Tyrosinase Inhibitory Activity of the Olive Oil Flavor Compounds

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A series of α,β-unsaturated aldehydes, otherwise known as (2*E*)-alkenals, characterized from the olive *Olea europaea* L. (Oleaceae) oil flavor was found to inhibit the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) catalyzed by mushroom tyrosinase, and the inhibition kinetics analyzed by a Lineweaver−Burk plot found that they are noncompetitive inhibitors. The inhibition mechanism presumably comes from their ability to form a Schiff base with a primary amino group in the enzyme. In addition, the hydrophobic alkyl chain length from the hydrophilic enal group seems to relate to their affinity to the enzyme, and this results in their inhibitory potency.

Keywords: *α,β-Unsaturated aldehydes; Olea europaea; tyrosinase inhibitory activity; Schiff base formation; noncompetitive inhibition*

INTRODUCTION

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO) (Mayer, 1987; Whitaker, 1995), is a copper-containing enzyme widely distributed in microorganisms, animals, and plants. Tyrosinase is responsible for browning in plants and is considered to be deleterious to the color quality of plant-derived foods and beverages. This unfavorable darkening from enzymatic oxidation generally results in a loss of nutritional and market values and has been of great concern (Friedman, 1996). Tyrosinase is responsible not only for browning in plants, but also melanization in animals. Recently, tyrosinase inhibitors have also become increasingly important in medicinal (Mosher et al., 1983) and cosmetic (Maeda and Fukuda, 1991) products in relation to hyperpigmentation. Hence, tyrosinase inhibitors should have broad applications.

In our continuing search for tyrosinase inhibitors from plants (Kubo, 1997), anisaldehyde (**1**) was found to inhibit the oxidation of L-DOPA by mushroom tyrosinase with an ID_{50} of 43 μg/mL (0.32 mM) (Kubo and Kinst-Hori, 1998a). Subsequently, several aromatic aldehydes such as benzaldehyde (**2**) and cuminaldehyde (**3**) (Kubo and Kinst-Hori, 1998b) were also characterized as tyrosinase inhibitors from edible plants. Their inhibition mechanism presumably comes from their ability to form a Schiff base with a primary amino group in the enzyme. Interestingly, the addition of an electron-donating group at the *para* position of benzaldehyde increase the inhibitory activity, presumably stabilizing the Schiff base. For example, the ID_{50}s of anisaldehyde and cuminaldehyde are about 2.5- and 16-fold more potent than that of benzaldehyde, respectively. In addition to stabilizing the binding site, the hydrophobic electron-donating groups such as methoxy and isopropyl also seem to relate to their binding affinity by the enzyme. However, the role of these hydrophobic substituents is not yet understood.

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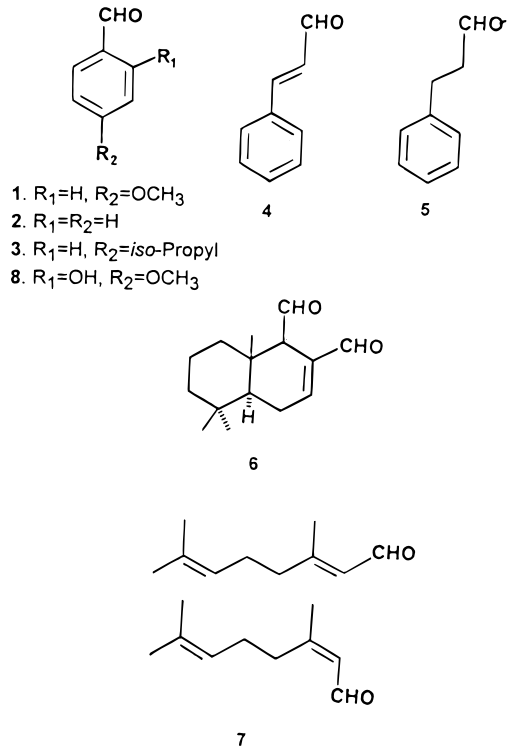

Figure 1. Chemical structures of aromatic aldehydes (**1**−**5** and **8**), polygodial (**6**), and citral (**7**).

We have recently reported that a series of aliphatic long-chain, (2*E*)-alkenals was characterized as antimicrobial agents from the olive oil flavor (Kubo, A., et al., 1995). Since α,β-unsaturated aldehydes are known, in general, to form a Schiff base (Schauenstein et al., 1977), these (2*E*)-alkenals can also be expected to inhibit tyrosinase, although most tyrosinase inhibitors reported so far are aromatic compounds (Pifferi et al., 1974; Passi and Nazzaro-Porro, 1981; Mayer, 1987; Conrad et al., 1994; Kubo, 1997). This assumption was supported by the observation that cinnamaldehyde (**4**) inhibited the oxidation of L-DOPA by mushroom tyrosinase with ID_{50} of 129 μg/mL (0.98 mM) while dihydrocinnamaldehyde

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(phenylpropionaldehyde) (**5**) did not show this inhibitory activity. The latter aldehyde does not form a stable Schiff base. In consequence, aldehydes which can form a Schiff base should show the inhibitory activity. If this is so, (2*E*)-alkenals may be a superior model for a structure and activity relationship (SAR) study because these molecules possess the same hydrophilic portion, the enal group, and thus understanding the role of the hydrophobic alkyl portion. In addition, a series of (2*E*)-alkenals as well as their related enal analogues such as polygodial (**6**) and citral (**7**) are readily available from our previous study (Kubo and Himejima, 1992) for comparison.

MATERIALS AND METHODS

General. General procedures were the same as those of previous work (Kubo et al., 1995a; Kubo and Kinst-Hori, 1998a,b).

Chemicals. A series of (2*E*)-alkenals and their corresponding saturated aldehydes, alcohols, and acids were available from our previous work (Kubo, A., et al., 1995). Polygodial was also available from our previous study (Kubo and Himejima, 1992). It should be noted that (2*E*)-alkenals were prepared up to (2*E*)-eicosenal, but the assay data were obtained unequivocally only up to (2*E*)-nonenal because of solubility problems of the (2*E*)-alkenals having more than 10 carbon atoms in the water-based test solution. Dimethyl sulfoxide (DMSO) and L-tyrosine were purchased from Sigma Chemical Co. (St. Louis, MO). L-DOPA and citral were obtained from Aldrich Chemical Co. (Milwaukee, WI). The chemicals purchased were used as received.

Enzyme Assay. The mushroom tyrosinase (EC 1.14.18.1) used for the bioassay was purchased from Sigma Chemical Co. Although mushroom tyrosinase differs somewhat from other sources (van Gelder et al., 1997), this fungal source was used for the entire experiment because it is readily available. All the samples were first dissolved in DMSO and used for the experiment at 30 times dilution. The preliminary assay was tested at 167 μg/mL, unless otherwise specified. Tyrosinase catalyzes a reaction between two substrates, a phenolic compound and oxygen, but the assay was carried out in air-saturated solutions. The enzyme activity was monitored by dopachrome formation at 475 nm up to the appropriate time (usually not exceeding 10 min). The extent of inhibition by the addition of samples is expressed as the percentage necessary for 50% inhibition (ID_{50}).

The assay was performed as previously described (Masamoto et al., 1980) with slight modifications. First, 1 mL of a 2.5 mM L-DOPA or L-tyrosine solution was mixed with 1.8 mL of 0.1 M phosphate buffer (pH 6.8) and incubated at 25 °C for 10 min. Then, 0.1 mL of the sample solution and 0.1 mL of the aqueous solution of the mushroom tyrosinase (138 units) were added in this order to the mixture. This solution was immediately monitored for the formation of dopachrome by measuring the linear increase in optical density at 475 nm.

The preincubation mixture consisted of 1.8 mL of 0.1 M phosphate buffer (pH 6.8), 0.6 mL of water, 0.1 mL of the samples solution (equivalent amount of ID_{50}), and 0.1 mL of the aqueous solution of the mushroom tyrosinase (138 units). The mixture was preincubated at 25 °C for 5 min. Then 0.4 mL of 6.3 mM L-DOPA solution was added, and the reaction was monitored at 475 nm for 2 min.

RESULTS AND DISCUSSION

Tyrosinase inhibitory activity of a series of (2*E*)-alkenals was tested, and the results are listed in Table 1. As expected, these α,β-unsaturated aldehydes inhibited the oxidation of L-DOPA by mushroom tyrosinase. The result reveals that the aromatic nucleus is not essential to elicit the inhibitory activity since it can be replaced by conjugated double bonds. In general, (2*E*)-alkenals showed a dose-dependent inhibitory effect on the oxidation of L-DOPA. Noticeably, increasing the chain length resulted in increased inhibitory activity, similar to their antimicrobial activity (Kubo, A., et al., 1995). The result indicates that (2*E*)-alkenals of more carbon atoms than nine can be expected to exhibit more potent inhibitory activity and that (2*E*)-alkenals binding involves interactions with hydrophobic groups in the enzyme active site. However, the (2*E*)-decenal and (2*E*)-undecenal tested are hardly soluble in the water-based test solution. This caused variations with OD readings which were essential in determining the ID_{50}. Therefore, their ID_{50} could not be established unequivocally. Among the compounds tested unequivocally, (2*E*)-nonenal was found to be the most potent inhibitor with an ID_{50} of 90 μg/mL (0.64 mM). Therefore, the emphasis of further study was placed on (2*E*)-octenal and (2*E*)-nonenal.

Table 1. Tyrosinase Inhibitory Activity of (2*E*)-Alkenals and Aromatic Aldehydes[a]

compounds tested	ID_{50} (mM)	mode of inhibition
(2*E*)-alkenal		
C_5	3.6	*c*
C_6	4.3	*c*
C_7	1.3	noncompetitive
C_8	1.1	noncompetitive
C_9	0.64	noncompetitive
C_{10}	*b*	*c*
C_{11}	*b*	*c*
citral	1.5	noncompetitive
benzaldehyde (**2**)	0.82	noncompetitive[d]
anisaldehyde (**1**)	0.32	noncompetitive[d]
cuminaldehyde (**3**)	0.05	noncompetitive[d]
cinnamaldehyde (**4**)	0.98	noncompetitive
p-hydroxybenzaldehyde	1.2	competitive[d]
2-hydroxy-4-methoxybenzaldehyde (**8**)	0.03	mixed[e]
2-hydroxy-5-methoxybenzaldehyde	3.2	*c*
vanillin	70[d]	
isovanillin	28	*c*
o-vanillin	26	*c*

[a] With respect to the oxidation of L-DOPA by mushroom tyrosinase. [b] The data could not be obtained precisely. [c] Not tested. [d] Kubo and Kinst-Hori, 1998b. [e] Kubo and Kinst-Hori, 1999.

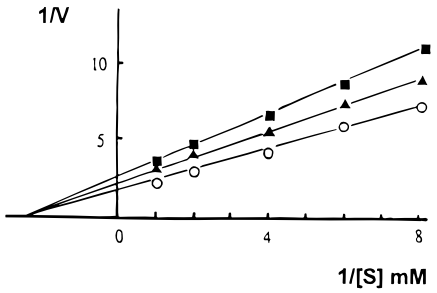

Figure 2. Lineweaver−Burk plots of mushroom tyrosinase and L-DOPA without (○) and with (2*E*)-octenal [(▲): 0.4 mM and (■): 0.8 mM]. 1/*V*: 1/△475 nm/min.

The inhibition kinetics of (2*E*)-octenal were analyzed by a Lineweaver−Burk plot as shown in Figure 2. The three lines, obtained from the uninhibited enzyme and from the two different concentrations of (2*E*)-octenal, intersected on the horizontal axis. This result indicates that (2*E*)-octenal was a noncompetitive inhibitor for the oxidation of L-DOPA by mushroom tyrosinase. Similar kinetics data were also obtained with (2*E*)-heptenal and (2*E*)-nonenal. In addition, preincubation of the enzyme in the presence of 1.1 mM (2*E*)-octenal and in the absence of L-DOPA did not decrease the enzyme activity

significantly. Similar preincubation data was also observed with (2*E*)-heptenal and (2*E*)-nonenal. The results suggest that (2*E*)-heptenal, (2*E*)-octenal, and (2*E*)-nonenal are inhibitors rather than inactivators of the enzyme (Kahn and Andrawis, 1985). It should be borne in mind that most of the enzyme used is *met*-tyrosinase, known as the resting form of the enzyme. It appears that aliphatic (2*E*)-alkenals form a Schiff base with a primary amino group in the enzyme rather than binding to the binuclear copper active center, similar to aromatic aldehydes (Kubo and Kinst-Hori, 1998a,b). This was supported by the fact that their corresponding saturated aldehydes (alkanals) did not show significant inhibitory activity. It should be noted that alkanals do not form a stable Schiff base but still show some inhibitory activity. This can be explained by their hydrophobic interaction with the enzyme—presumably by disrupting the tertiary structure of the enzyme—similar to toluene reported previously (Conrad et al., 1994). The inhibitory activity exerted by (2*E*)-alkenals could be on the basis of the assumption that the enzyme [**E**] is complexed with a (2*E*)-alkenal as a inhibitor [**I**] and the resulting complex [**EI**] is inactive. As expected, their corresponding alcohols (alkanols) and acids did not exhibit significant inhibitory activity (data not shown). Similar to alkanals, alkanols and fatty acids likely disturb the tertiary structure of the enzyme and apparently reflect inferior inhibitory activity.

Tyrosinase contains a strongly coupled binuclear copper active site and functions both as a monophenolase and as an *o*-diphenolase (Lerch, 1987; Sánchez-Ferrer et al., 1995). The discussion so far described is on the basis of the experiment using L-DOPA as a substrate. Therefore, the activity aforementioned is *o*-diphenolase inhibitory activity of mushroom tyrosinase. It should be mentioned that the lag time is known for the oxidation of monophenolic substrates such as L-tyrosine and can be extended by monophenolase inhibitors such as tropolone (Kahn and Andrawis, 1985). Interestingly, (2*E*)-alkenals did not lengthen the lag phase at all. On the basis of this observation, it can be concluded that (2*E*)-alkenals do not inhibit monophenolase activity. However, it should be repeated that most of the enzyme used is the *met*-form of tyrosinase by which L-tyrosine is not oxidized (Espín et al., 1998).

The results obtained so far indicate that the maximum inhibitory activity depends on the hydrophobic alkyl (tail) chain length from the hydrophilic enal group (head). In the case against microorganisms, the antimicrobial activity disappeared after the chain length reached the maximum activity (the so-called "cutoff" effect) (Kubo, I., et al., 1995b). However, the cutoff could not be observed experimentally with tyrosinase inhibitory activity of (2*E*)-alkenals, because the assay data of (2*E*)-alkenals of more carbon atoms than 10 could not be obtained unequivocally because of their solubility limitation in the water-based test solution. However, it appears that these (2*E*)-alkenals ($>C_9$) did not exhibit more potent inhibitory activity than that of (2*E*)-nonenal, as shown in Table 2. Nevertheless, the hydrophobic alkyl group obviously plays an important role in the inhibitory activity, since increasing the chain length of the carbon tail resulted in increased inhibitory activity. (2*E*)-Alkenals with a longer alkyl group may be better associated with the hydrophobic protein pocket close to the binuclear active site (Tanford, 1980; Wilcox et al., 1985; Conrad et al., 1994). The result obtained

Table 2. Inhibition Percent of (2*E*)-Alkenals Tested[a]

compounds tested	inhibition %
(2*E*)-alkenal	
C_5	36
C_6	31
C_7	54
C_8	56
C_9	66
C_{10}	24[b]
C_{11}	15[b]
C_{12}	19[b]
C_{13}	21[b]

[a] With respect to the oxidation of L-DOPA by mushroom tyrosinase. [b] The data could not be obtained unequivocally because of their solubility problem in the water-based test solution.

may give a hint to their interaction with the tertiary structure of the enzyme, but this remains unclear since the structure of mushroom tyrosinase used for this study has not yet been established. Nevertheless, it appears now that (2*E*)-alkenals inhibit the oxidation of L-DOPA by mushroom tyrosinase as noncompetitive inhibitors and that the hydrophobic alkyl portion relates to their inhibition activity. The inhibition mechanism of (2*E*)-alkenals comes from their ability to form a Schiff base with a primary amino group in the enzyme, similar to aromatic tyrosinase inhibitory aldehydes (Kubo and Kinst-Hori, 1998a,b). It should be noted, however, that a cyclic α,β-unsaturated aldehyde sesquiterpene, polygodial (**6**), did not inhibit the oxidation of L-DOPA by mushroom tyrosinase. This antifungal sesquiterpene dialdehyde is know to form a Schiff base, but its hydrophobic decaline moiety may not associate well with the protein pocket in the enzyme. On the other hand, citral (**7**) inhibited this oxidation with an ID_{50} of 227 μg/mL (1.5 mM). This acyclic monoterpene aldehyde holds a key position among fragrance and flavor chemicals and occurs as cis and trans isomers (Bauer et al., 1990). The difference in the inhibitory effect of these isomers remains unclear. However, it may not be illogical to assume that the trans isomer has a more potent inhibitory activity than that of the cis congener since it is supposed to form a more stable Schiff base.

We have previously characterized several aromatic aldehydes—such as benzaldehyde and cinnamaldehyde derivatives—as tyrosinase inhibitors from various plants. These aromatic aldehydes were found to inhibit the oxidation of L-DOPA by mushroom tyrosinase (Table 1), presumably by forming a Schiff base with a primary amino group in the enzyme (Kubo and Kinst-Hori, 1998a,b). Interestingly, aldehydes that form a more stable Schiff base seem to have a more potent inhibitory activity. As far as benzaldehyde derivatives are concerned, there are three factors to increase inhibitory activity. For example, 2-hydroxy-4-methoxybenzaldehyde (**8**), the most potent inhibitor among the inhibitors we have characterized from botanical sources so far, shows its ID_{50} as low as 4.3 μg/mL (0.03 mM). The reason for the exceptionally potent inhibitory activity of this aldehyde can now be explained, at least in part, more precisely. That is, the Schiff base of this benzaldehyde derivative is stabilized by the inductive effect of the electron-donating methoxy group at the *para* position to the aldehyde group and the mesomeric effect by the hydroxyl group at the *ortho* position which can form a quasi-six-membered ring with the unshared pair of electrons on the nitrogen atom through intramolecular hydrogen bonding and produces a stable chelate

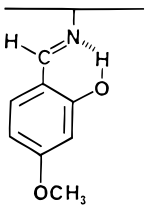

Figure 3. Chemical structure of Schiff base adduct of **8**.

structure, as illustrated in Figure 3. In addition, the binding affinity of this inhibitor by the enzyme is increased by association with the methoxy group. The mixed inhibition exerted by **8** may come from its ability to form a Schiff base with a primary amino group and to chelate copper in the enzyme (Kubo and Kinst-Hori, 1999). It appears now that the aldehydes, regardless of being aromatic or aliphatic, which can form a stable Schiff base are mushroom tyrosinase inhibitors with respect to the oxidation of L-DOPA and noncompetitive inhibitors, though aromatic aldehydes can offer a wider range of structural and electronic variations. It may not be illogical to assume that the primary amino group plays an important role in the tertiary structure of mushroom tyrosinase. For instance, it may be involved with the hydrogen bonding which is essential to maintain the tertiary structure of the enzyme. If this is so, the aldehydes may disrupt this tertiary structure by forming a Schiff base. This assumption can be supported by the previous report that the hydrogen-bonding interactions stabilize the *oxy*-form of *Streptomyces glaucescens* tyrosinase (Jackman et al., 1992). However, the conclusive interpretation remains to be clarified since the structure of tyrosinase used for this study has not yet been established.

Safety is a primary consideration for tyrosinase inhibitors, especially those in food and cosmetic products, which may be utilized in unregulated quantities on a regular basis. Olive oil has proven its safety through many years of human use and consumption. In addition, a similar (2*E*)-alkenal composition was also reported in the essential oil of grapefruit (Nursten and Williams, 1967) and coriander (Potter and Fagerson, 1990). This further substantiates its safety. Hence, the olive oil flavor compounds described herein may be considered as potential tyrosinase inhibitors for food and cosmetic products. However, (2*E*)-alkenals were reported to exhibit potent mutagenic activity (Marnett et al., 1985), though aromatic aldehydes such as cinnamaldehyde and benzaldehyde are known to be generally recognized as safe (GRAS). It seems that Schiff base formation is important to inhibit tyrosinase activity, but Michael-type 1,4-addition relates to mutagenic activity. The maximum nontoxic dose described (Marnett et al., 1985) is much lower than the concentrations which are needed to inhibit tyrosinase, and this may limit their application. Interestingly, (2*E*)-hexenal was described negatively for mutagenicity, a volatile compound modification of the test method (Andersen et al., 1994). If this is so, in addition to its broad spectrum of antimicrobial activity (Kubo, A., et al., 1995), this volatile compound may find applications on food, such as fumigation type. Although (2*E*)-alkenals, particularly (2*E*)-hexenal, were characterized as key flavor compounds in the olive oil, they are found only in minute amounts (Flath et al., 1973). These (2*E*)-alkenals are found in many plants and easily available in large quantities since they have been widely used as food flavors (Bauer et al., 1990). It is worthwhile to add that the olive oil itself did not inhibit tyrosinase, though (2*E*)-alkenals were characterized as a key flavor compound but in a minute amount. However, the steam distillates of commercial olive oils were found to exhibit some inhibitory activity, as expected.

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LITERATURE CITED

Andersen, R. A.; Hamilton-Kemp, T. R.; Hildebrand, D. F.; McCracken, C. T., Jr.; Collins, R. W.; Fleming, P. D. Structure-antifungal activity relationships among volatile C_6 and C_9 aliphatic aldehydes, ketones, and alcohols. *J. Agric. Food Chem*. **1994**, *42*, 1563−1568.

Bauer, K.; Garbe, D.; Surburg, H. *Common Fragrance and Flavor Materials*; VCH: Weinheim, 1990; pp 10−34.

Conrad, J. S.; Dawso, S. R.; Hubbard, E. R.; Meyers, T. E.; Strothkamp, K. G. Inhibitor binding to the binuclear active site of tyrosinase: Temperature, pH, and solvent deuterium isotope effects. *Biochemistry* **1994**, *33*, 5739−5744.

Espín, J. C.; García-Ruiz, P. A.; Tudela, J.; Varón, R.; García-Cánovas, F. Monophenolase and diphenolase reaction mechanisms of apple and pear polyphenol oxidases. *J. Agric. Food Chem*. **1998**, *46*, 2968−2975.

Flath, R. A.; Forrey, R. R.; Guadagni, D. G. Aroma components of olive oil. *J. Agric. Food Chem*. **1973**, *21*, 948−952.

Friedman, M. Food browning and its prevention: An overview. *J. Agric. Food Chem*. **1996**, *44*, 631−653.

Jackman, M. P.; Huber, M.; Hajnal, A.; Lerch, K. Stabilization of the oxy form of tyrosinase by a single conservative amino acid substitution. *Biochim. J*. **1992**, *282*, 915−918.

Kahn, V.; Andrawis, A. Inhibition of mushroom tyrosinase by tropolone. *Phytochemistry* **1985**, *24*, 905−908.

Kubo, A.; Lunde; C. S.; Kubo, I. Antimicrobial activity of the olive oil flavor compounds. *J. Agric. Food Chem*. **1995**, *43*, 1629−1633.

Kubo, I.; Himejima, M. Potentiation of antifungal activity of sesquiterpene dialdehydes against *Candida albicans* and two other fungi. *Experientia* **1992**, *48*, 1162−1164.

Kubo, I.; Yokokawa, Y.; Kinst-Hori, I. Tyrosinase inhibitors from Bolivian medicinal plants. *J. Nat. Prod*. **1995a**, *58*, 739−743.

Kubo, I.; Muroi, H.; Kubo, A. Structural functions of antimicrobial long-chain alcohols and phenolics. *Bioorg. Med. Chem*. **1995b**, *3*, 873−880.

Kubo, I. Tyrosinase inhibitors from plants. In *Phytochemicals for Pest Control*; Hedin, P., Hollingworth, R., Masler, E., Miyamoto, J., Thompson, D. Eds.; ACS Symposium Series 658; American Chemical Society: Washington, DC, 1997; pp 310−326.

Kubo, I.; Kinst-Hori, I. Tyrosinase inhibitors from anise oil. *J. Agric. Food Chem*. **1998a**, *46*, 1268−1271.

Kubo, I.; Kinst-Hori, I. Tyrosinase inhibitors from cumin. *J. Agric. Food Chem*. **1998b**, *46*, 5338−5341.

Kubo, I.; Kinst-Hori, I. 2-Hydroxy-4-methoxybenzaldehyde, a potent tyrosinase inhibitor from African medicinal plants. *Planta Med*. **1999**, *65*, 19−22.

Lerch, K. Molecular and active site structure of tyrosinase. *Life Chem. Rep*. **1987**, *5*, 221−234.

Maeda, K.; Fukuda, M. *In vitro* effectiveness of several whitening cosmetic components in human melanocytes. *J. Soc. Cosmet. Chem*. **1991**, *42*, 361−368.

Marnett, L. J.; Hurd, H. K.; Hollstein, M. C.; Levin, D. E.; Esterbauer, H.; Ames, B. N. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA 104. *Mutat. Res*. **1985**, *148*, 25−34.

Masamoto, Y.; Iida, S.; Kubo, M. Inhibitory effect of Chinese crude drugs on tyrosinase. *Planta Med*. **1980**, *40*, 361−365.

Mayer, A. M. Polyphenol oxidases in plants—Recent progress. *Phytochemistry* **1987**, *26*, 11−20.

Mosher, D. B.; Pathak, M. A.; Fitzpatrick, T. B. Vitiligo, etiology, pathogenesis, diagnosis, and treatment. In *Update*: *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 205−225.

Nursten, H. E.; Williams, A. A. Fruit aromas: survey of components identified. *Chem. Ind.* (*London*) **1967**, *12*, 486−497.

Passi, S.; Nazzaro-Porro, P. M. Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds. *Br. J. Dermatol.* **1981**, *104*, 659−665.

Pifferi, P. G.; Baldassari, L.; Cultrera, R. Inhibition by carboxylic acids of an *o*-diphenol oxidase from *Prunus avium* fruits. *J. Sci. Food Agric.* **1974**, *25*, 263−270.

Potter, T. L.; Fagerson, I. S. Composition of coriander leaf volatiles. *J. Agric. Food Chem.* **1990**, *38*, 2054−2056.

Sánchez-Ferrer, A.; Rodríguez-López, J. N.; García-Cánovas, F.; García-Carmona, F. Tyrosinase: A comprehensive review of its mechanism. *Biochim. Biophys. Acta* **1995**, *1247*, 1−11.

Schauenstein, E.; Esterbauer, H.; Zollner, H. *Aldehydes in Biological Systems*; Pion: London, 1977; pp 31−32.

Tanford, C. *The Hydrophobic Effect*: *Formation of Micelles and Biological Membranes*, 2nd ed.; John Wiley & Sons: New York, 1980.

van Gelder, C. W. G.; Flurkey, W. H.; Wichers, H. J. Sequence and structural features of plant and fungal tyrosinases. *Phytochemistry* **1997**, *45*, 1309−1323.

Whitaker, J. R. Polyphenol oxidase. In *Food Enzymes, Structure and Mechanism*; Wong, D. W. S., Ed.; Chapman & Hall: New York, 1995; pp 271−307.

Wilcox, D. E.; Porras, A. G.; Hwang, Y. T.; Lerch, K.; Winkler, M. E.; Solomon, E. I. Substrate analogue binding to the coupled binuclear copper active site in tyrosinase. *J. Am. Chem. Soc.* **1985**, *107*, 4015−4027.

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