JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Inhibitory Effects of (*S*)- and (*R*)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acids on Tyrosinase Activity

LIANGLI YU*

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, Colorado 80523

The inhibition of (*R*)-, (*S*)-, and (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acids (HTCCA) on mushroom tyrosinase was evaluated. All HTCCAs inhibited the tyrosinase activity. The ID_{50} values were 1.88, 1.84, and 1.88 for the (*R*)-, (*S*)-, and (±)-HTCCAs, respectively. The inhibition kinetics analyzed by Hanes−Woolf plots indicated that both (*R*)- and (*S*)-HTCCAs are competitive inhibitors of the tyrosinase, with K_i values of 0.83 and 0.61 mM, respectively. Dimethyl sulfoxide (DMSO) was also tested for its direct inhibitory activity against the tyrosinase and its potential influence on the tyrosinase inhibitory effects of (*R*)- and (*S*)-HTCCAs. DMSO, a widely used solvent for tyrosinase inhibitors, was found to dose-dependently inhibit the tyrosinase activity. Addition of DMSO in a tyrosinase digest containing either (*R*)- or (*S*)-HTCCA further dose-dependently reduced the tyrosinase activity. These data indicated a potential to use a HTCCA as a tyrosinase inhibitor in food, cosmetic, and medicinal products and a need to improve the solvent system for the studies of tyrosinase inhibitions.

KEYWORDS: 6-Hydoxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; tyrosinase; inhibitor; Trolox; DMSO

INTRODUCTION

Tyrosinase (EC.1.14.18.1), also known as the polyphenol oxidase, is a copper-containing multifunctional oxidase that catalyzes both the hydroxylation of monophenols to diphenols and the oxidation of *o*-diphenols to *o*-quinones. Tyrosinase widely exists in plants and animals and is involved in the formation of melanin pigments (*1*−*3*). In the food industry, tyrosinase is a very important enzyme in controlling the quality and economics of fruit and vegetable storage and processing including fruit pulp manufacturing (*4*, *5*). Tyrosinase catalyzes the oxidation of phenolic compounds to the corresponding quinone and is responsible for the enzymatic browning of fruits and vegetables. In addition to the undesirable color and flavor, the quinone compounds produced in the browning reaction may irreversibly react with the amino and sulfhydryl groups of proteins. The quinone−protein reaction decreases the digestibility of the protein and the bioavailability of the essential amino acids including lysine and cysteine. Tyrosinase inhibition may be a potential approach to prevent and control the enzymatic browning reactions and improve the quality and nutritional value of food products.

Tyrosinase also plays an important role in the developmental and defensive functions of insects. Tyrosinase is involved in melanogenesis, wound healing, parasite encapsulation, and sclerotization in insects (*6*−*8*). Recently, the development of tyrosinase inhibitors has become an active alternative approach to control insect pests.

In addition, it is well-recognized that tyrosinase inhibitors are important for their potential applications in medical and cosmetic products that may be used to prevent or treat pigmentation disorders (*3*, *9*, *10*). Tyrosinase inhibitors may result in a reduction in melanin biosynthesis and are used in cosmetic products for perpigmentation-related concerns including the formation of freckles. Tyrosinase may also be a target for developing medicines to treat hypopigmentation-related problems, such as albinism and piebaldism (*3*). Other biological functions of tyrosinase include the neuromelanin formation in human brain and the melanoma specific anticarcinogenic activity (*1*). Recent research indicated that tyrosinase could play a role in dopamine neurotoxicity and contribute to the neurodegeneration associated with Parkinson's disease (*11*).

The discovery and characterization of new tyrosinase inhibitors are necessary for their potential applications in improving food quality and nutritional value, controlling insect pests, and preventing pigmentation disorders and other melanin-related health problems in human beings. The mushroom tyrosinase has been widely used as the target enzyme in screening and characterizing the potential inhibitors. In the present study, inhibitory effects of (*S*)- and (*R*)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acids (HTCCA) on mushroom tyrosinase activity were examined and compared.

* To whom correspondence should be addressed. Tel: (970)491-5676. Fax: (970)491-7252. E-mail: yu@cahs.colostate.edu.

10.1021/jf0208379 CCC: $25.00
Published on Web 03/14/2003

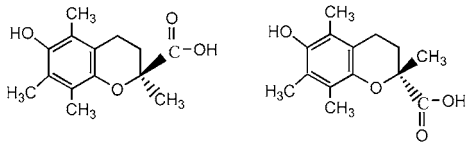

Figure 1. Stereo structures of (*R*)- and (*S*)-HTCCAs.

MATERIALS AND METHODS

Materials. (*S*)-, (*R*)-, and (±)-HTCCAs (98% pure for both), the mushroom tyrosinase (EC.1.14.18.1), and L-3,4-dihydroxyphenylalanine (L-DOPA) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Reaction Conditions for Kinetic Analysis. The tyrosinase reaction mixtures contained 0.5−2.0 mM L-DOPA, 0.1 M phosphate buffer (pH 6.8), and certain levels of (*R*)- or (*S*)-HTCCA in a total volume of 3.0 mL (*1*, *6*). A total of 125 U of tyrosinase was added to initiate the enzymatic reactions. The reactions were carried out at 25 °C. The increase in the absorbance at 475 nm, which accompanies the oxidation of the L-DOPA, was measured at 0.5, 1.0, 1.5, and 2.0 min of the enzymatic reaction. The effects of (*R*)- or (*S*)-HTCCA on the tyrosinase activity were determined at final concentrations of 0.45 and 0.90 mM, respectively. The initial velocity (v_i) of each reaction was determined by a linear least squares determination of the slope of the line resulting from the plot of the absorbance at 475 nm vs time. The kinetics analyses of the reactions were obtained by using Hanes−Woolf plots ($[S]/v_i$ vs $[S]$) (*12*), while the K_i for each HTCCA was obtained from the secondary plot of the apparent K_m against the inhibitor concentrations. One unit (U) of the tyrosinase activity was defined as the amount of the enzyme to increase 0.001 of the absorbance at 475 nm under the experimental condition (*1*).

Determination of the ID_{50}. ID_{50} is the required concentration of (*R*)- or (*S*)-HTCCA to reduce the tyrosinase activity to 50% of that in the control containing no inhibitor under the same experimental conditions. Eight levels of each HTCCA were used to obtain the ID_{50} value against the tyrosinase. The enzymatic reaction mixture contained 0.8 mM L-DOPA, 0.1 M phosphate buffer (pH 6.8), 67 U/mL of the tyrosinase, and a certain concentration of a HTCCA. The tested concentrations of each HTCCA were 0, 0.19, 0.39, 0.58, 0.88, 1.22, 1.70, and 2.19 mM in a final total volume of 1.85 mL. The absorbance at 475 nm was measured against a pure water blank at 0.5 and 1 min of the reaction and used to calculate the tyrosinase activity. The ID_{50} of each HTCCA was obtained by plotting the percent tyrosinase activity remaining under the experimental condition against HTCCA concentration. Triplicate reactions were carried out for each level of a HTCCA.

RESULTS AND DISCUSSION

HTCCAs are water soluble derivatives of vitamin E, containing the essential structural components observed in identified tyrosinase inhibitors. There are a pair of enantiomers of HTCCA, with a *R* or *S* configuration on the C2 (**Figure 1**). HTCCA is a water soluble analogue of vitamin E and has been used as a water soluble antioxidant standard in a number of research studies (*3*, *14*). In the present study, (*R*)- and (*S*)-HTCCA were examined and compared for their inhibitory effects against the mushroom tyrosinase. Both (*R*)- and (*S*)-HTCCA are competitive inhibitors of the tyrosinase (**Figures 2** and **3**). Under the experimental conditions, the apparent K_m and V_m of the L-DOPA oxidation reaction catalyzed by the tyrosinase was 0.34 mM and 0.21 ΔA_{475nm}/min, respectively. The K_i values for both (*R*)- and (*S*)-HTCCA were obtained from the secondary plots of the K_m^{app} of each inhibitor at each level against the inhibitor concentration (**Figure 4A**,**B**). The K_i of (*R*)-HTCCA against

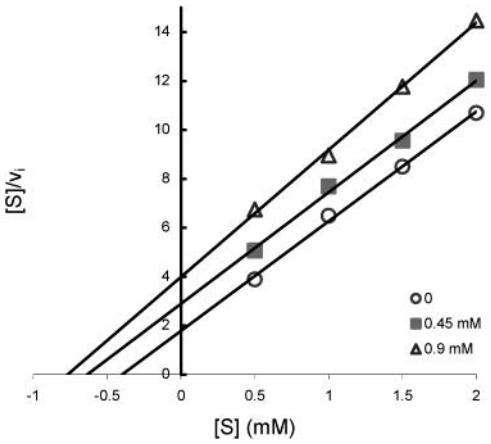

Figure 2. Hanes–Woolf plots of mushroom tyrosinase and L-DOPA without (○) and with (*S*)-HTCCA (□) 0.45 and (△) 0.90 mM. $[S]/v_i$ = mM min $(\Delta_{A475nm})^{-1}$.

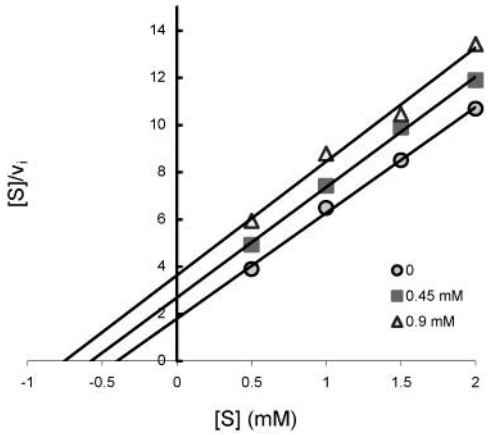

Figure 3. Hanes–Woolf plots of mushroom tyrosinase and L-DOPA without (○) and with (*R*)-HTCCA (□) 0.45 and (△) 0.90 mM. $[S]/v_i$ = mM min $(\Delta_{A475nm})^{-1}$.

mushroom tyrosinase was 0.83 mM, which was greater than the K_i (0.61 mM) of the (*S*)-HTCCA under the same experimental conditions (**Table 1**). These results indicated that the (*R*)-HTCCA has stronger tyrosinase affinity than the (*S*) enantiomer, while the tyrosinase affinities of both HTCCA isomers are lower than that of L-DOPA, suggesting tyrosinases may have stereoselectivity in binding substrates or inhibitors. It was also noted that the L-DOPA concentration range (0.5−2.0 mM) used in this study was greater than K_m, and further kinetic studies using broader concentration ranges of L-DOPA (0.25−5 K_m) may be performed to more accurately determine the inhibitory kinetics of HTCCA against the tyrosinase if necessary (*15*).

The inhibitory capacities of the (*R*)-, (*S*)-, and (±)-HTCCA were determined within 1 min of the reactions when the enzymatic reactions have reached their steady states and expressed as the ID_{50} values. The ID_{50} is the level of each HTCCA isomer required to reduce the tyrosinase activity to 50% of that in the control containing no inhibitor under the same experimental conditions. No significant difference in the ID_{50} values among the (*R*)-, (*S*)-, and (±)-HTCCA against the tyrosinase was observed in this study (**Table 1**). The results from this research showed the potential utilization of (*R*)-, (*S*)-, and (±)-HTCCA as tyrosinase inhibitors in food, cosmetic, and medicinal products. It needs to be noted that the measurements of the ID_{50} values were not done at the equilibrium of each

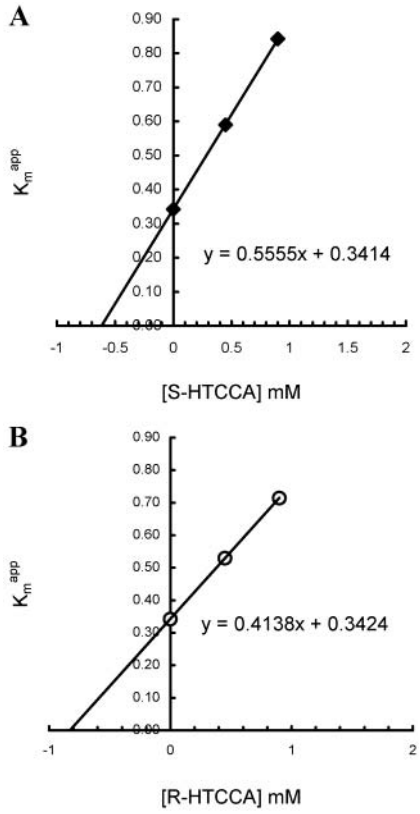

Figure 4. Secondary plot of K_m^{app} as a function of inhibitor concentration. [(*S*)-HTCCA] and [(*R*)-HTCCA] stand for the concentrations of (*S*)- and (*R*)-HTCCAs, respectively.

Table 1. Tyrosinase Inhibitory Activity of HTCCAs

compd	ID_{50}[a] (mM)	K_i (mM)	mode of inhibition
(*R*)-HTCCA	1.88	0.83	competitive
(*S*)-HTCCA	1.84	0.61	competitive
(±)-HTCCA	1.88	N/A[b]	N/A

[a] ID_{50} is the initial concentration of a HTCCA to reduce the tyrosinase activity to 50% of that in the control containing no inhibitor under the same experimental condition. [b] N/A stands for not tested.

reaction, since melanin pigments are water insoluble and precipitate from the reaction mixture upon formation. The mushroom tyrosinase was selected for the present study due to its availability. However, it has to be kept in mind that the tyrosinases from individual sources may differ in their structures and properties (*4*). In addition, because L-DOPA was used as a substrate in this study, the inhibitory activities of all of the HTCCA isomers discussed in this paper are against the diphenolase activity of mushroom tyrosinase.

The effects of dimethyl sulfoxide (DMSO), a solvent widely used to dissolve or help to dissolve tyrosinase inhibitors (*1*, *4*), on the tyrosinase activity and the tyrosinase inhibitory capacities of (*R*)- and (*S*)-HTCCA were also determined in this research, since we observed the potential influences of DMSO in tyrosinase activity and the inhibitory effects of both HTCCA enantiomers against mushroom tyrosinase in our preliminary experiments. As compared to the control containing no inhibitor, DMSO suppressed the tyrosinase activity at tested levels, 100 and 200 μL in a total volume of 3.0 mL (**Figure 5**). The inhibition of DMSO on the mushroom tyrosinase was dose-dependent. Additions of DMSO at the two levels in the

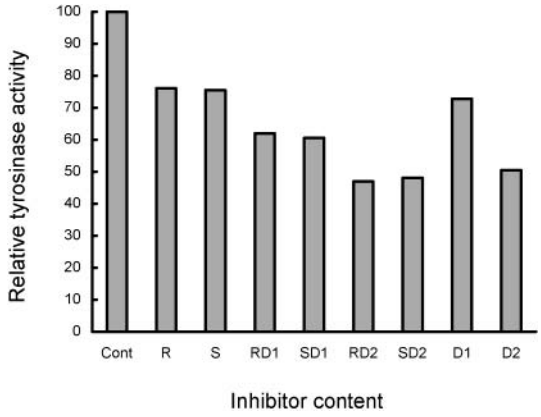

Figure 5. Effects of DMSO on mushroom tyrosinase and the inhibitory activities of (*R*)- and (*S*)-HTCCAs. Cont = the control containing no inhibitor or DMSO; *R* and *S* stand for (*R*)- and (*S*)-HTCCAs at a concentration of 0.90 mM, respectively; D1 and D2 stand for the final levels of DMSO at 100 and 200 μL in a total volume of 3.0 mL, respectively; RD1 and SD1 = 0.90 mM (*R*)- or (*S*)-HTCCAs and 100 μL of DMSO in a total volume of 3.0 mL, while RD2 and SD2 = 0.90 mM (*R*)- or (*S*)-HTCCAs and 200 μL of DMSO in a total volume of 3.0 mL.

tyrosinase digests containing (*R*)- or (*S*)-HTCCA resulted in further inhibitions of the tyrosinase activity (**Figure 5**). The influence of the DMSO on the inhibitory effects of both (*R*)- and (*S*)-HTCCA against the tyrosinase was also dose-dependent. These data suggest that it is essential to identify an alternative approach for evaluating the hydrophobic compounds for their potential tyrosinase inhibitory activity in an aqueous system.

In summary, this research evaluated the kinetic properties and the capacities of both (*R*)- and (*S*)-HTCCA for their potentials to inhibit mushroom tyrosinase activity. The results indicated the potential to use the HTCCA as tyrosinase inhibitors in food, cosmetic, and medicinal products, since HTCCA is a water soluble vitamin E analogue and may have less safety concerns. This research also indicated the possible interactions between DMSO and other chemicals for their inhibitory activities against a tyrosinase, suggesting that an improved solvent system is needed for future research in the tyrosinase inhibitions.

LITERATURE CITED

(1) Chen, Q.-X.; Kubo, I. Kinetics of mushroom tyrosinase inhibition by quercetin. *J. Agric. Food Chem.* **2002**, *50*, 4108−4112.
(2) Zawistowski, J.; Biliaderis, C. G.; Eskin, N. A. M. Polyphenol oxidase. In *Oxidative Enzymes in Foods*; Robinson, D. S., Eskin, N. A. M., Eds.; Elsevier Applied Science: New York, 1991; pp 217−273.
(3) Pawelek, J. M.; Körner, A. M. The biosynthesis of mammalian melanin. *AMSCA* **1982**, *70*, 136−145.
(4) Lee, H.-S. Tyrosinase inhibitors of *pulsatilla cernua* root-derived materials. *J. Agric. Food Chem.* **2002**, *50*, 1400−1403.
(5) Whitaker, J. R. Polyphenol oxidase. In *Food Enzyme: Structure and Mechanism*; Wong, D. W. S., Ed.; Chapman & Hall: New York, 1995; pp 271−307.
(6) Lee, S.-E.; Kim, M.-K.; Lee, S.-G.; Ahn, Y.-J.; Lee, H.-S. Inhibition effects of *cinnamomum cassia* bark-derived materials on mushroom tyrosinase. *Food Sci. Biotechnol.* **2000**, *9*, 330−333.
(7) Sugumaran, M. Molecular mechanism for culticular sclerotization. *Adv. Insect Physiol.* **1988**, *21*, 179−231.
(8) Barrett, F. M. Wound-healing phenoloxidase in larval cuticle of Calpodes ethlius (Lepidoptera: Hesperiidae). *Can. J. Zool.* **1984**, *62*, 834−838.

(9) Maeda, K.; Fukuda, M. In vitro effectiveness of several whitening cosmetic components in human melanocytes. *J. Soc. Cosmet. Chem*. **1991**, *42*, 361−368.
(10) Mosher, D. B.; Pathak, M. A.; Fitzpatrick, T. B. Vitiligo: ethology, pathogenesis, diagnosis, and treatment. In *Updates: Dermatology in General Medicine*; Fitzpatrick, T. B., Eisen, A. Z., Wolff, K., Freedberg, I. M., Austen, K. F., Eds.; McGraw-Hill: New York, 1983; pp 373−398.
(11) Xu, Y.; Stokes, A. H.; Freeman, W. M.; Kumer, S. C.; Vogt, B. A.; Vrana, K. E. Tyrosinase mRNA is expressed in human substantia nigra. *Mol. Brain Res*. **1997**, *45*, 159−162.
(12) Yu, L.; Robyt, J. F. Effects of water soluble constitunents of clove on the activities of glucansucrase and the potential for the inhibition of dental plaque formation. *Carbohydr. Lett.* **1998**, *3*, 9−16.
(13) Yu, L.; Haley, S.; Perret, J.; Harris, M.; Wilson, J.; Qian, M. Free radical scavenging properties of wheat extracts. *J. Agric. Food Chem*. **2002**, *50*, 1619−1624.
(14) Giulivi, C.; Cadenas, E. Inhibition of protein radical reactions of ferrylmyoglobin by water-soluble analogue of vitamin E, trolox C. *Arch. Biochem. Biophys*. **1993**, *303*, 152−158.
(15) Copeland, R. A. *Enzymes*, 2nd ed.; Wiley-VCH: New York, 2000; pp 124−128.

Received for review July 29, 2002. Revised manuscript received January 20, 2003. Accepted February 6, 2003. This study was supported by the Colorado Agricultural Experiment Station and Pharmanutrients Company (Lake Bluff, IL).

JF0208379