 45 min. The solubilized microsomes were then centrifuged at 100,000 g for 45 min, and the supernatant was used in the spectrophotometric assays described below. Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

HPLC Analysis of SAC/SACS. To detect SAC and SACS in urine, the urine was first fractionated to remove many contaminating peaks (Fig. 2). Briefly, urine (0.5 ml) was acidified with 25 µl 35% perchloric acid and then centrifuged for 5 min in a Beckman Ti-6 centrifuge (Beckman Coulter, Inc., Fullerton, CA) at 3000 rpm to remove precipitated proteins. The resulting supernatant was filtered with an Acrodisc LC-13 membrane filter (Pall Gelman Sciences, Ann Arbor, MI) before fractionation by HPLC. HPLC analyses were carried out using a Gilson dual pump gradient-controlled system (Gilson, Inc., Middleton, WI) fitted with a semi-preparative Beckman ODS 5-µm reverse-phase C₁₈ column (10 x 250 mm). UV detection was used at 220 nm on a Beckman 166 detector. Injection volume was 250 µl carried out by a Gilson 234 autosampler. The mobile phase on pump A was 1% acetonitrile (ACN), pH 2.5, and pump B contained 75% ACN, pH 2.5. Flowrate was 3 ml/min.

SAC, and SACS were eluted using a gradient method with an initial concentration/extraction procedure. The reaction yield was 91% and was ≥95% pure as determined by HPLC with UV detection at 220 nm. Identity of NASBC was also confirmed by ¹H NMR, and the chemical shifts match well with what has been reported in the literature (Glass et al., 1989).

Animals. Male Sprague-Dawley rats (240-300 g) were purchased from Sasco Inc. (Omaha, NE). Rats were maintained on a 12-h light/dark cycle and allowed food and water ad libitum. Rats were housed individually in metabolic cages (Nalgene, Rochester, NY). Rats were injected with either 200 or 400 mg/kg (1242 or 2484 µmol/kg) SAC i.p. These doses were chosen because they were similar to what was used in the chemoprevention studies (Sumiyoshi and Wargovich, 1990; Hatono et al., 1996) and the dose that showed protection against acetaminophen hepatotoxicity in mice (Wang et al., 1996).

For the in vitro studies, rat liver microsomes containing 20% glycerol were prepared as previously described (Sausen and Elfarra, 1990) and stored at -80°C until use. In some experiments, microsomes were solubilized using a buffer comprised of 0.1M KH₂PO₄, 0.1 M KCl, 5 mM EDTA containing 1% Emulgen 911 at pH 7.4 by stirring on ice for 45 min. The solubilized microsomes were then centrifuged at 100,000 g for 45 min, and the supernatant was used in the spectrophotometric assays described below. Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

HPLC Analysis of SAC/SACS. To detect SAC and SACS in urine, the urine was first fractionated to remove many contaminating peaks (Fig. 2). Briefly, urine (0.5 ml) was acidified with 25 µl 35% perchloric acid and then centrifuged for 5 min in a Beckman Ti-6 centrifuge (Beckman Coulter, Inc., Fullerton, CA) at 3000 rpm to remove precipitated proteins. The resulting supernatant was filtered with an Acrodisc LC-13 membrane filter (Pall Gelman Sciences, Ann Arbor, MI) before fractionation by HPLC. HPLC analyses were carried out using a Gilson dual pump gradient-controlled system (Gilson, Inc., Middleton, WI) fitted with a semi-preparative Beckman ODS 5-µm reverse-phase C₁₈ column (10 x 250 mm). UV detection was used at 220 nm on a Beckman 166 detector. Injection volume was 250 µl carried out by a Gilson 234 autosampler. The mobile phase on pump A was 1% acetonitrile (ACN), pH 2.5, and pump B contained 75% ACN, pH 2.5. Flowrate was 3 ml/min.

SAC, and SACS were eluted using a gradient method with an initial concentration/extraction procedure. The reaction yield was 91% and was ≥95% pure as determined by HPLC with UV detection at 220 nm. Identity of NASBC was also confirmed by ¹H NMR, and the chemical shifts match well with what has been reported in the literature (Glass et al., 1989).
tized SAC and SACS started at an initial concentration of 30% B where it was held for 6 min. It was then increased to 70% B over 4 min where it was maintained for 5 min. Retention times of SAC and SACS were 13.7 and 18.9 min, respectively. Standard curves for SAC and SACS quantitation were prepared in urine, fractionated, and derivatized as described above. Limits of detection were 6.2 and 2.8 nmol/ml for SAC and SACS, respectively; correlation coefficients of \( r^2 > 0.99 \) were obtained.

**NASAC/NASACS Analyses.** To assess the amount of SAC excreted as its mercapturate and corresponding sulfoxide, a GC method was developed adapted from de Rooij et al. (1996). Briefly, NASBC (150 \( \mu \)l of a 2 mg/ml solution prepared in water) was added to 1 ml of urine as internal standard. To precipitate urine proteins, 6 M HCl (125 \( \mu \)l) was added, followed by centrifugation in a Beckman TJ-R tabletop centrifuge at 3000 rpm to remove precipitated proteins. The supernatant was transferred to a new tube, and the NASAC was extracted with two 3-ml portions of ethyl acetate. Extraction efficiency of NASAC from urine using this method was \( \approx 96\% \) whereas very little NASACS could be extracted by this method. The ethyl acetate extracts were combined and were evaporated to dryness under N\(_2\) before the chamber was opened and the sample was evaporated to dryness under N\(_2\) again. This residue was dissolved in 50 \( \mu \)l of methanol and 50 \( \mu \)l of ethyl acetate, and an aliquot (2 \( \mu \)l) was analyzed by GC.

NASAC was found to be thermally unstable under GC conditions and could not be accurately or directly analyzed for; therefore, a method to chemically reduce it to NASAC was developed. Briefly, to the remaining aqueous phase from the above extraction, another aliquot (150 \( \mu \)l) of NASBC was added. The sample was then reduced in volume to approximately 200 \( \mu \)l in a Savant SC110 SpeedVac. Acetonitrile (800 \( \mu \)l) was added to the sample followed by the addition of 1 \( \mu \)l of titanium (IV) chloride and 2 mg of sodium iodide (Pennington and Byrnes, 1995). The sample was then heated in a dry heating block at 50°C for 1 h. Water (2 \( \mu \)l) was added to the sample, and it was extracted with two 5-ml portions of ethyl acetate. The extracts were evaporated to dryness, derivatized with ethereal diazomethane, and analyzed by GC in the same manner described above for NASAC.

**Gas Chromatographic Analyses.** Analyses of the methylated samples were carried out on a HP 5890 Series II gas chromatograph fitted with a DB-1 capillary GC column (J&W Scientific Inc., Folsom, CA) using flame ionization detection. The injector temperature was 220°C, and detector temperature was 250°C. Initial temperature was 50°C where it was held for 1 min. The temperature was then increased at a rate of 25°C/min to 200°C where it was held for 3 min. The temperature was then increased to 250°C at a rate of 10°C/min where it was held for 5 min, resulting in a total run time of 20 min. Retention time of methylated NASAC was 11.4 and 18.4 min for the methylated internal standard, NASBC. A standard curve containing both NASAC and NASACS was prepared in urine and extracted with ethyl acetate. The ethyl acetate extracts were derivatized and analyzed for NASAC as described above. The aqueous layer containing NASACS was reduced with TiCl\(_4\) to NASAC and worked up as described above. Limits of detection for both compounds were 49 nmol/ml. GC-mass spectrometry was carried out on a HP 6890 series mass selective detector fitted with a HP-5 capillary GC column (Hewlett Packard, Palo Alto, CA) with a similar method to that used for the GC analyses.

**In Vitro Metabolism of SAC and NASAC.** Rates of SAC and NASAC oxidation by rat liver microsomes were measured using the spectrophotometric NADPH oxidation assay (Sauser et al., 1993). The assays were carried out on a Beckman DU 640 spectrophotometer fitted with a kinetic package and a Peltier temperature controller. Briefly, to a cuvette, 100 \( \mu \)l of NADPH (0.1 mM, final concentration) was added, followed by the addition of 200 \( \mu \)l of solubilized rat liver microsomes (0.3–0.7 mg of protein). The incubation mixtures were allowed to equilibrate at 37°C for 2 min before the reactions were started by the addition of 5 mM substrate. Additional solubilization buffer was added to bring the total reaction volume to 1 ml. Concurrent controls were also run simultaneously with buffer rather than substrate to correct for any nonsubstrate-dependent loss of NADPH. Reactions were monitored every 30 s to 60 min with the addition of 150 \( \mu \)l 2% perchloric acid. HPLC analyses were carried out on the system used for the fractionation of SAC/SACS equipped with a Beckman Ultrasphere ODS 5-\( \mu \)m column (4.6 x 250 mm) using UV detection at 225 nm. The mobile phase on pump A was 1% ACN, pH 2.5 with TFA, and the mobile phase on pump B was 25% ACN, pH 2.5, with TFA. The gradient started at a concentration of 10% B for 5 min. The percentage B increased to 75% over 4 min where it was held for 4 min. The gradient then

**Fig. 2. Process of sample preparation for the analysis of SAC and its potential metabolites.**
decreased back to the initial concentration of 10% B over 4 min where it was maintained for a total run time of 20 min. The retention time of NASAC was 14.8 min. Retention time of NASACS was 3.5 min, and the limit of detection was 7.3 nmol/ml.

**Statistics.** Statistical analyses were carried out using the SigmaStat software program (SPSS Inc., Chicago, IL). Comparisons of means were done by one-way analysis of variance. When significant differences were determined from analysis of variance, the Fisher least significant difference test was used to determine which means were significantly different. Significance level was set at $p \leq 0.05$.

### Results

Development of HPLC and GC methods allowed us to monitor the in vivo metabolism of SAC (Figs. 1 and 2). Analysis of urine samples from rats dosed with SAC revealed several peaks that were not detected in the urine collected from the same animal before treatment. In all treatment cases, the major metabolite detected was NASAC, followed by NASACS (Table 1). Along with coelution of the new peaks to the reference compounds, the identity of NASAC in urine of rats treated with SAC was also confirmed by GC-mass spectrometry (data not shown). The mass spectrum exhibited the expected molecular ion at $m/z$ 217. Other characteristic fragments were detected at 176, 158, and 144 probably corresponding to M-CH$_2$CH = CH$_2$, M-NHCOCH$_3$ or M-COOCH$_3$, and M-SCH$_2$CH$_2$ = CH$_2$, respectively. The NASAC fragmentation pattern is consistent with that previously described in literature (Jandke and Spiteller, 1987). The results also showed that little compound was excreted as the parent compound, SAC, and its corresponding sulfoxide, SACS, over the 24-h time period monitored. The latter two compounds combined accounted for less than 1.5% of the recovered dose in all treatments (Table 1). The amount of sulfoxides (SACS and NASACS) detected comprised nearly 20% of the recovered dose (except in the animals pretreated with methimazole), suggesting that the FMO reaction is quite favorable in vivo. For all treatments, nearly 40 to 50% of the SAC dose was recovered in the 24-h time period (Table 1), and most of the recovered dose (70–80%) was excreted within the first 8 h.

No significant decrease was observed in the percentage of dose when the 400 mg/kg dose was compared with the 200 mg/kg dose (Table 1). These results suggest that the metabolic pathways were not quite saturated, even at the 400 mg/kg dose.

Pretreatment with the alternative FMO substrate, methimazole, caused a significant decrease in the percentage dose of SACS and NASACS excreted in urine in the 0 to 8 h period when compared with the corresponding time period of the 200 mg/kg SAC treatment (Table 1). Similarly, there was a significant decrease in SACS and NASACS urine excretion in the animals pretreated with methimazole over the 24-h collection period (Fig. 3, A and B).

Pretreatment with the β-lyase and transaminase inhibitor, AOAA, increased the SAC excretion in the 8- to 24-h time period compared with the 200 mg/kg SAC at the corresponding time but had no effect on the total amount excreted over 24 h. No other effects on SACS,
NASAC, and NASACS were observed when compared with animals treated with 200 mg/kg SAC alone.

To help elucidate whether SAC is first N-acetylated to NASAC before sulfoxidation or is oxidized to SACS before N-acetylation, the in vitro metabolism of equimolar concentration of SAC and NASAC was monitored spectrophotometrically by examining concurrent oxidation of NADPH. The specific activity of the reaction was 0.88 ± 0.10 and 0.03 ± 0.03 (means ± S.D.) nmol/mg of protein/min for SAC and NASAC, respectively. Thus, the results show that SAC is metabolized at a rate that is at least 20 times faster than that of NASAC (Fig. 4). In fact, there was very little additional loss of NADPH in the incubations containing NASAC over the control incubations that contained no substrate. The fact that NASAC was a poor substrate was also confirmed by HPLC, using nonsolubilized rat liver microsomes and NADPH. Only a trace amount of NASACS was detected in these incubations (data not shown). However, SACS could be easily detected in similar liver microsomal incubations with SAC (data not shown).

Discussion

The results presented in this manuscript show that rats treated in vivo with the natural garlic component, SAC, excrete large amounts of NASAC and its corresponding sulfoxide into their urine. Only small amounts of SAC or SACS are excreted. As indicated above, other investigators have already shown SAC metabolism to NASAC. However, this is the first study that developed GC and HPLC methods to quantitate the in vivo formation of sulfoxides of SAC and NASAC and examined the role of FMOs in these reactions. Indeed, the methods developed in this study could be used to quantitate SAC metabolism to SACS and NASACS in humans.

In the current study, the NASAC detected in rat urine comprised 30 to 40% of the dose, and the SAC detected comprised approximately 1% of the dose when rats were given either 200 or 400 mg/kg SAC i.p. This is consistent with a previous study done in rats (Nagae et al., 1994), although in that study the SAC doses used were up to 50 mg/kg, and SAC was administered either by gavage or intravenously.

Methimazole pretreatment significantly affected the amount of SACS and NASACS formed by rats given SAC (200 mg/kg; 1242 µmol/kg). Since methimazole is an alternative substrate for FMOs with $K_m$ values for the different isoforms ranging from 2 to 34 µM (Duescher et al., 1994), the dose we used in this study was expected to inhibit SAC and NASAC sulfoxidations. The significant effects observed provide evidence that FMOs are the enzymes involved in SACS and NASACS formation. Although methimazole can be oxidized by cytochrome P450s to give rise to N-methylimidazolone through a sulfenic acid metabolite that has been shown to inhibit cytochrome P450s (Kedderis and Rickert, 1985), the $K_m$ for this reaction is 23 mM (Lee and Neal, 1978), which is much higher than the dose used in this study. Also, this inhibition of cytochrome P450s would probably have little effect on SAC metabolism as Ripp et al. (1997) showed that the general P450 inhibitor, 1-benzylimidazole, did not decrease SACS formation in vitro.

Pretreatment of rats with AOAA before they were given SAC had no significant effect on the metabolite excretion pattern observed. The AOAA dose used in this study has been shown to cause nearly 70 to 90% inhibition of hepatic and renal cysteine conjugate $\beta$-lyase activities and was also shown to protect against 5- (1,2-dichlorovinyl)-l-cysteine nephrotoxicity (Elfarra et al., 1986). Since AOAA inhibits pyridoxal enzymes such as $\beta$-lyases and transaminases, it was expected to increase the amount of the competing sulfoxidation reaction. However, this effect was not observed, probably due to the fact that the N-acetylation pathway was not yet saturated. This is supported by the fact that there is not a significant decrease in the percentage of NASAC excreted between the 200 and 400 mg/kg dose.

Our in vitro results comparing the sulfoxidation of SAC and NASAC demonstrate that NASAC is a poor substrate for FMOs compared with SAC. NASAC appears not to be a good substrate for P450s as indicated by the results of the in vitro assays using nonsolubilized rat liver microsomes. Lack of P450 involvement in the metabolism of SAC was observed previously by Ripp et al. (1997) using rabbit liver microsomes. In these studies, the P450 inhibitor, 1-benzylimidazole, did not decrease SACS formation and solubilization with Emulgen 911, a procedure that inactivates P450s but does not affect FMOs, slightly increased the rate of SAC sulfoxidation in rabbit liver microsomes. Collectively, our results suggest that SACS is more likely to be formed and then N-acetylated in vivo to yield NASACs rather than for NASACs to be formed by the sulfoxidation of SAC. However, considering that NASAC is the major metabolite of SAC, both metabolic pathways could be operative in vivo.

The doses that we used in the current study are the same as the doses used by Sumiyoshi and Wargovich (1990), which reduced the incidences of 1,2-dimethylhydrazine-induced colon tumors in mice. The mechanisms by which SAC exerts its anticancer and antioxidant effects are still unknown. Induction of glutathione $S$-transferase-$\alpha$ and glutathione $S$-transferase-$\mu$ was observed when SAC (1.8 mmol/kg/day) was administered to rats for 3 days (Hatono et al., 1996) and may be one mechanism by which SAC exerts its chemoprotective effects. SAC has also been shown to have antioxidant properties and to inhibit nuclear factor kappa B in human T cells (Geng et al., 1997). However, the roles of SAC metabolites in these biological effects of SAC are presently unknown.

The formation of SACS from SAC has been previously suggested, based upon the use of paper chromatography (Kaye et al., 1972). Our results provide further evidence for SAC sulfoxidation. The finding that nearly 20% of the recovered dose of SAC is in the sulfoxide form (SACS and NASACS) suggests that sulfoxidation plays an important role in the overall metabolism of SAC. Because several halogenated hydrocarbons and their corresponding glutathione- and cysteine $S$-conjugates are metabolized to sulfoxides in vivo (Barnsley et al., 1969; Elfarra, 1995), our results suggest that FMOs might also be involved in these metabolic reactions.
References


