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Physical, Chemical, and Biological Properties of S-Allylcysteine, an Amino Acid Derived from Garlic

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Physical, chemical, and biological properties of S-allylcysteine (SAC) were investigated. SAC showed stable properties under tested conditions, and its acute/subacute toxicity was very minor in mice and rats (LD_{50} value >54.7 mM/kg po; >20 mM/kg ip). The pharmacokinetics of SAC was investigated after oral administration of garlic supplement containing SAC to human volunteers. SAC from garlic consumption was rapidly absorbed from the gastrointestinal tract, however, the half-life and excretion time were more than 10 h and 30 h, respectively.

KEYWORDS: Garlic; S-allylcysteine; toxicity; stability; pharmacokinetics

INTRODUCTION

Although garlic (*Allium sativum* L.) has been used for remedies and food for more than a thousand years, most people used garlic based on their experiences without any knowledge about relationships between biological activities and constituents of garlic or its transformation products (*1*−*3*). Because garlic's characteristic odor is attributed to its sulfur-containing compounds (*1*−*3*), researchers interested in garlic have focused their attention nearly exclusively on these compounds. The discovery of allicin in 1944 ignited intense investigation of the sulfur-containing compounds in garlic and its unique sulfur chemistry (*4*−*6*). Numerous sulfur-containing compounds derived from garlic have since been identified (**Figure 1**) , and their biological activities have been investigated (*2*, *3*).

Recently, S-allylcysteine (SAC), a sulfur-containing amino acid derived from garlic, has been reported to have antioxidant activity (*7*−*10*), anti-cancer promoting activity (*11*−*17*), anti-hepatopathic activity (*18*, *19*), and neurotrophic activity (*20*, *21*). SAC content in the intact garlic is small (not more than 30 μg/g-fresh weight), however, this compound is produced in a soaking preparation through hydrolysis of γ-glutamyl-S-allyl-cysteine which exists in raw garlic as a precursor of SAC (*3*). The SAC formed in preparation was stable throughout the remainder of 2 years. Thus, SAC is one of the chemically and biologically remarkable sulfur-containing compounds derived from garlic. Nagae et al. have reported the pharmcokinetics of SAC in experimental animals (*22*, *23*). Although numerous compounds derived from garlic have been investigated in the last 50 years to determine the benefits of garlic for human health, there has been very little comprehensive evaluation of compounds derived from garlic, including SAC.

Therefore, this paper addresses the physical, chemical, and biological properties of SAC derived from garlic, which might have an important role in the benefits of garlic to human health.

MATERIALS AND METHODS

Chemicals. SAC was synthesized and purified according to a recent paper (*22*), and was purchased from Nihonrigakakuyakuhin-kogyo Co., Ltd. (Tokyo, Japan). Aged garlic extract was used for a SAC-containing garlic supplement (Wakunaga Pharm. Co., Ltd., Osaka, Japan). All chemicals for analysis and/or experiment were of analytical grade and were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animals. Six-week-old Crj wistar rats and six-week-old Crj CD-1 mice (Japan Charls River Inc.) were used for experiments. The animals were fasted for 18 h before and 6 h after the drug administration.

Study of Solubility. Solubility was determined by the volume of solvent to dissolve one gram of SAC at 20 °C. Fine powdered SAC was placed into appropriate glassware and an appropriate volume of solvent was added. This mixture was shaken vigorously and kept at 20 °C for 30 min. Additional solvent was added and kept at the same conditions if insoluble substance remained in the resulting solution. Solubility in various solvents was tested using water, #1 solution for disintegration test on JP (2.0 g of NaCl and 7.0 mL of HCl in 1000 mL of purified water), and several organic solvents. Solubility at various pH levels was tested using Carmody's buffer (buffering ability: pH 2−12). Various pH solutions were prepared by appropriate mixing of

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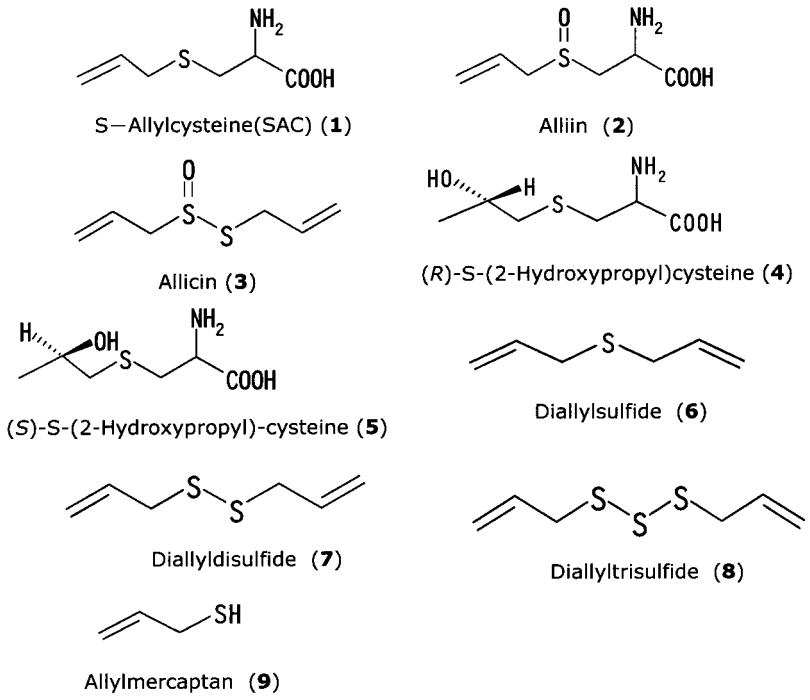

Figure 1. Structure of sulfur-containing compounds derived from garlic

the following A and B solutions: solution A, 0.05 M citric acid and 0.2 M boric acid; Solution B, 0.1 M sodium monophosphate.

Hygroscopicity. One gram of fine powdered SAC was placed into a weighing bottle, spread at the thickness of not more than 2 mm, and then kept at 25 °C on a glass desiccator which controlled the relative humidity using a saturated base solution (see footnote on **Table 3**). Bottles were taken out of the desiccator and weighed at 7, 14, and 28 days after the beginning of storage. Hygroscopicity under each humidity condition was calculated by weight difference between the before storage and after storage measurements.

Melting Range and Thermal Analysis. Melting range was measured according to the Japanese Pharmacopeia (JP). Thermal analysis was performed by thermogravimetry−differential thermal analysis using a TG/DTA6200 (Seiko Instruments, Chiba, Japan).

Dissociation Constant. Dissociation constant was estimated by obtained titration curve using 0.05 N HCl and 0.05 N NaOH test solutions.

Partition Constant. The partition between Carmody's buffer or JP Solution #1 (see Study of Solubility) and 1-octanol was calculated by measurement of SAC content in the 1-octanol phase before and after mixing with each buffer or solution. Fine powered SAC was mixed with 1-octanol and shaken vigorously. This mixture was centrifuged at about 2000*g* for 10 min, and then the supernatant was filtered using a membrane filter (pore size: 0.45 μm). To 10-mL aliquots of the obtained filtered 1-octanol solution, in capped, 50-mL centrifuge tubes, was added the same volume of each buffer or solution. The tubes were then shaken vigorously at room temperature for 10 min. The SAC content in 1-octanol phase, before and after mixing with buffers and solutions, was measured by HPLC using the following conditions: column, YMC pack ODS AQ-313; mobile phase, mixture of water/1% (v/v) phosphate/acetonitrile (5:10:85, v/v); controlled flow to elute the SAC at about 11 min; detection, UV 220 nm absorption (0.08 aufs).

Optical Rotation. A 10-g aliquot of SAC was dissolved in 6 N HCl, and from this was made 50 mL by the same solution. Optical rotation was measured at 20 °C using a digital polarimeter JASCO DIP-360 (Nihonbunko, Tokyo, Japan).

X-ray Powder Diffractometry. X-ray powder diffractometry was carried out for the sample shown in **Table 5**, and polymorphism of SAC was evaluated by obtaining 2θ angles of each sample. X-ray analysis was performed on Shimadzu Techno-Research Inc. (Kyoto, Japan) using X-ray diffractometer XD-D1 (Shimadzu Co., Kyoto, Japan).

Stability under Light Irradiation. Stability under light irradiation was performed in UV and fluorescent light. SAC was dissolved in purified water, 1 N HCl, and 0.1 N NaOH to make a 0.2 g/mL solution. The fine powdered SAC was spread on a 70-mm-diameter Petri dish at the thickness of not more than 3 mm. Crystal samples or samples of each solution were irradiated by UV light (not less than 200W*hr/cm^2) or fluorescent light (not less than 120 million Lux) at 20 °C. Dark control samples were prepared in the same manner, wrapped with aluminum foil, and placed beside the test samples. SAC content in each sample after irradiation was measured by HPLC method as follows: column, YMC pack ODS AQ-313; mobile phase, mixture of acetonitrile/water/0.1% phosphate solution (7:2:1) containing 3.6 g of SDS per 1000 mL of mixture, detection, UV 220 nm absorbance; flow rate, controlled flow to elute the SAC at about 8 min.

Stability under Alkaline, Acid, Heated, and Long-Term Conditions. SAC was tested for its stability under alkaline, acid, and heated conditions, as well as for its long-term stability. One gram of SAC was dissolved in 50 mL of 6 N HCl or 1 N NaOH, and stored at 50 °C for 5 or 6 days. Each sample was analyzed by the same HPLC method as described above. Observed transformed or decomposed products on HPLC profiles were isolated by chromatographic methods using silica gel and identified by NMR, IR, and MS. Fine powdered SAC was heated at 105 °C for 24 h, and this sample was analyzed by the same HPLC method as above. Stability of SAC under heat was evaluated by obtained chromatoprofiles. SAC (50 g) was packed in polyethylene bags and heat-sealed. The polyethylene bags were wrapped in aluminum sheets and heat-sealed. They were stored at 25 °C and 75% humidity. Stability of SAC was determined by the following items for suitable time periods using the same HPLC method as above: character (color, odor, appearance, optical rotation, melting range, and pH); identification (amino group, sulfur, and IR spectrum); purity (analogues: cysteine, cystine); loss on drying, and content.

Preparation of (R)-S-(2-hydroxypropyl)-cysteine and (S)-S-(2-hydroxypropyl)-cysteine ((R)S2HPC and (S)S2HPC). (R)S2HPC and (S)S2HPC, identified transformation products generated under strong acidic conditions, were synthesized to confirm chemical structure by comparing the spectra of NMR, IR, and MS. NaH (0.6 g) suspended in mineral oil (13.8 mmol) was placed in a 100-mL volumetric flask, and the mineral oil was removed using ether. DMSO (10 mL) was added under a nitrogen gas stream. A 1.26-g portion of powdered cysteine (10.4 mmol) and 0.63 mL of propylenoxide (11.7 mmol) were added and this mixture was stirred at room temperature for 2 h.

Table 1. Evaluation and Test Results of Synthesized SAC

test	specifications in house	results[a] lot 1	lot 2
description			
(1) appearance	white crystalline or crystalline powder	crystalline powder	
(2) odor	characteristic odor	characteristic odor	
identification			
(1) amino group	positive to ninhydrin reaction	same as standard	
(2) sulfur	positive to nitroprusside reaction	same as standard	
purity			
(1) optical rotation	+4.0 – +5.0°	+4.17°	+4.20°
(2) bromide	not more than 0.1%	not more than 0.1%	
(3) heavy metals	not more than 20 ppm	not more than 20 ppm	
(4) arsenic	not more than 2 ppm	not more than 2 ppm	
analogues			
(1)cystine	not more than 1.0%	not more than 1.0%	
(2)cysteine	not more than 0.1%	not more than 0.1%	
loss on drying	not more than 0.1%	0.0%	0.0%
residue on ignition	not more than 0.5%	0.01%	0.1%
melting range	222–225 °C	223.2 °C	222.0–222.4 °C
assay	98.5%–101.5%	99.7%	99.8%

[a] Lot 1 was used for physical and chemical properties. Lot 2 was used for toxicity studies.

Resulting (R)S2HPC and (S)S2HPC was purified by silica gel chromatography using $CHCl_3$/MeOH/H_2O (6:4:1) as elution solvent. Chemical structures of the compounds obtained were confirmed by MS, ^{1}H NMR, and ^{13}C NMR.

Pharmacokinetic Study. Healthy volunteers (ages 38, 45, and 46) were given 500 mg of aged garlic extract in capsule form with water (for dosage of SAC, see footnote on **Figure 7**). Their diets were controlled to eliminate consumption of the allium species for 2 days before the test and 1 day after the test. Blood samples (ca. 10 mL) were collected from the brachiocephalic vein of volunteers using heparinized syringes. Blood collected before administration of the garlic extract was used for the control blood sample. About 10 mL of blood was collected by same manner as above after administration (1 h, 5 h, and 23 h). Collected blood samples were centrifuged at about 1000*g* for 10 min, and the plasma fraction was collected for analytical test samples.

Preparation of Test Solution for SAC Analysis in Blood Samples. To 1 mL of obtained plasma sample or blood cell fraction sample, 1 mL of methanol was added, and this was mixed vigorously, and centrifuged at 2000*g* for 5 min. The supernatant was collected; 1 mL of 50(v/v)% methanol was added to the residue, the solution was mixed vigorously and centrifuged at 2000*g* for 5 min. The supernatants were combined, and the solvent was removed under reduced pressure. A 0.2-mL aliquot of elution solvent of HPLC was added to the residue, which was dissolved and filtered using a membrane filter (pore size 0.45 μm). The filtrate was used for sample preparation.

Stability of SAC in Blood Samples. Stability of SAC in the plasma fraction or red blood fraction was evaluated by recovery tests from these fractions. Standard SAC was dissolved in saline, and the concentration was adjusted to 200 μg/mL. This solution (50 μL) was added to 0.5 mL of whole blood/plasma samples and kept at room temperature. Aliquots of these mixtures were collected each time and the SAC content in each mixture was tested using HPLC and the analysis method described above.

HPLC Analysis of Blood Sample. Determination of SAC content in blood samples was carried out by OPA-labeled postcolumn ion-exchanging HPLC using a Shimazdu LC-6A gradient HPLC system and Aminopack column (4.6 mm i.d. × 120 mm, anion exchange column, Tosho, Japan). HPLC conditions were as follows: detection, excitation wavelength 350 nm, emission wavelength 455 nm (*7*).

Acute Toxicity. Tested SAC was dissolved/suspended in vehicle (0.5% CMC solution) and administered by oral (po) and intraperitoneal (ip) routes. Doses were 2100 to 15000 mg/kg, and volumes of administration were 6 to 44 mL/kg-body weight. Five animals of both genders were used for one group. Experimental items were observation of general state and LD_{50} values.

Subacute Toxicity. Tested SAC was dissolved/suspended in vehicle (0.5% CMC solution) and administered by po route. Dose levels were 250, 500, 1000, and 2000 mg/kg, and volumes of administration were 10 mL/kg-body weight. Ten animals of both genders were used for one group. Experimental items were observation of general state, hematological examination, biochemical examination, urinalysis, and pathological examination. Biochemical examination were performed at Fukuyama Rinsyo Co., Ltd. (Fukuyama, Japan).

Table 2. Solubility of SAC in Various Solvents and Various pH

solvent		volume of solvent (mL)
water		14.7
10% of HCl		5.0
0.1N NaOH		11.0
methanol		1053
ethanol		>10000
acetonitrile		>10000
ethyl acetate		>10000
Carmody's buffer	pH 2.41	13.1
	pH 3.65	14.2
	pH 5.84	14.0
	pH 8.29	13.4
	pH 9.68	13.0
	pH 10.47	12.5

RESULTS

Physical and Chemical Properties. Synthesized/purchased SAC was evaluated according to the testing method in the Japanese Pharmacopeia before performing the physical, chemical, and biological studies, and evaluated results are shown in **Table 1**. The solubility of SAC in various solvents and at various pH levels are shown in **Table 2**. Solubility was determined by the volume of solvent to dissolve one gram of SAC at 20 °C. **Table 3** shows hygroscopicity at varying humidity. Weight increases were observed in some humidity conditions, however, differences were only slight. Therefore, SAC has not shown hygroscopic ability and critical hygroscopicity was not estimated. The melting range of SAC was 223.3−223.6 °C according to the testing method in the Japanese Pharmacopoeia. The TG-DTA curve of thermal analysis showed 223.7 °C as the melting point of SAC (**Figure 2**). SAC might be decomposed at the melting point considering that the weight decreased at the peak of heat absorption when melted.

Dissociation constant on acidic region was not determined because the point of inflection was not clearly observed on the acidic phase (rough estimation pK = 2.2), however, dissociation

Table 3. Hygroscopicity of SAC in Various Levels of Humidity[a]

humidity (%)	storage period (days)	weight increase (%)
93	7	0.03
	14	0.09
	28	0.17
81	7	0.03
	14	0.07
	28	0.07
71	7	0.02
	14	0.06
	28	0.13
51	7	0.02
	14	0.05
	28	0.08

[a] Each humidity was controlled using the following saturated basic solution: 51%, $Ca(NO_3)_2 \cdot 4H_2O$; 71%, $NH_4Cl+KNO_3$; 81%, $(NH_4)_2SO_4$; 93%, $NH_4H_2PO_4$.

constant on the basic region was determined to be 9.1 by obtained titration curve (**Figure 3**). Isoelectric point (pI) was determined at 5.6. The pH of aqueous SAC solution was 5.56 at 24.5 °C and this result corresponded to the pI result. The partition between Carmody's buffer or JP Solution #1 and 1-octanol was measured. The partition coefficient could not be determined because all of the SAC was recovered from the aqueous solution (**Table 4**). Optical rotation of SAC was measured at 20 °C and it was +4.41. X-ray powder diffractometry was carried out for the crystal of intact powder and some treated powders (**Table 5**). As there was no change on any of the X-ray powder diffraction patterns (**Figure 4**), no polymorphism of SAC was observed.

Stability and Transformation Product. Table 6 shows the results of the stability of SAC in aqueous solution. Recovery of SAC from each tested solution was almost 100%, and no peaks were observed except that of SAC on chromatoprofiles. Storage under more severe conditions was performed to detect the transformation/decomposition products. Storage in strong acidic conditions (dissolved in 6 N HCl and stored at 50 °C for 5 days) was performed, and a new peak was observed on the HPLC chromatoprofile. This product was isolated from the storage mixture by silica gel chromatography, and then the chemical structure was analyzed using IR, MS, and NMR. The isolated compounds were identified compounds (R)S2HPC and (S)S2HPC. Furthermore, compounds (R)S2HPC and (S)S2HPC were synthesized and compared with the discovered isolated compound to confirm the chemical structure. Obtained spectrums of the synthesized compound corresponded to spectrums of the isolated compound. Spectrum data of the isolated compound follow: mass spectrum, M/Z = 180 (chemical ionization); ^{1}H NMR spectrum (in D_2O): 1.24 ppm (6H, d, CH_3), 2.66−2.80 ppm (4H, ddd, S−CH_2), 3.02−3.17 ppm (4H, m, C(OH)−CH_2−S), 3.88−3.97 ppm (4H, m, CH(OH) and CH of alpha carbon); ^{13}C NMR spectrum (DMSO-*d*): 22.35 ppm and 22.49 ppm (R/S, CH_3−), 33.02 ppm and 33.21 ppm (R/S, −CH_2−S−), 40.36 ppm and 40.53 ppm(R/S, S−CH_2−), 53.00 ppm and 53.08 ppm (R/S,−CH(OH)−), 65.77 ppm and 65.93 ppm (R/S, CH of alpha carbon), 173.34 ppm (C═O); IR spectrum, 3200−3600 cm^{-1} (s, broad, −OH group).

Upon storage under basic conditions (2 N NaOH, 50 °C, 6 days), new peaks, which might be transformation products, were observed on the HPLC chromatoprofile, and the solution took on the characteristic smell and appearance of sulfide. Retention time of one peak was the same as diallylsulfide on HPLC analysis (data not shown). This product was isolated from the storage mixture by silica gel chromatography and then the chemical structure was analyzed using NMR. The isolated compound was diallylsulfide. Obtained spectrums were compared with those of authentic diallylsulfide. Following are the data obtained: ^{1}H NMR spectrum (in $CDCl_3$): 3.32−3.35 ppm (2H, dd, CH_2−S), 5.13−5.23 ppm (2H, m, CH_2═C-,), 5.77−5.89 ppm (1H, m, C═CH−C−S); ^{13}C NMR spectrum (in $CDCl_3$): 42.29 ppm (CH_2−S), 118.42 ppm (CH_2═C−), 133.47 ppm (C═CH−C−S).

The retention time of another peak corresponded to allylmercaptan, however, the substance corresponding to this peak was not recovered. Therefore the compound of this peak was not identified clearly.

Furthermore, no transformation products were observed after the crystal samples were heated at 105 °C for 24 h, although the color of sample changed to a slightly yellowish color. **Table 7** show the stability of SAC under light irradiated conditions. Although surface color of the crystal samples changed to a slightly yellowish color, no other significant differences were observed in the samples. Also, no significant differences were observed in the aqueous samples. All of the samples were stable under the storage conditions. Results of the long-term stability test at 25 °C are shown in **Table 8**. SAC was shown to be a very stable compound because no changes were noted under the various storage conditions.

Evaluation of Safety. Acute toxicity of SAC in mice and rats was determined by po and ip administration route on a single dose. **Table 9** shows the LD_{50} values of the tested animals. Although a decrease in spontaneous motor activities was observed in the general state observation, this sign in survivors disappeared about 24 h after administration. Most death cases in both animals occurred within 24 h after po and ip administration. Necropsy of dead animals indicated slight congestion of the lung in some groups of mice and rats. No notable pathological changes associated with SAC were found in the organs of the animals sacrificed after surviving the 7 days.

Although some of the mice in the high dosage group of both genders had slightly rough hair, no other significant observations were noted in the general state observations of the subacute toxicity test. Body weight changes in male and female rats orally administered SAC for one month are shown in **Figure 5** and **Figure 6**, respectively. The ratios of body weight gain were suppressed on high dosages. Significant differences were observed at 500−2000 mg/kg dosages at 2 days after administration. Body weight increased on the 250 mg/kg dosage of male rats, and significant differences were observed 18 days after administration. Body weight changes in female rats were similar to those of their male counterparts, however, difference of body weight changes on each dosage group of female were very little as compared to those of the male. Food consumption was decreased depending on the dosage in both genders. Consumption amounts were recovered in order of dosage amount, and the recovery period of female groups was faster than that of male groups (data not shown).

Table 10 shows the results of urinalysis in rats. Increasing of pH and decreasing of urobilinogen in males were observed, and significant differences were observed in the 1000 mg/kg and 2000 mg/kg groups. Decreasing of protein and urobilinogen were observed in the female group. **Table 11** shows the results of the hematological examination. Several tested items, such as mean corpuscular volume (MCV), hematocrit (Ht), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), decreased depending on the dosage, with significant differences seen in both genders. **Table 12** shows the results of the biochemical examination. Decreases in blood urea nitrogen (BUN), creati-

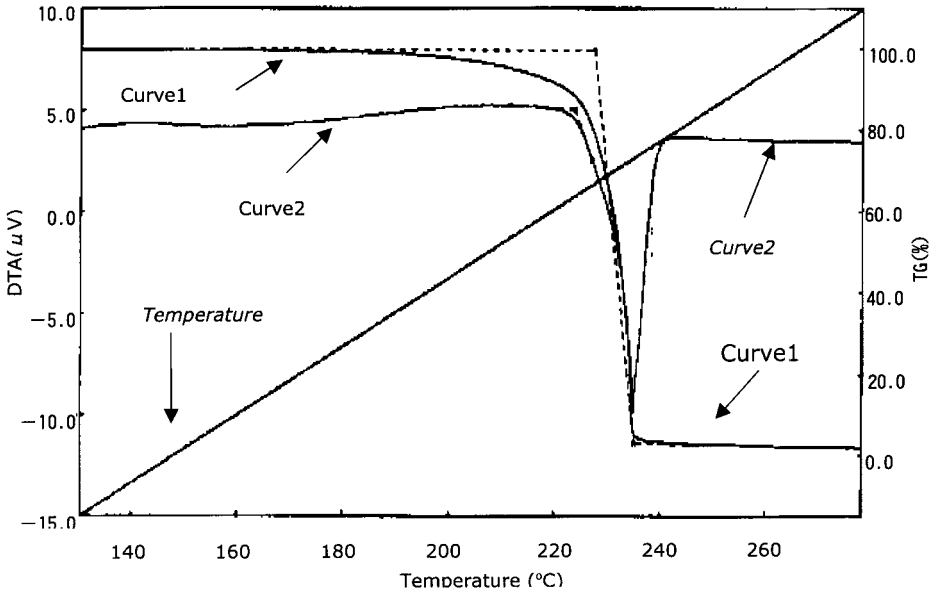

Figure 2. Profile of SAC on thermogravimetry–differential thermal analysis (TG-DTA). Curve 1, TG curve; Curve 2, DTA curve. Temperature was increased at 10 °C/min for 30–150 °C, and at 2 °C for 150–280 °C.

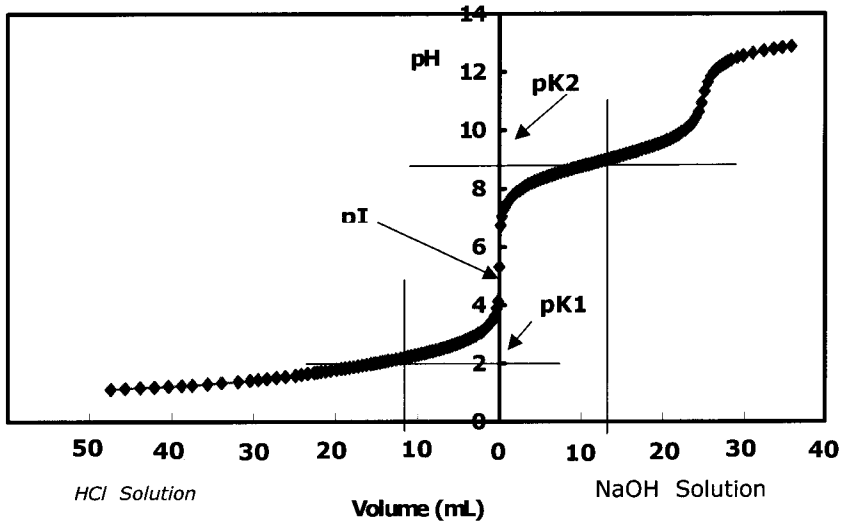

Figure 3. Titration curve of SAC.

Table 4. Recovery of SAC on Partition Test

buffer[a]	pH	content in buffer (%)
1	2.60	102.2
2	3.78	101.0
3	5.71	101.9
4	9.31	101.2
5	9.71	101.5
6	10.52	102.1
7	1.20	102.1

[a] Buffers 1–6 were prepared using Carmody's buffer, and buffer 7 was solution # 1 on JP.

Table 5. List of Tested Samples for X-ray Analysis

sample	treatment
1	intact SAC powder
2	recrystallized SAC from water
3	recrystallized SAC from 70% ethanol
4	heated SAC at 105 °C for 25 h and irradiated UV at not less than 200Whr/m^2

nine, and total protein were observed in both genders with significance on high dosage. Gender differences were observed, such as increases in albumin and K^+ in the male group, and increases in alkaline phosphatase (ALP), total cholesterol, total lipid, and glucose in the female group. Observation on necropsy of surviving rats follows: an adhesion of liver on 2000 mg/kg of males, two hypertrophy of the liver on 2000 mg/kg of males, a white surface of spleen on 2000 mg/kg of both genders, an atrophy of pancreas on 500 and 2000 mg/kg of males and 1000 and 2000 mg/kg of females, an atrophy of seminal vesicles on 2000 mg/kg, and two atrophy of thymus on 2000 mg/kg of females. However, the incidences for all of these effects were not significantly different from those of the controls in both genders.

Pharmacokinetic Study. Figure 7 shows the concentration of SAC in plasma after oral administration of garlic supplement by healthy volunteers. T_{max} of SAC was around 1 h after administration and $T_{1/2\beta}$ was estimated to be greater than 10 h. These results were the same as those from preliminary examinations (data not shown). Stability of SAC in the plasma fraction and red blood fraction was tested. Recovery of SAC from the

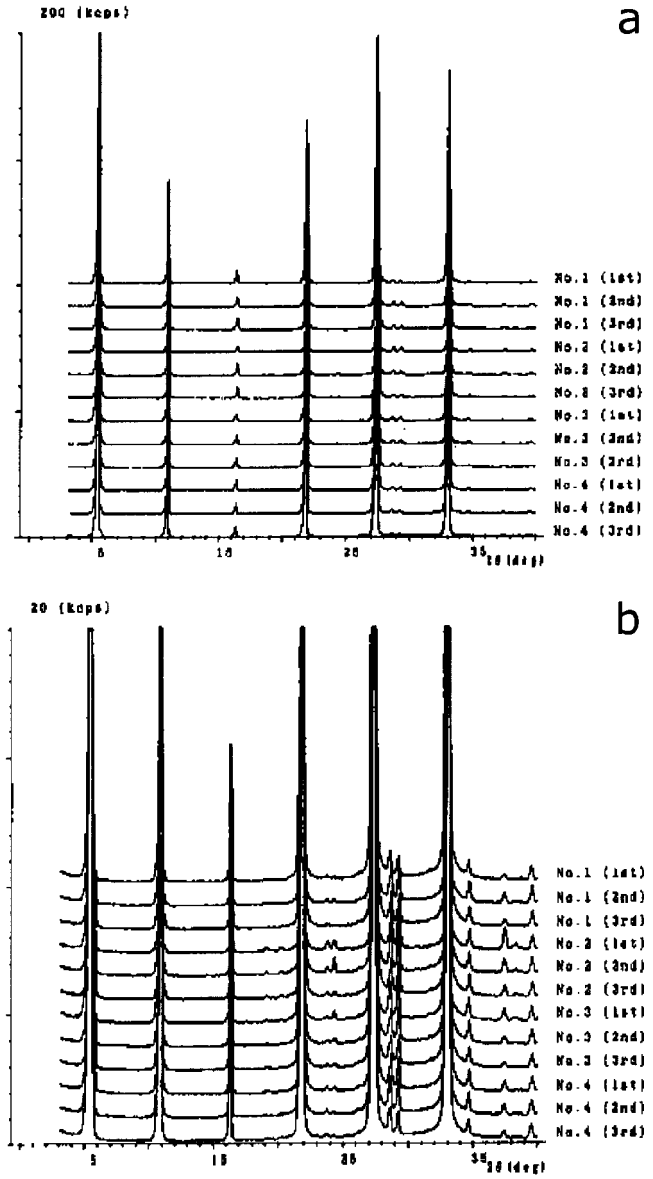

Figure 4. X-ray powder diffraction patterns of SAC. (a) Intensity scale 200 (kcps), (b) intensity scale 20 (kcps).

Table 6. Stability of SAC in Aqueous Solution

storage condition[a]	recovery(%)
crystal	100.0
water solution	100.7
1 N HCl solution	100.1
1 N NaOH solution	100.6

[a] Each sample was kept at 50 °C for 5 days.

plasma fraction was almost 100% at 3 h after the addition of SAC to this fraction; however, recovery from the red blood fraction was 87% at 3 h (data not shown).

DISCUSSION

Although a lot of compounds derived from garlic have been isolated and their biological activities have been investigated, little is known about the physical, chemical, and biological properties of isolated compounds. SAC is one such compound that has received only limited scientific evaluation. This paper presents physical, chemical, and biological properties of SAC, a sulfur-containing amino acid derived from garlic. In aqueous solvents, SAC dissolved easily, but it was found to be slightly soluble or insoluble in organic solvents, except for methanol. Solubility in basic solutions was higher than in acidic solutions, although the difference was small within the tested pH range. Hygroscopicity was tested at several humidity conditions. There were very little increases in the weight of tested samples (not more than 0.17% on 93% RH), therefore, SAC has almost no hygroscopicity ability. Critical hygroscopicity also could not be estimated from these results. TG-DTA curve showed that the melting point was 223.7 °C, and the weight decreased at the peak of heat absorption to melt the SAC. Therefore, the melting point of SAC might be the decomposition point.

Table 7. Stability of SAC under the Light Irradiated Conditions

storage sample		content (%) UV light[a]	content (%) fluorescent light[b]
crystal	sample	101.4	99.2
	dark control	99.9	98.5
water	sample	99.5	98.0
	dark control	100.2	100.2
1 N HCl	sample	99.6	102.3
	dark control	99.9	98.5
0.1 N NaOH	sample	100.3	100.4
	dark control	99.2	99.9

[a] Not less than 200 W*hrs/m^2. [b] Not less than 120 million Lux.

SAC is a very stable compound as seen from the results of stability test conditions, although storage samples showed a slight change into a yellowish color, but no transformation or decomposition products were observed. Under strong acidic and basic conditions, transformation/decomposition products were observed. It was well-known that numerous transformed/decomposed compounds appear after cleavage of the C−S bond in the sulfur chemistry of garlic. Observation of the existence of allylmercaptan and allylsulfide indicate that the cleavage of the C−S bond and subsequent complicated reactions proceed under basic storage conditions. However, evidence of cleavage of the C−S bond was not observed under acidic storage conditions. These observations indicate that SAC in garlic preparations would be absorbed without any decomposition from changes in gastrointestinal pH after administration.

Toxicity studies of garlic and garlic preparations have been conducted (*24*, *25*). As a food, garlic shows very low toxicity, and aged garlic extract exhibits no histopathological changes in various organs and tissues (*26*, *27*). Although more than 50 years of investigation has led to the isolation and biological evaluation of numerous compounds in garlic, very little research has been done regarding their safety. The LD_{50} of allicin, a well-known compound derived from garlic, is 60 mg/kg intravenously (iv) and 120 mg/kg subcutaneously (sc) in mice (*6*). Feeding of garlic oil (100 mg/kg, intragastrically) after 24 h of fasting was found to be lethal by causing acute pulmonary edema (*28*). Garlic oil is mainly composed of allylpolysulfides, such as diallylsulfide, diallyl disulfide, diallyltrisulfide, and so on. These hydrophobic compounds were presumed to be responsible for the benefits of garlic, and numerous studies have been performed both in vivo and in vitro. Investigating these compounds at a normal level of daily intake, such as a few cloves, may not be a problem. However, evaluation in more detail should be required if these compounds are to be used for pharmaceutical or medical applications. Also, hydrophilic compounds derived from garlic, such as alliin and glutamyl peptide, do not have in vivo safety data. The present results confirm the low toxicity of SAC, because the LD_{50} in both animals upon po

Table 8. Long Term Stability Test

	storage period(months)						
	0	3	6	9	12	18	48
appearance							
color	significant differences from initial properties were not observed.						
smell							
optical rotation[a]	+4.67	+4.38	+4.47	+4.50	+4.57	+4.60	+4.39
melting range(°C)	223.3–223.6	222.7–223.0	222.6–223.0	222.9–223.5	223.1–223.6	223.2–223.7	223.0–223.9
pH	5.63	5.53	5.51	5.56	5.61	5.61	5.45
analogues							
cystine	<1%	<1%	<1%	<1%	<1%	<1%	<1%
cysteine	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%
loss on drying	0.01%	0.01%	0.03%	0.04%	0.06%	0.04%	0.05%
identification[a]	All of tested items were identified.						
content(%)	100.2	99.8	100.3	99.9	100.4	100.4	100.2

[a] Identified item: amino group, sulfur, IR spectrum.

Table 9. LD_{50} Values of SAC in Rat and Mice

		LD_{50} values(mg/kg)[a,b]			
route	sex	mice		rat	
po	male	8890	(7600–10220)	10940	(9500–12660)
	female	9390	(8600–10130)	9500	(8080–11150)
ip	male	6910	(6020–8000)	3340	(2890–4090)
	female	3650	(2640–4410)	3340	(2930–3850)

[a] LD_{50} values were calculated by Probit method. [b] 95% confidence limits are expressed in parentheses

administration was over 8.8 g/kg (54.7 mM/kg). The LD_{50} on ip administration of SAC (>20 mM/kg) was at a level similar to that of essential L-amino acids on ip administration in rats, such as Ile (52 mM/kg), Val (46 mM/kg), Leu (41 mM/kg), Phe (32 mM/kg), Met (29 mM/kg), Thr (26 mM/kg), His·HCl (23 mM/kg), Lys·HCl (22 mM/kg), Arg·HCl (18 mM/kg), and Try (8 mM/kg) (*29*). A gender difference was observed in the acute toxicity test upon ip administration to mice, and the LD_{50} in males was about 1.7 times higher than that in females. Body weight, food consumption, and water consumption decreased depending on dosage in both genders in the subacute toxicity test; these observations were more remarkable in males than in females. Recovery was also delayed in the high dosage groups. Urobilinogen in urine is part of a reabsorbed metabolite of bilirubin generated by intestinal bacteria. Therefore, decreased levels of urobilinogen in urinalyses suggests that SAC might have some effect on the intestinal flora at high dosages. Increases in serum glucose, total cholesterol, and total protein were observed in biochemical analysis of the high dosage female group. It could be suggested that a high dosage of SAC induces atrophy of the pancreas and decreases insulin secretion. Indeed, atrophy of the pancreas was observed on necropsy of the female group. However, biochemical analysis revealed no difference in these items in the male group other than atrophy of pancreas at high dosages. Promotion of kidney function by increasing kidney cell number might be indicated because decreased BUN and creatinine in serum and decreased protein in urine were observed. Also, increased liver and kidney weights were observed. All of these negative effects of SAC were observed only at very high dosages, such as more than 500 mg/kg. Therefore, the subacute toxicity test revealed nontoxic doses for rats as much as 250 mg/kg under experimental conditions.

The pharmacokinetic study of SAC in humans was performed by oral administration of garlic preparation, which contains SAC. The peak corresponding to SAC was absent from the chromatoprofile of each volunteer before administration of the

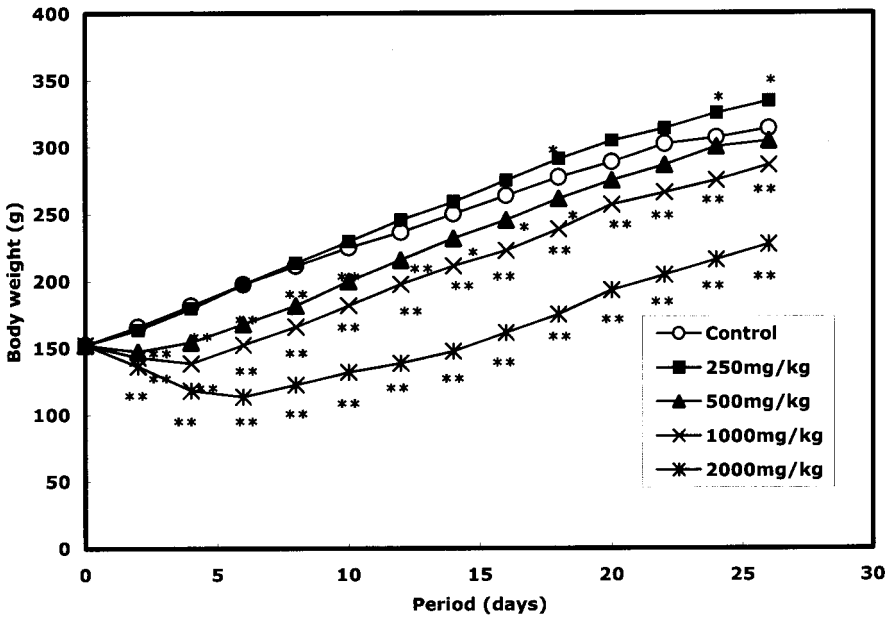

Figure 5. Body weight changes in male rats treated orally with SAC for 1 month. Values represent the average of 10 rats, and asterisks indicate significant difference from control group (*, $p < 0.05$; **, $p < 0.01$).

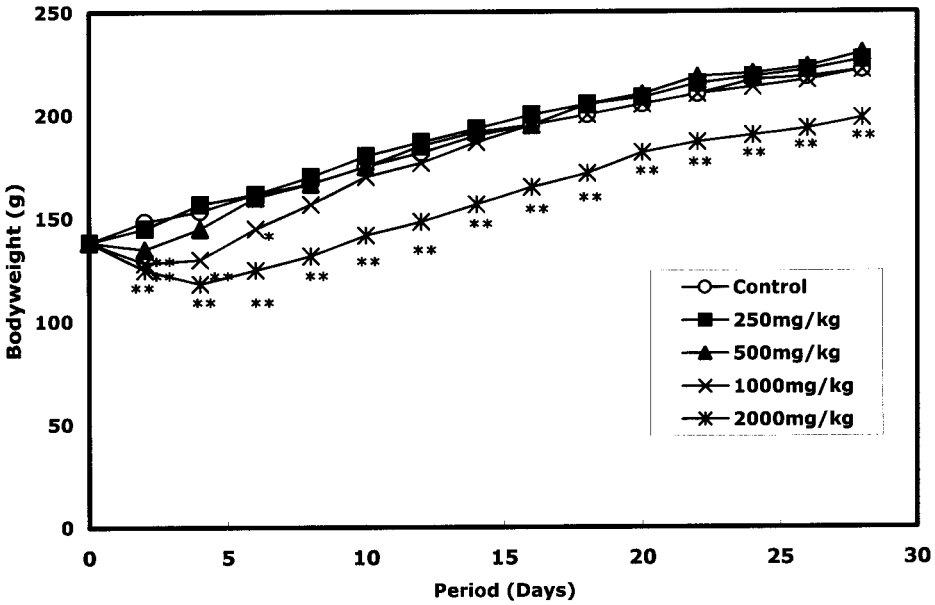

Figure 6. Body weight changes in female rats treated orally with SAC for 1 month. Values represent the average of 10 rats, and asterisks indicate significant difference from control group (*, $p < 0.05$; **, $p < 0.01$).

Table 10. Results of Urinalysis on Rats Treated Orally with SAC for One Month[a]

			pH						protein				
sex	dose (mg/kg)	no. of rats	6.0	6.5	7.0	7.5	8.0	8.5	−	±	+	++	+++
male	control	10	3	2	3	2	0	0	0	0	4	5	1
	250	10	2	0	4	2	2	0	0	0	4	5	1
	500	10	2	0	2	5	1	0	0	0	5	5	0
	1000	10	0	1	2	6	1	0*	0	1	4	5	0
	2000	10	0	4	0	2	3	1	0	1	7	2	0
female	control	10	1	0	3	4	2	0	0	3	5	2	0
	250	10	0	0	2	8	0	0	2	8	0	0	0**
	500	10	1	1	4	4	0	0	2	6	2	0	0*
	1000	9	0	1	1	5	2	0	0	6	2	1	0
	2000	10	2	0	2	6	0	0	0	4	4	2	0

		glucose		ketone body		bilirubin		occult blood			urobilinogen		
sex	dose (mg/kg)	−	+	−	±	−	+	−	±	+	0.1	1	2
male	control	10	0	10	0	10	0	9	1	0	0	2	8
	250	10	0	6	4	10	0	7	3	0	0	6	4
	500	10	0	10	0	10	0	6	2	2	1	5	4
	1000	10	0	9	1	10	0	9	1	0	1	7	2*
	2000	10	0	8	2	10	0	8	2	0	0	10	0**
female	control	10	0	10	0	10	0	8	1	1	0	7	3
	250	10	0	10	0	10	0	9	2	0	6	4	0**
	500	10	0	10	0	10	0	10	0	0	4	4	2
	1000	9	0	9	0	9	0	7	2	0	1	6	2
	2000	10	0	8	2	10	0	9	1	0	0	8	2

[a] Asterisks indicate significant difference (*, $p < 0.05$; **, $p < 0.01$) from the control by Mann–Whitney's U-test.

garlic preparation. To investigate the pharmacokinetic behaviors of SAC in human in this study, the diets consumed in this experimental period might be assumed to have no effect for analysis of SAC in plasma. Additionally, the peak corresponding to SAC was observed in some chromatoprofiles in our follow-up investigation under non-diet-control conditions (data not shown, several volunteers consumed garlic in their diets, but the amount of garlic consumed was not quantified). The half-life of SAC in humans after oral administration was more than 10 h, and clearance time (C_0) was estimated to be more than 30 h. These results appeared similar to experimental results tests in dogs, where the half-life was found to be about 10 h and clearance time (C_0) was more than 24 h, but they differ from experimental results from tests in murine (*22*, *23*). Using GC−MS, Jandke et al. (*30*) identified *N*-acetyl-S-allylcysteine as a metabolite of SAC in human urine after oral administration of garlic. However, using the HPLC method reported by Nagae et al. (*22*) in this study, *N*-acetyl-S-allylcysteine was not identified in human urine samples (data not shown). Total SAC content in the blood of volunteers at T_{max} was roughly calculated to be about 450 μg (content on T_{max}, 23 ng/ mL plasma; body weight, 65 kg; volume of total blood, 1/13 of body weight), and this calculation suggests high bioavailability of SAC in humans. Also, it can be speculated that SAC may be reabsorbed by the kidney as Nagae et al. mentioned (*22*). Allylmercaptan was identified as a transformation product generated from some sulfur-containing compounds in garlic when the garlic was mixed with blood (*31*). In this experiment, di- or trisulfide

Table 11. Results of Hematological Examination on Rats Treated Orally with SAC for One Month[a]

sex	dose (mg/kg)	no. of rats	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Ht (%)	Hb (g/dL)
male	control	10	7.67 ± 0.21	5.38 ± 1.58	45.2 ± 1.4	14.6 ± 0.4
	250	10	7.61 ± 0.36	6.44 ± 2.61	44.8 ± 2.0	14.4 ± 0.7
	500	10	7.37 ± 0.32*	6.79 ± 2.36	43.3 ± 1.3**	13.9 ± 0.4**
	1000	10	7.50 ± 0.41	4.96 ± 1.60	42.5 ± 1.5**	13.5 ± 0.5**
	2000	10	7.39 ± 0.60	4.30 ± 1.69	41.0 ± 2.7**	12.9 ± 0.9**
female	control	10	7.47 ± 0.43	4.04 ± 0.76	43.5 ± 1.9	14.3 ± 0.6
	250	10	7.58 ± 0.45	4.98 ± 1.85	43.3 ± 2.6	14.2 ± 0.6
	500	10	7.67 ± 0.39	4.33 ± 1.31	43.0 ± 2.6	13.9 ± 0.7
	1000	9	7.72 ± 0.43	4.12 ± 1.59	41.5 ± 1.7*	13.3 ± 0.7**
	2000	10	7.42 ± 0.38	3.85 ± 1.19	39.3 ± 2.1**	12.3 ± 0.7**

sex	dose (mg/kg)	MCV (μ^3)	MCH (pg)	MCHC (%)	PLT (10^4/mm^3)	retic (0/00)
male	control	59.2 ± 1.4	19.1 ± 0.4	32.3 ± 0.7	100.0 ± 18.5	21.2 ± 9.6
	250	58.9 ± 1.7	18.9 ± 0.7	32.1 ± 0.8	98.6 ± 18.4	29.2 ± 12.0
	500	58.8 ± 2.1	18.9 ± 0.6	32.1 ± 0.7	107.4 ± 14.9	41.7 ± 21.9*
	1000	56.7 ± 1.7**	18.0 ± 0.8**	31.8 ± 0.7	92.9 ± 14.5	50.3 ± 16.6**
	2000	55.5 ± 1.7**	17.5 ± 0.6**	31.5 ± 0.4**	95.3 ± 15.3	93.4 ± 40.3**
female	control	58.2 ± 1.7	19.1 ± 0.7	32.8 ± 0.7	95.4 ± 21.8	27.6 ± 16.2
	250	57.2 ± 1.7	18.7 ± 0.7	32.7 ± 0.9	98.9 ± 20.3	26.7 ± 6.7
	500	56.0 ± 1.4**	18.1 ± 0.6**	32.2 ± 0.6	101.8 ± 14.6	34.8 ± 14.4
	1000	53.7 ± 1.3**	17.2 ± 0.5**	32.0 ± 0.6*	98.5 ± 12.4	41.6 ± 17.0
	2000	53.0 ± 1.4**	16.6 ± 0.4**	31.4 ± 0.8**	106.0 ± 14.9	78.3 ± 37.0**

[a] Data presents the means ± SD, and asterisks indicate significant difference (*, $p < 0.05$; *, $p < 0.01$) from the control by Student's t-test or Aspin–Welch method after the F-test.

Table 12. Results of Biological Examination on Rats Treated Orally with SAC for One Month

sex	dose (mg/kg)	no. of rats	GOT (IU)	GPT (IU)	ALP (IU)	LHD (IU)	CPK (IU)	T. Chol. (mg/dL)
M	control	10	64.7 ± 8.5	27.6 ± 5.0	315 ± 84	260 ± 57	206 ± 65	68 ± 17
	250	10	64.6 ± 11.5	28.7 ± 5.4	292 ± 55	218 ± 50	182 ± 52	67 ± 11
	500	10	60.7 ± 5.0	32.0 ± 5.6	280 ± 51	227 ± 73	194 ± 39	73 ± 9
	1000	10	87.1 ± 73.4	55.1 ± 59.6	294 ± 61	206 ± 70	212 ± 67	67 ± 21
	2000	10	91.8 ± 96.4	71.0 ± 107.7	318 ± 75	247 ± 105	198 ± 35	75 ± 15
F	control	10	58.5 ± 4.4	27.5 ± 3.8	181 ± 29	197 ± 45	128 ± 27	84 ± 16
	250	10	56.6 ± 5.9	37.2 ± 7.6**	231 ± 46**	261 ± 151	155 ± 41	94 ± 18
	500	10	60.3 ± 8.4	40.2 ± 8.5**	220 ± 35*	252 ± 83	164 ± 30**	103 ± 15*
	1000	9	57.9 ± 8.2	39.7 ± 11.0*	221 ± 65	263 ± 75	145 ± 24	102 ± 18*
	2000	10	77.6 ± 54.8	60.1 ± 51.1	240 ± 64*	204 ± 87	136 ± 26	89 ± 11

sex	dose (mg/kg)	T. Lip. (mg/dL)	BUN (mg/dL)	creatinine (mg/dL)	glucose (mg/dL)	T. P. (g/dL)	albumin (g/dL)
M	control	249 ± 34	19.9 ± 1.6	0.42 ± 0.06	136 ± 10	5.99 ± 0.22	3.33 ± 0.12
	250	250 ± 30	17.7 ± 3.2	0.40 ± 0.05	133 ± 26	5.84 ± 0.20	3.38 ± 0.12
	500	258 ± 31	15.7 ± 2.2	0.40 ± 0.07	129 ± 23	5.79 ± 0.21	3.48 ± 0.08**
	1000	246 ± 49	16.1 ± 1.8	0.35 ± 0.05*	128 ± 25	5.80 ± 0.15*	3.57 ± 0.11**
	2000	282 ± 45	16.0 ± 3.9	0.30 ± 0.00**	140 ± 24	5.72 ± 0.18**	3.45 ± 0.17
F	control	287 ± 45	21.7 ± 3.2	0.50 ± 0.05	118 ± 10	6.45 ± 0.22	3.69 ± 0.15
	250	319 ± 49	17.7 ± 3.5*	0.41 ± 0.06*	120 ± 13	6.18 ± 0.24*	3.58 ± 0.14
	500	344 ± 32**	18.0 ± 3.2*	0.39 ± 0.07**	121 ± 10	6.20 ± 0.14**	3.60 ± 0.12
	1000	365 ± 52**	15.1 ± 2.8**	0.38 ± 0.07**	143 ± 13**	6.10 ± 0.32*	3.61 ± 0.18
	2000	312 ± 26	15.7 ± 2.4**	0.33 ± 0.05**	130 ± 9*	6.03 ± 0.23**	3.81 ± 0.26

sex	dose (mg/kg)	A/G ratio	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{2+} (mg/dL)	IP (mg/dL)
M	control	1.26 ± 0.13	140.6 ± 1.6	4.32 ± 0.46	9.47 ± 0.37	7.73 ± 0.58
	250	1.38 ± 0.12*	141.0 ± 2.3	4.73 ± 0.75	9.35 ± 0.36	7.84 ± 0.69
	500	1.52 ± 0.06**	140.7 ± 1.9	4.82 ± 1.11	9.40 ± 0.35	7.70 ± 0.52
	1000	1.61 ± 0.09**	140.7 ± 1.6	4.98 ± 0.87*	9.27 ± 0.42	7.96 ± 0.51
	2000	1.53 ± 0.15**	141.0 ± 1.9	5.25 ± 1.11*	9.21 ± 0.21	8.55 ± 1.05*
F	control	1.33 ± 0.09	141.8 ± 1.3	3.44 ± 0.42	9.85 ± 0.33	6.91 ± 0.53
	250	1.39 ± 0.10	140.7 ± 1.6	4.22 ± 0.95*	9.82 ± 0.45	7.64 ± 0.96
	500	1.38 ± 0.08	141.5 ± 1.6	3.66 ± 0.39	9.87 ± 0.24	7.37 ± 0.61
	1000	1.47 ± 0.15*	141.7 ± 1.8	3.89 ± 0.70	9.69 ± 0.32	7.81 ± 0.97*
	2000	1.75 ± 0.18**	140.6 ± 1.5	3.65 ± 0.26	9.69 ± 0.17	7.57 ± 0.43**

Data present the means ± SD, and asterisks indicate significant difference (*, $p < 0.05$; **, $p < 0.01$) from the control by Student's *t*-test or Aspin–Welch method after the F-test.

reacted with blood rapidly but monosulfide did not, and allylmercaptan was identified as the transformation product. These results indicate that SAC, the monosulfide compound, might be stable in the blood. Indeed, recovery of SAC in plasma and red cell fraction was high. Considering SAC shows such high bioavailability, reabsorption, and stability in blood, it may

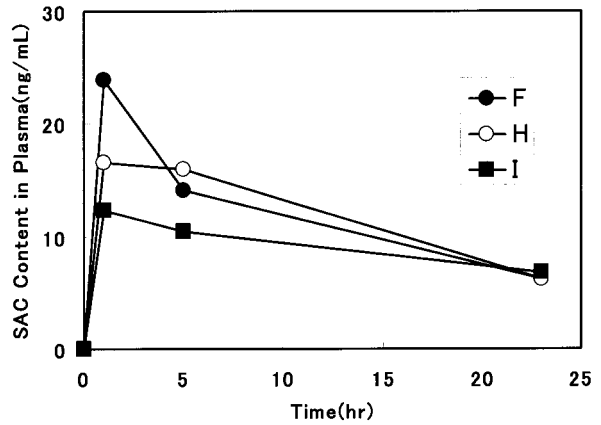

Figure 7. SAC content in human volunteers orally consuming garlic supplement containing SAC. Volunteer F: age, 46; sex, male; body weight, 64 kg; SAC consumed, 0.82 mg). Volunteer H: age, 38; sex, male; body weight, 63 kg; SAC consumed, 0.67 mg). Volunteer I: age, 45; sex, male; body weight, 65 kg; SAC consumed, 0.67 mg.

be efficiently carried to target sites, and high dosages may not be required for humans to obtain health benefits.

The peak corresponding to alliin was not clearly identified in the HPLC analysis of the blood samples after oral administration of alliin (35.5 mg derived from enteric-coated garlic, data not shown). Alliin in urine was not tested. There is a possibility that alliin was not detected in the blood samples because it was transformed to allicin in the intestinal tract, or it may have rapidly reacted with trace materials in the intestine, or it may have been rapidly absorbed or metabolized.

Hatono (*17*) and Moriguchi (*20*) investigated the relationships between the structure and biological activities of SAC and its analogues. The S-allyl group had the highest potency for colon cancer prevention and was safer than the other groups, such as S-methyl, S-propyl, S-propenyl, and these disulfide derivatives (*17*). The importance of the S-allyl group on the survival of neurons was investigated by using analogues or derivatives of SAC and γ-glutamyl-S-alk(en)yl cysteine, then only compounds containing the S-allyl group were effective. Additionally, this investigation revealed the necessity of the allyl group being attached to the sulfur atom for manifestation of neurotrophic activity (*20*). The results of their investigation indicated that the S-allyl group plays important roles in disease prevention, neurotrophic activity, and other biological activities. SAC is one of the characteristic compounds derived from garlic, and this compound is generated in the processing of garlic. It is stable in the blood and also has several unique biological activities as reported. Also, this compound shows low toxicity. Therefore, SAC could be used as a compliance marker for pharmacological investigations of garlic preparations containing SAC that are used for medical or neutraceutical applications for disease prevention.

ABBREVIATIONS USED

SAC, S-allylcysteine; LD_{50} value, median lethal dose; po, per os; ip, intraperitoneal; HPLC, high-performance liquid chromatography; UV, ultra violet; aufs, absorbance units full scale; NMR, nuclear magnetic resolution; IR, infrared; MS, mass spectrometry; (R)S2HPC, (R)-S-(2-hydroxypropyl)-cysteine; (S)S2HPC, (S)-S-(2-hydroxypropyl)-cysteine; MeOH, methanol; DMSO, dimethyl sulfoxide; NaH, sodium hydride; $CHCl_3$, chloroform; G, gravity; OPA, *o*-phthalaldehyde; CMC, carboxymethyl cellulose; h, hour; TG-DTA, thermogravimetry−differential thermal analysis; MCV, mean corpuscular volume; Ht, hematocrit; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BUN, blood urea nitrogen; ALP, alkaline phosphatase; RH, relative humidity; T_{max}, time to the reach the maximum plasma concentration; $T_{1/2}$, biological half-life

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