DOI:10.1021/jf9007554

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Antityrosinase and Antimicrobial Activities of *trans*-Cinnamaldehyde Thiosemicarbazone

YU-JING ZHU,[†,‡,∥] KANG-KANG SONG,[†,∥] ZHI-CONG LI,[†,§] ZHI-ZHEN PAN,[†] YUN-JI GUO,[†,§] JING-JING ZHOU,[†] QIN WANG,[†] BO LIU,*[,‡] AND QING-XI CHEN*[,†,§]

[†]Key Laboratory of Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Science, Xiamen University, Xiamen 361005, China, [‡]Agricultural Bioresource Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350003, China, and [§]Key Laboratory for Chemical Biology of Fujian Province, Xiamen University, Xiamen 361005, China. [∥] These authors contributed equally to this work.

Tyrosinase (EC 1.14.18.1) is a key enzyme in pigment biosynthesis of organisms. *trans*-Cinnamaldehyde thiosemicarbazone, a derivative of benzaldehyde thiosemicarbazone, was synthesized as an inhibitor of tyrosinase. The inhibitory effects of this compound on the activity of mushroom tyrosinase were investigated. The results showed that *trans*-cinnamaldehyde thiosemicarbazone could potently inhibit both monophenolase activity and diphenolase activity of tyrosinase. For monophenolase activity, *trans*-cinnamaldehyde thiosemicarbazone could not only lengthen the lag time but also decrease the steady-state rate. For diphenolase activity, the IC_{50} value was determined to be 5.72 μM. Kinetic analyses showed that *trans*-cinnamaldehyde thiosemicarbazone was a reversible and mixed type inhibitor on this enzyme. The inhibition constants (K_I and K_{IS}) were determined to be 4.45 and 8.85 μM, respectively. Furthermore, the antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphyloccocus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Agrobacterium tumefaciens* was investigated. The results showed that *trans*-cinnamaldehyde thiosemicarbazone was more effective against *B. subtilis* and *S. aureus* with the same minimum inhibitory concentration (MIC) of 50 μg/mL and with the same minimum bactericidal concentration (MBC) of 50 μg/mL.

KEYWORDS: Tyrosinase; *trans*-cinnamaldehyde thiosemicarbazone; antityrosinase activity; inhibition kinetics; antimicrobial activity

INTRODUCTION

Tyrosinase (EC 1.14.18.1), a copper-containing multifunctional oxidase, is widely distributed in microorganisms, animals, and plants (*1*). Certain fruits and vegetables usually incur browning during handling, processing, and storage after harvest. Enzymatic browning is a major factor because browning contributes to quality loss in foods and beverages (*2*). Browning usually impairs the sensory properties of products because of the associated changes in color, flavor, and softening, which results in shorter shelf life and decreased market value (*3*). Therefore, tyrosinase inhibitors have been established as important constituents of depigmentation agents (*4*) and have potential uses as food preservatives (*5*).

So far, some aromatic aldehydes have been put to use as food additives in food processing (*6*, *7*). They can be used as spices and food preservatives. In our previous papers, cuminaldehyde (*6*) and salinaldehyde (*7*) were found to have inhibitory effects on mushroom tyrosinase. It was reported that phenyl thioureas and alkyl thioureas could exhibit moderate to weakly depigmenting activity. Recently, benzaldehyde thiosemicarbazone derivatives have been discoverd as the inhibitors of PO from insects (*8*).

Cinnamaldehyde, a low molecular weight cinnamic acid analogue with relatively broad distribution in plants, has been shown various activities such as antitumor, antifungal, cytotoxic, and mutagenic (*9*, *10*). In addition, cinnamaldehyde had inhibitory effects on alcohol dehydrogenase and glutathione *S*-transferase activities in human melanoma cells, and had antityrosinase and anti-ATPase activities (*11*). The aim of this present work is, therefore, to carry out kinetic studies of the inhibition on the monophenolase and diphenolase activities of tyrosinase by *trans*-cinnamaldehyde thiosemicarbazone, a new derivative of benzaldehyde thiosemicarbazone, to evaluate the kinetic parameters and inhibition constants characterizing the system and investigate the inhibition mechanism. Furthermore, we found that *trans*-cinnamaldehyde thiosemicarbazone had bacteriostatic activity. All of these data may provide the basis for developing novel tyrosinase inhibitors and searching for new potent food preservatives or insecticides.

MATERIALS AND METHODS

Reagents. Tyrosinase (EC 1.14.18.1) from mushroom was the product of Sigma Chemical Co. (St. Louis, MO, USA). The specific activity of the

*To whom correspondence should be addressed. Tel/Fax: +86 592 2185487. E-mail: chenqx@xmu.edu.cn (Q.-X.C.); laeptb@163.com (B.L.).

© 2009 American Chemical Society

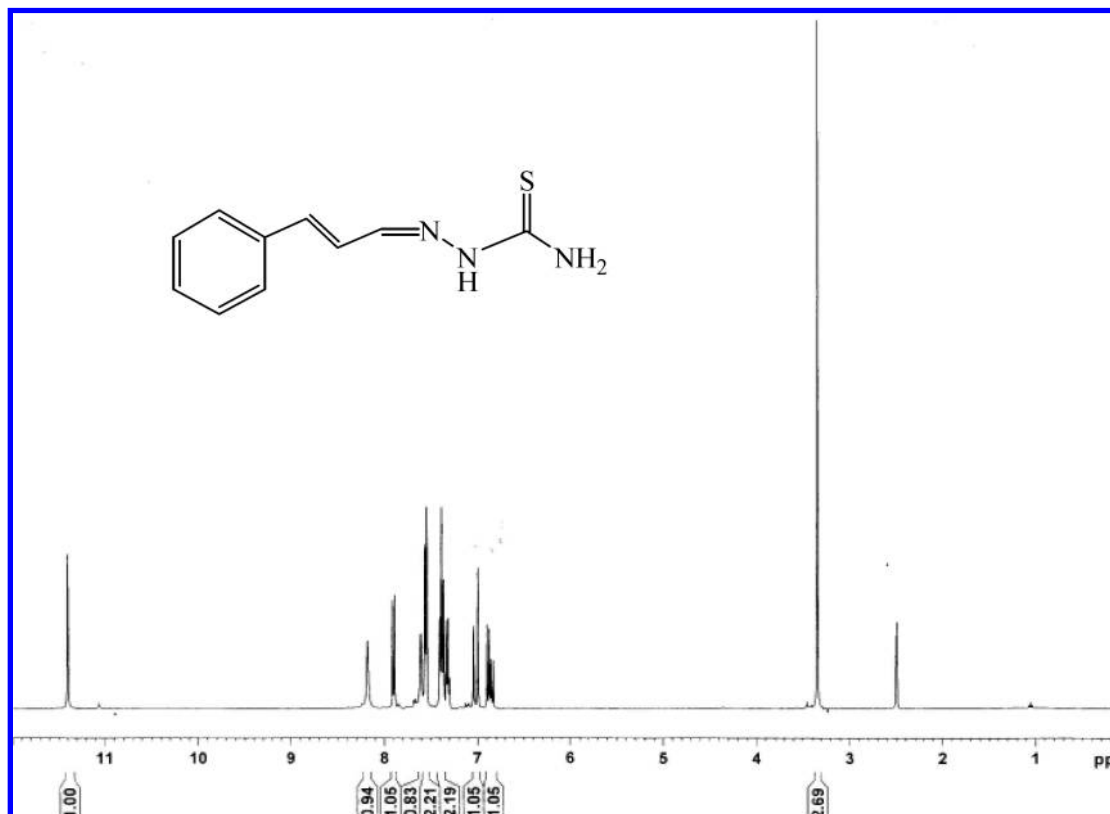

Figure 1. Chemical structure and the ^{1}H NMR spectra of *trans*-cinnamaldehyde thiosemicarbazone.

enzyme is 6680 U/mg. Dimethylsulfoxide (DMSO), L-3,4-dihydroxyphenylalanine (L-DOPA), and L-tyrosine (L-Tyr) were obtained from Aldrich (St. Louis, MO). *Bacillus subtilis*, *Escherichia coli*, *Staphyloccocus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Agrobacterium tumefaciens* were collected from a colony preserved at −80 °C in Fujian Academy of Agricultural Sciences. All other reagents were local and of analytical grade. The water used was redistilled and ion-free.

Synthesis of *trans*-Cinnamaldehyde Thiosemicarbazone. This compound was prepared by a simple one-step reaction of cinnamaldehyde with thiosemibazide in an acidic solution of ethanol and water. A mixture of cinnamaldehyde (20 mM) with thiosemibazide (20 mM) in 60 mL of ethanol with 4 mL of acetic acid solution was refluxed for 2−5 h and then cooled to room temperature. The yellow precipitates were collected and washed with cold ethanol. The products were purified by recrystallization from ethanol and were identified by ESI−MS and ^{1}H NMR data. ESI−MS data were obtained on a Bruker ESQUIRE-LC. ^{1}H NMR data were acquired on a 400 MHz NMR spectrometer (AV400) from Bruker.

Antityrosinase Assay. The antityrosinase activity assay was performed as reported by Chen et al. (*12*). In this investigation, L-Tyr was used as the substrate for the monophenolase activity assay, and L-DOPA was used as the substrate for the diphenolase activity assay. The reaction media (3 mL) for the activity assay contained 2.0 mM L-Tyr or 0.5 mM L-DOPA in 50 mM Na_2HPO_4−NaH_2PO_4 buffer (pH 6.8). The final concentration of tyrosinase was 33.33 μg/mL for monophenolase activity and 6.67 μg/mL for *o*-diphenolase activity. The reaction was carried out at a constant temperature of 30 °C. *trans*-Cinnamaldehyde thiosemicarbazone was first dissolved in DMSO. The final concentration of DMSO in the test solution was 3.3%. Controls, without inhibitor but containing 3.3% DMSO, were routinely carried out (*13*). The extent of inhibition by the addition of the sample was expressed as the percentage necessary for 50% inhibition (IC_{50}). The inhibition type was assayed by a Lineweaver−Burk plot, and the inhibition constant was determined by the second plots of the apparent K_m/V_m or $1/V_m$ versus the concentration of the inhibitor.

Antimicrobial Assay. The antimicrobial assay was carried out in tryptone beef extract agar, at pH 7.2, with an inoculum of 1−2 × 10^5 cells/mL. The antimicrobial activity of *trans*-cinnamaldehyde thiosemicarbazone was determined using the agar well diffusion method following a published procedure with slight modifications (*14*, *15*). Briefly, culture medium was inoculated with the given microorganism by spreading the bacterial inoculum in the media. Wells (7 mm diameter) were punched in the agar and filled with *trans*-cinnamaldehyde thiosemicarbazone with different concentrations. Control wells, containing neat DMSO (negative control) and standard antibiotic streptomycin sulfate (1000 U/mL) for the tested bacteria, were also run parallel in the same plate. Bacteria were incubated at 37 °C for 24 h. Antimicrobial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were tested by broth macrodilution methods (*16*). Briefly, serial 2-fold dilutions of the test compounds were prepared in DMSO, and 30 μL of each dilution was added to 3 mL of the above medium with the same inoculum of 1−2 × 10^5 cells/mL and under the same culture conditions. After the cultures were incubated at 37 °C for 24 h, MIC was determined as the lowest concentration of the test compound that demonstrated no visible growth. After the determination of the MIC, 100-fold dilutions with drug-free medium from each tube showing no turbidity were incubated at 37 °C for 48 h. The MBC was the lowest concentration of the test compound that showed no visible growth in the drug-free cultivation.

RESULTS

Synthesis of *trans*-Cinnamaldehyde Thiosemicarbazone. The yellow precipitates were collected and washed with cold ethanol. Yield 95.6%. ^{1}H NMR (DMSO-d_6, TMS, 400 MHz): δ (ppm) 11.40 (HN, s), 7.91 (CH=N, d), 8.18, 7.61 (NH_2, d), 7.9−7.38 (C_6H_5, 5H, m), 7.02 (CH=C, d), 6.87 (CH-C, q). ESI−MS: m/z (100%) = 228 (M + Na^+, DMSO) (see **Figure 1** for the structure).

Effect of *trans*-Cinnamaldehyde Thiosemicarbazone on the Monophenolase Activity of Mushroom Tyrosinase. When the enzymatic oxidation reaction used L-Tyr as the substrate, the time course is shown as curve 1 in **Figure 2a**. For the first minute, absorptions increased slowly. After some time, the curve rose mostly linearly and obtained an invariable slope. There was a

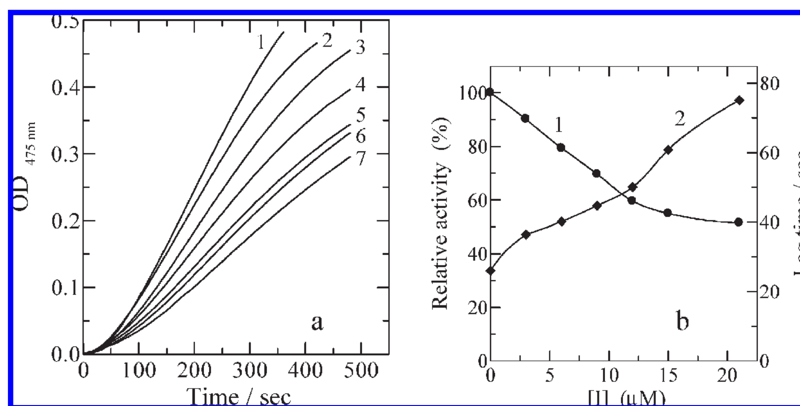

Figure 2. Inhibition effects of *trans*-cinnamaldehyde thiosemicarbazone on the monophenolase activity of mushroom tyrosinase. (**a**) Progress curves for the oxidation of L-Tyr by the enzyme. The concentrations of *trans*-cinnamaldehyde thiosemicarbazone for curves 1−7 were 0, 3, 6, 9, 12, 15, and 21 μM, respectively. (**b**) Effects of *trans*-cinnamaldehyde thiosemicarbazone on the steady-state rate of monophenolase activity (curve 1) and the lag period of mushroom tyrosinase (curve 2) for the oxidation of tyrosine. The concentrations of the enzyme and the substrate (L-Tyr) were 6.67 μg/mL and 0.5 mM, respectively.

marked lag period, characteristic of monophenolase activity, which was estimated by extrapolation of the curve to the abscissa. The system reached a constant rate (the steady-state rate) after the lag period. The effects of *trans*-cinnamaldehyde thiosemicarbazone on the oxidation of tyrosine by tyrosinase were studied. The kinetic courses of the oxidation of the substrate in the presence of different concentrations of the compound are shown as curves 2−7 in **Figure 2a**. The results showed that *trans*-cinnamaldehyde thiosemicarbazone could slow down the reaction velocity of the monophenolase of mushroom tyrosinase. The lag time and the steady-state activity were determined, and the results are shown in **Figure 2b**. The steady-state rate (v_{ss}) decreased quickly, and the lag time extended with increasing concentrations of *trans*-cinnamaldehyde thiosemicarbazone. When the concentration of the compound reached 21 μM, the lag time extended from 25 to 76 s, about 3 times, and the steady-state rate decreased by 40%. The results indicated that *trans*-cinnamaldehyde thiosemicarbazone could inhibit the monophenolase activity of tyrosine not only by decreasing the steady rates of the enzyme but also by prolonging the lag period.

Effect of *trans*-Cinnamaldehyde Thiosemicarbazone on the Diphenolase Activity of Mushroom Tyrosinase. When using L-DOPA as the assay substrate of diphenolase activity, the progress curve of enzyme reaction was a family of lines passing through the origin with different slopes that indicated diphenolase activity. The results showed that the lag period did not exist in the progress of the enzyme catalyzing the oxidation of L-DOPA. In our investigation, *trans*-cinnamaldehyde thiosemicarbazone was used as an inhibitor on the activity of mushroom tyrosinase for the oxidation of L-DOPA. The inhibitory concentration effect on the diphenolase activity of mushroom tyrosinase was assayed. Diphenolase activity decreased with increasing inhibitor concentrations. When the concentration of the inhibitor reached 9.0 μM, enzyme activity was inhibited by 60% (**Figure 3**), indicating that *trans*-cinnamaldehyde thiosemicarbazone exhibited a potent inhibitory effect on diphenolase activity with dose-dependence. The IC_{50} value of *trans*-cinnamaldehyde thiosemicarbazone on the diphenolase activity of the enzyme obtained was 5.72 μM.

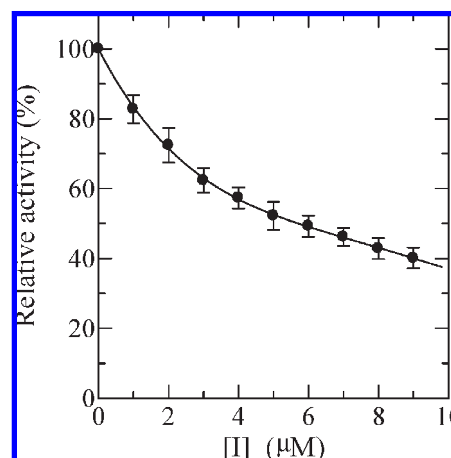

Figure 3. Effect of *trans*-cinnamaldehyde thiosemicarbazone on the diphenolase activity of mushroom tyrosinase.

Inhibitory Mechanism of *trans*-Cinnamaldehyde Thiosemicarbazone on the Diphenolase Activity of Mushroom Tyrosinase. The inhibition mechanism of *trans*-cinnamaldehyde thiosemicarbazone on mushroom tyrosinase for the oxidation of L-DOPA was first studied. The plots of the remaining enzyme activity versus the concentrations of enzyme in the presence of different concentrations of *trans*-cinnamaldehyde thiosemicarbazone gave a family of straight lines, which all passed through the origin (**Figure 4**). Increasing the inhibitor concentration resulted in the descending of the slope of the line, indicating that the inhibition by *trans*-cinnamaldehyde thiosemicarbazone on diphenolase was reversible. The presence of *trans*-cinnamaldehyde thiosemicarbazone

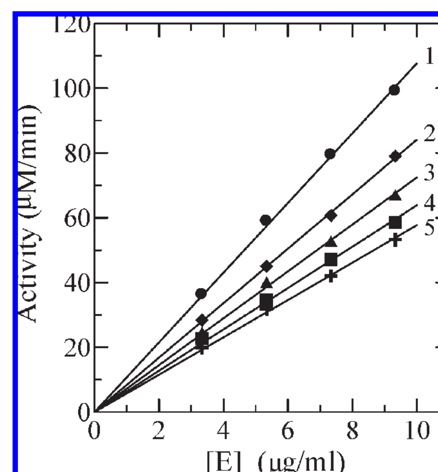

Figure 4. Effects of tyrosinase concentration on its activity for the oxidation of L-DOPA at different concentrations of *trans*-cinnamaldehyde thiosemicarbazone. The concentrations of *trans*-cinnamaldehyde thiosemicarbazone for curves 1−5 were 0, 1, 2, 3, and 4 μM, respectively.

did not bring down the amount of the efficient enzyme but just resulted in the inhibition of enzyme activity.

Determination of the Inhibition type of *trans*-Cinnamaldehyde Thiosemicarbazone on the Enzyme Activity of Mushroom Tyrosinase. Inhibition type of *trans*-cinnamaldehyde thiosemicarbazone on the diphenolase activity of the enzyme has been investigated, and the results are illustrated in **Figure 5**. With increasing concentration, of *trans*-cinnamaldehyde thiosemicarbazone, K_m increased, and V_{max} decreased. Double-reciprocal plots yielded a family of straight lines intersecting at the second quadrant. Therefore, the inhibition belonged to mixed-I type, namely, the competitive effect being stronger than the uncompetitive effect, which indicated that this compound inhibited the enzyme–substrate complex more weakly than did the free enzyme.

According to mixed type inhibition kinetics, the kinetic parameters for inhibitor can be analyzed by using the following equations:

In the absence of inhibitors, the kinetic function is

$$v_0 = \frac{V_m[S]}{K_m + [S]} \quad (1)$$

In the presence of mixed type inhibitor, the kinetic function is

$$v_i = \frac{V_m[S]}{K_m\left(1 + \frac{[I]}{K_I}\right) + [S]\left(1 + \frac{[I]}{K_{IS}}\right)} \quad (2)$$

The inhibition rate:

$$i\,\% = \left(1 - \frac{v_i}{v_0}\right) \times 100\% = \frac{K_m \frac{[I]}{K_I} + [S]\frac{[I]}{K_{IS}}}{K_m\left(1 + \frac{[I]}{K_I}\right) + [S]\left(1 + \frac{[I]}{K_{IS}}\right)} \times 100\% \quad (3)$$

When i % reaches to 50%, [I] = IC_{50}. Therefore,

$$\frac{1}{2} = \frac{K_m \frac{IC_{50}}{K_I} + [S]\frac{IC_{50}}{K_{IS}}}{K_m\left(1 + \frac{IC_{50}}{K_I}\right) + [S]\left(1 + \frac{IC_{50}}{K_{IS}}\right)} \quad (4)$$

Equation 4 is converted to

$$IC_{50} = \frac{K_m + [S]}{\frac{K_m}{K_I} + \frac{[S]}{K_{IS}}} \quad (5)$$

The values of K_I and K_{IS} were determined to be 4.45 μM and 8.85 μM, respectively. According to the experiment conditions and eq 5, we can obtain the theoretical value of IC_{50} as follows: IC_{50} = (614 + 500)/(614/4.45) + (500/8.85) = 5.728 μM, which is very close to the test value (5.72 μM).

Antimicrobial Activity of *trans*-Cinnamaldehyde Thiosemicarbazone. The antimicrobial activities of *trans*-cinnamaldehyde thiosemicarbazone on *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumonia*, and *A. tumefaciens* were first studied. The results are showed in **Figure 6** and **Table 1**. In the test, the bacteriostatic activities were assayed by taking 1000 U/mL streptomycin sulfate as the control. It was found that *trans*-cinnamaldehyde thiosemicarbazone had significant inhibition against *S. aureus* and *A. tumefaciens*. Meanwhile, the compound could inhibit the proliferation of *B. subtilis* and *E. coli* in different extents, but less than the corresponding control. No effect was found in the tests by using *trans*-cinnamaldehyde thiosemicarbazone against *P. aeruginosa* and *K. pneumonia*. DMSO had no obvious inhibition on the proliferation of these six different kinds of bacteria.

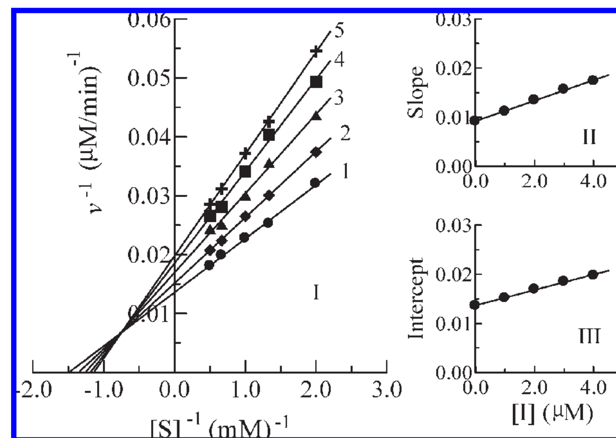

Figure 5. Lineweaver–Burk plot for the determination of the inhibitory mechanism of *trans*-cinnamaldehyde thiosemicarbazone on mushroom tyrosinase (I). The concentrations of *trans*-cinnamaldehyde thiosemicarbazone for curves 1–5 were 0, 1, 2, 3, and 4 μM, respectively. (II) and (III) represent the plot of slope and intercept versus the concentration of *trans*-cinnamaldehyde thiosemicarbazone for determining the inhibition constants K_I and K_{IS}, respectively.

We also used a broth dilution method to test the antimicrobial activities of *trans*-cinnamaldehyde thiosemicarbazone against the six bacteria mentioned above. The results obtained are listed in **Table 2**. *trans*-Cinnamaldehyde thiosemicarbazone was effective against the *B. subtilis*, *E. coli*, *S. aureus*, and *A. tumefaciens*, among which the antimicrobial activity against *B. subtilis* and *S. aureus* was more effective with the same MIC of 50 μg/mL and with the same MBC of 50 μg/mL, while the MIC and the MBC against *E. coli* were 50 μg/mL and 100 μg/mL, respectively.

DISCUSSION

Tyrosinase shows two enzymatic activities, *ortho*-monophenoloxidase and polyphenoloxidase activity and accepts many phenols and catechols as substrates (*17*). Up to now, although a large number of tyrosinase inhibitors have been reported, most of them could not be used because of their lower individual activities or side effects. For example, 2-(4-hydroxyphenoxy)-tetrahydro-6-(hydroxylmethyl)- 2*H*-pyran-3,4,5-triol (arbutin or arbutoside) (IC_{50} = 30 mM) and kojic acid (IC_{50} = 23 μM) were not demonstrated as they are clinically efficient. So far, tropolone is one of the most strong tyrosinase inhibitors reported (IC_{50} = 0.4 μM), but the serious side effect limits its use in medicine (*18*). Cinnamaldehyde is present in various human foods, including beverages, ice cream, sweets, and chewing gum and is reported with an IC_{50} of 1.05 mM against tyrosinase (*19*), which means that it has broad applications in medicinal and cosmetic whitening agents. In this work, *trans*-cinnamaldehyde thiosemicarbazone could inhibit both the diphenolase activity and the monophenolase activity of mushroom tyrosinase. The inhibitory potency of *trans*-cinnamaldehyde thiosemicarbazone (IC_{50} = 5.72 μM) on diphenolase activity was more potent than that of cinnamaldehyde. Similarly, Xie et al. (*7*) found the substitute benzaldehyde thiosemicarbazones had about 1000 times higher activities than benaldehyde and its analogues for inhibition on phenoloxidase of *Plutella xylostella* L.

There are two copper ions in the active center of tyrosinase, and a lipophilic long-narrow gorge exists close to the active center (*16*). Liu et al. (*20*) found that arylethylidene thiosemicarbazide and its analogue compounds showed high tyrosinase inhibition, which suggested that the sulfur atom and nitrogen atom in the molecule exhibited strong affinity for copper ion.

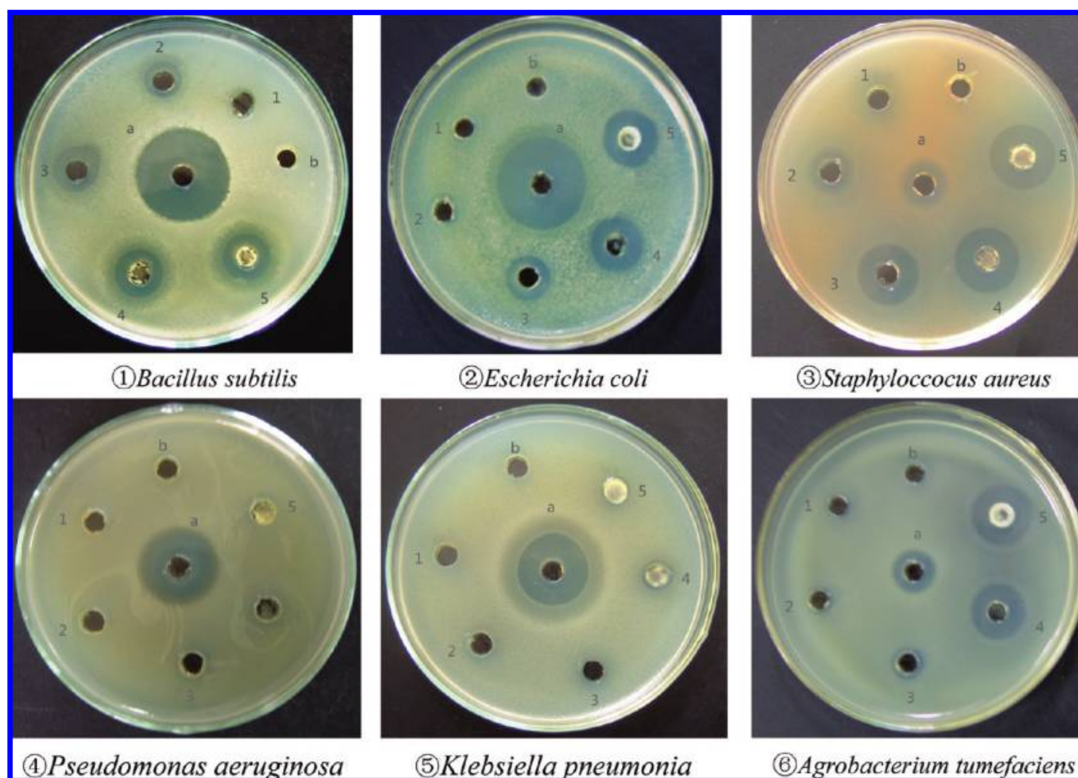

Figure 6. Antimicrobial activity of *trans*-cinnamaldehyde thiosemicarbazone at different concentrations. The concentrations of *trans*-cinnamaldehyde thiosemicarbazone for dishes 1−5 were 0.625, 1.25, 2.5, 5, and 10 mg/mL, respectively. a, positive control with 1000 U/mL of streptomycin sulfate for bacterium; b, negative control with DMSO.

Table 1. Antimicrobial Activity of *trans*-Cinnamaldehyde Thiosemicarbazone[a]

concentration (mg/mL)	*Bacillus subtilis*	*Escherichia coli*	*Staphyloccocus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Agrobacterium tumefaciens*
a	+++	+++	+	+++	+++	+
b	−	−	−	−	−	−
10	++	++	+++	−	±	+++
5	+	+	+++	−	±	++
2.5	+	+	++	−	−	+
1.25	+	+	+	−	−	+
0.625	±	+	±	−	−	±

[a] a, positive control with 1000 U/mL of streptomycin sulfate for bacterium; b, negative control with DMSO; +++, antimicrobial zone is above 12 mm in diameter; ++, antimicrobial zone is between 10 mm and 12 mm; +, antimicrobial zone is less than 10 mm; ±, antimicrobial zone is faint; −, no inhibition.

Table 2. MIC and MBC (μg/mL) of *trans*-Cinnamaldehyde Thiosemicarbazone

bacteria	MIC (μg/mL)	MBC (μg/mL)
Bacillus subtilis	50	50
Escherichia coli	50	100
Staphyloccocus aureus	50	50
Pseudomonas aeruginosa	400	500
Klebsiella pneumonia	>500	not tested
Agrobacterium tumefaciens	100	100

Therefore, it could be supposed that complexes would be formed between arylethylidene thiosemicarbazide compounds and tyrosinase when both substrates were mixed together in solution. In addition, the sulfur atom and nitrogen atom, especially the sulfur atom, of arylethylidene thiosemicarbazide compounds and the copper ion of tyrosinase could be the center of complexation, on the basis of the active center structure of tyrosinase. In the complex, the active center of tyrosinase could coordinate with two arylethylidene thiosemicarbazide molecules at the same time in two opposite directions. It was suggested that the intramolecule hydrogen bond was formed between the hydrogen atom on 3-N and nitrogen atom (2-N) in the arylethylidene thiosemicarbazide molecule, which was beneficial to decrease the molecular energy state.

As a derivative of benzaldehyde thiosemicarbazone, *trans*-cinnamaldehyde thiosemicarbazone might have the same inhibitory behavior on diphenolase activity of tyrosinase as arylethylidene thiosemicarbazide. It was investigated as a reversible competitive−uncompetitive mixed-I type inhibitor. The value of K_{IS} is about two times as great as K_I, indicating that the affinity of the inhibitor for the free enzyme is stronger than that of the inhibitor for the enzyme−substrate complex.

trans-Cinnamaldehyde thiosemicarbazone has antimicrobial activity against *B. subtilis*, *E. coli*, *S. aureus*, and *A. tumefaciens*. It was found that *trans*-cinnamaldehyde thiosemicarbazone could inhibit the proliferation of these four different kinds of microbes to different extents. The results showed that the antimicrobial activity of *trans*-cinnamaldehyde thiosemicarbazone was broad-spectrum. It can inhibit G^+ bacteria and G^- bacteria. Cinnamaldehyde as the raw material to synthesize *trans*-cinnamaldehyde thiosemicarbazone is an effective antibacterial agent. The reason *trans*-cinnamaldehyde thiosemicarbazone can inhibit the growth of microorganisms may be the same as that for cinnamaldehyde.

In conclusion, *trans*-cinnamaldehyde thiosemicarbazone could inhibit both the monophenolase activity and diphenolase activity of tyrosinase. Furthermore, we found that *trans*-cinnamaldehyde thiosemicarbazone had bacteriostatic activity. All of these data may provide the basis for developing novel tyrosinase inhibitors and developing new potent food preservatives or cosmetic additives.

ABBREVIATIONS USED

DMSO, dimethylsulfoxide; L-DOPA, L-3,4-dihydroxyphenylalanine; L-Tyr, L-tyrosine; IC_{50}, inhibitor concentrations leading to 50% activity lost; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; v_0, initial velocity in the absence of the inhibitor; v_i, initial velocity in the presence of the inhibitor; K_I, equilibrium constant of the inhibitor combining with the free enzyme; K_{IS}, equilibrium constant of the inhibitor combining with the enzyme−substrate complex.

LITERATURE CITED

(1) Whitaker, J. R. Polyphenol Oxidase. In *Food Enzymes, Structure and Mechanism*; Wong, D. W. S., Ed.; Chapman & Hall: New York, 1995; pp 271−307.
(2) Friedman, M. Food browning and its prevention: an overview. *J. Agric. Food Chem.* **1996**, *44*, 631–653.
(3) Martinez, M. V.; Whitaker, J. R. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* **1995**, *6*, 195–200.
(4) Likhitwitayawuid, K.; Sritularak, B. A new dimeric stilbene with tyrosinase inhibitory activity from *Artocarpus gomezianus*. *J. Nat. Prod.* **2001**, *64*, 1457–1459.
(5) Scotter, M. J.; Castle, L. Chemical interactions between additives in foodstuffs: a review. *Food Addit. Contam.* **2004**, *21*, 93–124.
(6) Chen, Q. X.; Song, K. K.; Wang, Q.; Huang, H. Inhibitory effects on mushroom tyrosinase by some alkylbenzaldehydes. *J. Enzyme Inhib. Med. Chem.* **2003**, *15*, 491–496.
(7) Huang, H.; Liu, X. D.; Chen, Q. X. Studies on mushroom tyrosinase activity inhibited by benzaldehyde family compounds. *J. Xiamen Univ. (Nature Sci.)* **2003**, *1*, 97–101.
(8) Xie, X. Y.; Jiang, L.; Xue, C. B.; Luo, W. C. Synthesis of substituted benzaldehyde and its activity for inhibition of the phenoloxidase of the diamondback moth *Plutella xylostella* (L.). *Huaxue Shiji* **2007**, *29* (1), 34–36.
(9) Kwon, B. M.; Lee, S. H.; Choi, S. U.; Park, S. H.; Lee, C. O.; Cho, Y. K. Synthesis and in vitro cytotoxicity of cinnamaldehydes to human solid tumor cells. *Arch. Pharm. Res.* **1998**, *21*, 147–152.
(10) Shaughnessy, D. T.; Setzer, R. W.; DeMarini, D. M. The antimutagenic effect of vanillin and cinnamaldehyde on spontaneous mutation at GC but not AT sites. *Mutat. Res.* **2001**, *480−481*, 55–69.
(11) Lee, H. S. Tyrosinase inhibitors of *Pulsatilla cernua* root-derived materials. *J. Agric. Food Chem.* **2002**, *50* (6), 1400–1403.
(12) Chen, Q. X.; Song, K. K.; Qiu, L.; Liu, X. D.; Huang, H.; Guo, H. Y. Inhibitory effects on mushroom tyrosinase by *p*-alkoxybenzoic acids. *Food Chem.* **2005**, *91*, 269–274.
(13) Chen, Q. X.; Liu, X. D.; Huang, H. Inactivation kinetics of mushroom tyrosinase in the dimethyl sulfoxide solution. *Biochemistry (Moscow)* **2003**, *68*, 644–649.
(14) Perez, C.; Pauli, M.; Bazerque, P. An antibiotic assay by the agar well diffusion method. *Acta Biol. Med. Exp.* **1990**, *15*, 113–115.
(15) Owais, M.; Sharad, K. S.; Shehbaz, A.; Saleemuddin, M. Antibacterial efficacy of *Withania somnifera* (ashwagandha), an indigenous medicinal plant against experimental murine salmonellosis. *Phytomedicine* **2005**, *12*, 229–235.
(16) Kubo, I.; Fujita, K. I.; Kubo, A.; Nihei, K. I.; Ogura, T. Antibacterial activity of coriander volatile compounds against *Salmonella choleraesuis*. *J. Agric. Food Chem.* **2004**, *52*, 3329–3332.
(17) Han, P.; Chen, C. Q.; Zhang, C. L.; Song, K. K.; Zhou, H. T.; Chen, Q. X. Inhibitory effects of 4-chlorosalicylic acid on mushroom tyrosinase and its antimicrobial activities. *Food Chem.* **2008**, *107*, 797–803.
(18) Ley, J. P.; Bertram, H. J. Hydroxy- or methoxy-substituted benzaldoximes and benzaldehyde-*o*-alkyloximes as tyrosinase inhibitors. *Bioorg. Med. Chem.* **2001**, *9*, 1879–1885.
(19) Gong S. Z.; Yang, Z.; Cheng, J. Calculating method and verification on IC_{50} value of tyrosinase inhibitor. *China Sufactant Deterg. Cosmet.* **2007**, *37* (3), 149−151; 155.
(20) Liu, J. B.; Yi, W.; Wan, Y. Q.; Ma, L.; Song, H. C. 1-(1-Arylethylidene) thiosemicarbazide derivatives: A new class of tyrosinase inhibitors. *Bioorg. Med. Chem.* **2008**, *16*, 1096–1102.

Received March 5, 2009. Revised manuscript received May 8, 2009. Accepted May 11, 2009. The present investigation was supported by the National Key Technology R & D Program of China (No. 2007BAD07B06), the National High Technology Research and Development Program ("863" Program) of China (2006AA10211), the Science and Technology Foundation of Fujian Province (No. 2007N0051), the Natural Science Foundation of China (No. 20832005) and the Postdoctoral Foundation of China (20080430787).