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Inhibition effects of benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol on the activity of mushroom tyrosinase

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Tyrosinase (EC 1.14.18.1) is the key enzyme of melanin synthesis and fruit-vegetable browning. The inhibition of benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol on mushroom tyrosinase was first investigated. The results shown that these three compounds could effectively inhibit the enzyme activity sharply and the inhibitory effects were determined to be reversible. Their inhibitor concentrations leading to 50% activity lost values were determined to be 1.5, 2.8, and 1.1 mM for monophenolase and 2.0, 0.6, and 0.8 mM for diphenolase, respectively. For the monophenolase activity, all of these three compounds were mixed-type inhibitors, however, only 4-phenyl-2-butanol obviously lengthened the lag time. For the diphenolase activity, benzylideneacetone and benzylacetone were mixed-type inhibitors, while 4-phenyl-2-butanol was a noncompetitive type inhibitor. In conclusion, these compounds exhibited potent antityrosinase activities. This research would provide scientific evidence for the use of benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol as antityrosinase agents.

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[**Key words:** Benzylideneacetone; Benzylacetone; 4-Phenyl-2-butanol; Mushroom tyrosinase; Inhibition kinetics]

Tyrosinase (EC 1.14.18.1), a kind of multiple functional oxidase containing di-copper catalytic core, is widely founded in animals, insects, and microorganisms (1–3). Tyrosinase participates in melanogenesis of animals. Melanogenesis is a critical physiological process (4) that leads to the formation and accumulation of melanin, which plays a crucial role in protecting the skins from UV-induced injuries (5,6). Tyrosinase is general considered as both monophenolase activity and diphenolase activity tyrosinase (7). It can catalyze the tyrosine hydroxylation to induce the production of 3,4-dihydroxy-L-phenylalanine (L-DOPA), and oxidize L-DOPA into dopaquinone. The dopaquinone changes into melanin after several spontaneous chemical reactions (8,9). Excessive melanin can lead to some skin diseases, such as age spots, melasma, and freckles. Melanin synthesis can be controlled through inhibiting the activity of tyrosinase. Therefore, the work of screening high-efficient tyrosinase inhibitors become a current research focus (10,11).

Tyrosinase inhibitor also was applied in agriculture including fresh-keeping and insecticide (12–14). Browning is a kind of enzymatic reaction controlled by tyrosinase. Some undesirable browning phenomena contributing to the loss of quality and visual value usually occurs in some fruits and vegetables (15,16). Besides, tyrosinase plays a critical role in insect cuticle tanning process (17,18). Some research groups have reported that the addition of tyrosinase inhibitors in insects' food can prohibit the formation of insects' exoskeletons and greatly enhance the mortalities of insects (19).

Many previous studies have reported that the inhibitor effects of aromatic compounds on tyrosinase activities (20–22). Benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol were a set of structural analogues which are similar to the tyrosinase substrate. Therefore we chose benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol and determined their inhibition effects on diphenolase and monophenolase activities of tyrosinase. Chen et al. (23) indicated that many aromatic compounds have low practical potential for their high toxicities. For example, the hydroquinone is a kind of antityrosinase agent with high toxicity (24). Benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol are known as safe aromatic compounds with low toxicities and have been widely used as food and cosmetic additives (25,26). In this paper, these three aromatic compounds' inhibition mechanism and inhibition type were justified and their inhibition constants were determined. All the data could be provided the basis for exploitation of novel high-efficient tyrosinase inhibitors and application on cosmetic areas and fresh-keeping.

MATERIALS AND METHODS

Reagents Mushroom tyrosinase (EC 1.14.18.1) purchased from Sigma–Aldrich (St. Louis, MO, USA). The activity of the enzyme is 5037 U/mg. L-Tyr and L-DOPA were the products of Aldrich (St. Louis). Benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol were obtained from Xiamen Doingcom Chemical Company Limited. The purities of these chemical compounds are 99.99%, 97.00% and

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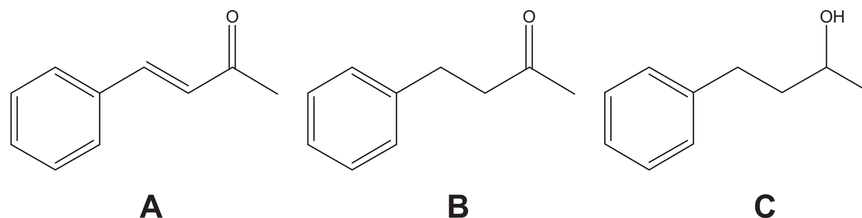

FIG. 1. Chemical structures of benzylideneacetone (A), benzylacetone (B) and 4-phenyl-2-butanol (C).

94.26%, respectively. All other reagents were local and of analytical grade. The water used was re-distilled and ion-free.

Enzyme activity assay Monophenolase and diphenolase activities of mushroom tyrosinase were determined by the method previous reported by using a Beckman UV-800 spectrophotometer (27).

Effects of inhibitors on the enzyme activity The inhibitory effects of inhibitors on diphenolase activity of tyrosinase were determined by the method previous reported (6). All the selected chemical compounds were firstly dissolved in dimethyl sulfoxide (DMSO) and diluted into DMSO solutions of the inhibitors in different concentrations. The final concentration of DMSO was controlled at 3.3% in the reaction media.

Determination of the inhibition type and inhibition constant The inhibition types of the inhibitors on monophenolase and diphenolase activities of mushroom tyrosinase were determined as follows. The concentrations of inhibitors were kept and the concentrations of substrates (L-Tyr for monophenolase activity assay and L-DOPA for diphenolase activity assay) were changed. The concentrations of L-Tyr and L-DOPA ranged from 0.20 to 1.00 mM and 0.25–0.67 mM, respectively. The final concentration of mushroom tyrosinase was 6.67 μg/ml in diphenolase activity assay and 33.3 μg/ml in monophenolase assay, respectively. The inhibition types of the inhibitors on the enzyme were assayed by the Lineweaver-Burk plot and the inhibition constants were determined by the plot of the intercept versus the concentrations of the inhibitors.

RESULTS

Effects of benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol on the diphenolase activity Benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol (Fig. 1 for the chemical structures) of different concentrations were firstly assayed for their effects on the oxidation of L-DOPA catalyzed by mushroom tyrosinase. The results obtained are shown in Fig. 2. With increasing concentrations of the inhibitors the diphenolase activities decreased. The inhibition was concentration-dependent and the values of the inhibitor concentrations leading to 50% activity lost (IC_{50}) of these three compounds were determined to be 2.0, 0.6 and 0.8 mM, respectively.

Inhibition mechanism Taking benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol as inhibitors, their inhibition mechanism on mushroom tyrosinase were investigated. All of inhibitors had same behavior. The relationship between remaining activity and enzyme concentration in the presence of benzylideneacetone is shown in Fig. 3. All the straight lines passed through the origin. With increasing inhibitor concentration, the slopes of line decreased. The results indicated that all of these three compounds were reversible inhibitors (28).

Inhibition types The inhibitor types of benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol on diphenolase activity were determined from Lineweaver–Burk double reciprocal plots and are shown in Fig. 4. The results show that increasing the concentration of benzylideneacetone or benzylacetone, the plots were got a group of lines intercepted in the second quadrant, indicating that both of benzylideneacetone and benzylacetone were mixed-type inhibitors. The values of equilibrium constant of the inhibitor combining with the free enzyme (K_I) and equilibrium constant of the inhibitor combining with the enzyme-substrate complex (K_{IS}) of benzylideneacetone were determined to be 1.73 mM and 3.89 mM, respectively (Fig. 4A). And that of benzylacetone were 0.39 mM and 1.33 mM, respectively. In comparison, when 4-phenyl-2-butanol was used as inhibitor, a group of straight lines had a common intercept on the 1/[S] axis with increasing the inhibitor concentration. This result illustrated in Fig. 4C showed that 4-phenyl-2-butanol was noncompetitive inhibitor. The inhibitor constant (K_I) of 4-phenyl-2-butanol was determined to be 1.02 mM.

Effects on the monophenolase activity Using benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol as inhibitors, the monophenolase activities of mushroom tyrosinase were determined with L-Tyr as substrate. In the present of different concentrations of inhibitors, the kinetics course of the oxidation of the substrate is shown in Fig. 5. The kinetic courses show that the steady-state rates were all sharply decreasing with the increasing concentration of the inhibitors. When the concentration of benzylideneacetone, benzylacetone, and 4-phenyl-2-butanol reached 5.0 mM, the remaining activities were determined to be

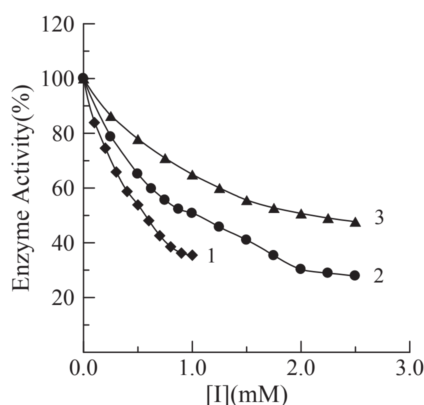

FIG. 2. The inhibitory effect of benzylideneacetone (A), benzylacetone (B), and 4-phenyl-2-butanol (C) on the activity of diphenolase.

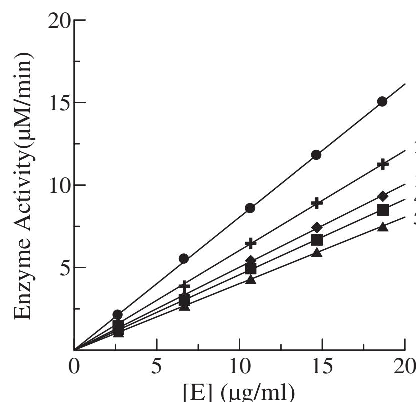

FIG. 3. The inhibitory mechanism of benzylideneacetone on the diphenolase activity of mushroom tyrosinase was reversible. The concentrations of benzylideneacetone for curves 1–5 are 0, 0.625, 1.25, 1.875, and 2.5 mM, respectively.

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