

芳香族香料对酪氨酸酶的抑制及生物学效应研究

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芳香族香料对酪氨酸酶的抑制及生物学效应研究  
Study on the Inhibitory Effects to Tyrosinase and  
Bioactivity by Aromatic Fragrance

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## 中文摘要

酪氨酸酶 (EC. 1. 14. 18. 1) 是一种含铜的氧化还原酶, 它具有单酚酶活性和二酚酶活性, 是生物体合成黑色素的限速酶<sup>[1-3]</sup>, 广泛存在于微生物、动植物及人体中。其抑制剂可广泛应用于美白化妆品、果蔬保鲜、杀虫等方面。

本论文围绕三大部分进行, 第一部分是探讨蘑菇酪氨酸酶的抑制剂对单酚酶和二酚酶催化反应的抑制机理。研究了芳香族香料对蘑菇酪氨酸单酚酶和二酚酶效应, 研究它们的抑制机理, 并测定它们的抑制类型和抑制常数。第二部分以小鼠B16黑色素瘤细胞为研究对象, 研究了芳香族香料对细胞增殖率、体内酪氨酸酶活力和对细胞黑色素生成的影响。第三部分是以大肠杆菌、枯草芽胞杆菌、金黄色葡萄球菌、串珠镰刀杆菌、茄形镰刀菌、尖孢镰刀菌和炭疽杆菌作为实验菌种, 研究抑制剂的抑菌效应。研究结果和内容如下:

- (1) 研究抑制剂对蘑菇酪氨酸二酚酶的抑制作用时, 分别研究了苯丙醛、苯丙醇、肉桂醇、肉桂酸乙酯、苜叉丙酮、苜基丙酮、4-苯基-2-丁醇、覆盆子酮、4-(4-羟基苯基)-3-丁烯酮-2、玫瑰烷和2-甲基-5-苯基-2, 4-戊二烯醛对蘑菇酪氨酸二酚酶的抑制作用。研究表明, 这十一个化合物对蘑菇酪氨酸二酚酶具有不同程度的抑制作用, 并测得它们对二酚酶抑制的  $IC_{50}$  值。研究了它们对酪氨酸二酚酶的抑制机理, 研究结果表明这十一个化合物对酪氨酸酶均为可逆抑制。在研究这十一个化合物对酪氨酸二酚酶抑制类型得到如下结果: 苯丙醛、覆盆子酮和4-(4-羟基苯基)-3-丁烯酮-2的抑制类型为反竞争; 苯丙醇、肉桂醇、苜叉丙酮和苜基丙酮的抑制类型为混合I型竞争; 肉桂酸乙酯、4-苯基-2-丁醇和2-甲基-5-苯基-2, 4-戊二烯醛的抑制类型为非竞争; 玫瑰烷的抑制类型为竞争型。并测定了这十一个化合物对酪氨酸二酚酶的抑制常数。
- (2) 研究抑制剂对蘑菇酪氨酸单酚酶的抑制作用时, 分别研究了苯丙醇、肉桂醇、苜叉丙酮、苜基丙酮、4-苯基-2-丁醇、覆盆子酮、4-(4-羟基苯基)-3-丁烯酮-2、玫瑰烷和2-甲基-5-苯基-2, 4-戊二烯醛对蘑菇酪氨酸单酚酶的抑制作用。研究表明, 这九个化合物对蘑菇酪氨酸单酚酶具有不同程度的抑制作用, 并测得它们对单酚酶抑制的  $IC_{50}$  值。研究了苜叉丙酮、苜基丙酮、4-苯基-2-丁醇、玫瑰烷和2-甲基-5-苯基-2, 4-戊二烯醛对酪氨酸单酚酶抑制类型得到如下结果: 苜叉丙酮、苜基丙酮、4-苯基-2-丁醇、玫瑰烷的抑制类型为混合I型竞争; 2-甲基-5-苯基-2, 4-戊二烯醛的抑制类型为非竞争。并测定了这五个化合物对酪氨酸单酚酶的抑制常数。

- (3) 选择了玫瑰烷和2-甲基-5-苯基-2,4-戊二烯醛作为效应物,研究它们对小鼠B16细胞增殖率、体内酪氨酸酶活力和它们对细胞内黑色素生成的影响。研究表明,这两个化合物在实验0.02 mM/L浓度下对B16细胞没有毒性,玫瑰烷对细胞体内酪氨酸酶的活力和黑色素含量无显著的影响,2-甲基-5-苯基-2,4-戊二烯醛对细胞体内酪氨酸酶的活力和黑色素生成有一定的抑制作用。
- (4) 分别研究了如上十一种芳香族香料对大肠杆菌、枯草芽胞杆菌、金黄色葡萄球菌、串珠镰刀菌、茄形镰刀菌、尖孢镰刀菌和炭疽杆菌的抑制作用。结果表明,11种芳香族香料对真菌的抑制作用比对细菌的抑制作用强。在对三种细菌的抑制中,只有2-甲基-5-苯基-2,4-戊二烯醛和4-(4-羟基苯基)-3-丁烯酮和4-苯基-3-丁烯酮-2有抑制作用,其它几个化合物在50 mg/mL的浓度下均对所测三种细菌没有抑制作用。在对如上四种真菌的抑菌实验中,11种芳香族香料都表现出抑菌性,其中4-苯基-3-丁烯酮-2最强,4-(4-羟基苯基)-3-丁烯酮-2表现次之。3-苯丙醇和2-甲基-5-苯基戊醇在50 mg/mL的浓度下,均对炭疽杆菌没有抑制作用。4-苯基-3-丁烯酮-2在50 mg/mL的浓度下,对茄形镰刀菌没有抑制作用;4-(4-羟基苯基)-丁烯酮-2在50 mg/mL的浓度下,对串珠镰刀菌没有抑制作用。

**关键词:** 蘑菇酪氨酸酶; 芳香族香料; B16细胞; 生物学效应; 抑菌作用

## Abstract

Tyrosinase (EC 1.14.18.1) is a kind of redoxidase containing copper, which is of the activity of monophenolase and diphenolase. It is a key enzyme during the melanin biosynthesis and widely distributed in nature. Its inhibitors can be used widely in many fields including whitening agents, keeping fruits and vegetables fresh, insecticides.

In this paper, it mainly is of three parts. The first part is study the inhibitory effect of the tyrosinase inhibitors. It studied the inhibitory mechanism of aromatic fragrance on the activity of monophenolase and diphenolase, and their kinetic constants were determined. The second part, we chose mouse B16 melanoma cells as the research object, measured aromatic fragrance on cell proliferation rate, tyrosinase activity, melanin content. The third part is study their antibacterial actives on *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium Solani* and *Colletotrichum truncatum*. Contents and results listed as follows:

(1) When study inhibitory effect of diphenolase, the effects of 3-phenylpropionaldehyde、3-phenylpropanol、cinnamic alcohol、ethyl cinnamate、benzylideneacetone、benzylacetone、4-phenyl-2-butanol、raspberry ketone、4-(p-hydroxyphenyl)-3-buten-2-one、rosaphen and 2-methyl-5-phenyl-2,4-pentadienal on mushroom tyrosinase were studied respectively. Results showed that these eleven compounds could inhibit the diphenolase activity and also got their  $IC_{50}$  for diphenolase. The inhibitory mechanisms of these eleven compounds on the activity of diphenolase were studied, showing that they were all reversible inhibitions. The inhibition type of 3-phenylpropionaldehyde、raspberry ketone and 4-(p-hydroxyphenyl)-3-buten-2-one were anticompetitive; the inhibition type of 3-phenylpropanol、cinnamic alcohol、benzylideneacetone and benzylacetone were mixed; the inhibition type of ethyl cinnamate、4-phenyl-2-butanol and 2-methyl-5-phenyl-2,4-pentadienal were noncompetitive; The inhibition type of rosaphen was competitive and the inhibition constants of these eleven compounds had been determined.

(2) When study inhibitory effect of monophenolase, the effects of 3-phenylpropanol、cinnamic alcohol、benzylideneacetone、benzylacetone、4-phenyl-2-butanol、raspberry ketone、4-(p-hydroxyphenyl)-3-buten-2-one、rosaphen and 2-methyl-5-phenyl-2,4-pentadienal on mushroom tyrosinase were studied respectively. Results showed that these nine compounds could inhibit the monophenolase activity and also got their  $IC_{50}$  for monophenolase. The inhibition type of benzylideneacetone、benzylacetone、4-phenyl-2-butanol、rosaphen and 2-methyl-5-phenyl-2,4-pentadienal were studied. The inhibition types of benzylideneacetone、



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