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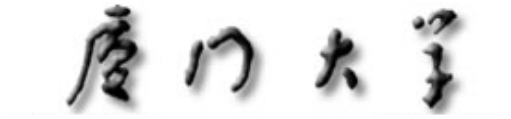

硕士学位论文

抑制剂对蘑菇酪氨酸酶的抑制效应及抗菌活性

Effects of inhibitors on Mushroom Tyrosinase and Their Antimicrobial Activity

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中 文 摘 要

酪氨酸酶（EC.1.14.18.1）是结构复杂的多亚基的含铜氧化还原酶，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，广泛存在于微生物、动植物及人体中。其抑制剂广泛应用于化妆品美白、果蔬保鲜及杀虫等方面。本论文从两大方面研究了抑制剂对酪氨酸酶的抑制活性和抗菌效应。第一部分选择了肉桂酸及其衍生物、肉桂醛及其衍生物、肉桂酸甲酯、曲酸和氰基化合物等五大类抑制剂，研究了它们对酪氨酸酶的抑制机理；第二部分以大肠杆菌、枯草芽孢杆菌、金黄色葡萄球菌和白色假丝酵母菌作为试验菌种，研究抑制剂的抗菌效应。

1) 肉桂酸、4-羟基肉桂酸和 4-甲氧基肉桂酸对酪氨酸酶具有单酚酶和二酚酶的抑制活性，实验结果表明，它们使单酚酶活力下降 50%的抑制剂浓度(IC_{50})分别为 0.58、0.27 和 0.50 mmol/L。使二酚酶活力下降 50%的抑制剂浓度(IC_{50})分别为 2.1、0.5 和 0.42 mmol/L。研究它们对二酚酶的抑制类型，肉桂酸和 4-甲氧基肉桂酸属于非竞争性抑制，抑制常数分别为 1.994 mmol/L 和 0.458 mmol/L，4-羟基肉桂酸为竞争性抑制剂，抑制常数为 0.244 mmol/ L。抗菌实验结果表明，肉桂酸、4-羟基肉桂酸和 4-甲氧基肉桂酸对大肠杆菌、枯草芽孢杆菌和金黄色葡萄球菌的最低抑制浓度（MIC）均为 500 μg/mL，最低杀菌浓度（MBC）均为 1000 μg/mL，对白色假丝酵母菌，肉桂酸和 4-羟基肉桂酸的 MIC 为 500 μg/mL，最低杀菌浓度（MFC）均为 1000 μg/mL，而 4-甲氧基肉桂酸的 MIC 为 250 μg/mL，MFC 为 500 μg/mL。

2) 肉桂醛和 4-甲氧基肉桂醛使酪氨酸酶单酚酶活力下降 50%的抑制剂浓度(IC_{50})分别为 0.64 和 0.39 mmol/L。二酚酶活力下降 50%的浓度(IC_{50})分别为 0.51 和 0.71 mmol/L。两者均为非竞争性抑制剂，抑制常数分别为 0.581 和 0.700 mmol/L。测定对大肠杆菌、枯草芽孢杆菌和金黄色葡萄球菌的 MIC 和 MBC，结果表明，肉桂醛为 250 和 500 μg/mL，4-甲氧基肉桂醛为 125 和 250 μg/mL。对白色假丝酵母菌的 MIC 和 MFC，肉桂醛为 1000 和 2000 μg/mL，4-甲氧基肉桂醛为 62.5 和 125 μg/mL。

3) 研究肉桂酸甲酯对蘑菇酪氨酸酶的抑制活性，结果表明，对单酚酶和二酚酶，它的 IC_{50} 分别为 0.92 mmol/L 和 1.65 mmol/L，是非竞争性抑制剂。对大肠杆

菌的 MIC 和 MBC 为 500 μg/mL 和 1000 μg/mL，对枯草芽孢杆菌和金黄色葡萄球菌的 MIC 和 MBC 为 250 μg/mL 和 500 μg/mL，对白色假丝酵母菌的 MIC 和 MFC 为 1000 μg/mL 和 2000 μg/mL。

4) 测定曲酸对蘑菇酪氨酸酶单酚酶活力下降 50%的抑制剂浓度(IC_{50})为 32 μmol/L，对蘑菇酪氨酸酶二酚酶活力下降 50%的抑制剂浓度(IC_{50})为 20 μmol/L。研究其抑制类型，结果表明，曲酸对二酚酶活力表现为可逆混合型抑制作用，对游离酶的抑制抑制常数(K_I) 和对酶-底物络合物的抑制常数(K_{IS})分别为 13.0 和 100.0 μmol/L。抗菌实验结果表明，曲酸对大肠杆菌、枯草芽孢杆菌和金黄色葡萄球菌的 MIC 为 1000 μg/mL，当浓度为 2000 μg/mL 时，不能对大肠杆菌、枯草芽孢杆菌和金黄色葡萄球菌达到杀灭的效果。对白色假丝酵母菌的 MIC 和 MFC 分别为 1000 μg/mL 和 2000 μg/mL。

5) 对氰基苯酚和 3,4-二羟基氰苯对蘑菇酪氨酸酶均有单酚酶和二酚酶的抑制活性，对单酚酶的 IC_{50} 分别为 0.22 mmol/L 和 9.2 μmol/L，对二酚酶的 IC_{50} 分别为 0.80 mmol/L 和 13.5 μmol/L。研究它们的抑制类型，结果表明，对氰基苯酚为竞争性抑制剂，3,4-二羟基氰苯为非竞争性抑制剂。而通过测定这两者对大肠杆菌、枯草芽孢杆菌和金黄色葡萄球菌的抗菌效应，结果表明，对氰基苯酚的 MIC 和 MBC 为 500 和 1000 μg/mL，3,4-二羟基氰苯为 1000 和 2000 μg/mL。对白色假丝酵母菌的 MIC 和 MFC，对氰基苯酚为 500 和 1000 μg/mL，3,4-二羟基氰苯为 1000 和 2000 μg/mL。

关键词：蘑菇酪氨酸酶；抑制剂；抑制效应；抗菌活性

Abstract

Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme, which is of the activity of monophenolase and diphenolase, is widely distributed in microorganisms, animals and plants. Its inhibitors can be used widely in many fields including whitening agents, keeping fruits and vegetables fresh, insecticides. Our research in the present paper is composed of two parts. In the first part, we chose cinnamic acid and its derivants, cinnamaldehyde and its derivants, methyl cinnamate, kojic, 4-cyanophenol and 3,4-dihydroxybenzonitrile as research objects, studied the inhibitory mechanism of analogs of them on the activity of monophenolase and diphenolase, and their kinetic constants were determined. In the second part, we studied the antimicrobials effect of these inhibitors.

The effects of cinnamic acid, 4-hydroxy cinnamic acid and 4-methoxy cinnamic acid on mushroom tyrosinase were studied. Their IC_{50} for monophenolase were listed as: 0.58、0.27 and 0.50 mmol/L, IC_{50} for diphenolase were listed as 2.1、0.5 and 0.42 mmol/L. Through the study of inhibitory mechanism, the results showed, cinnamic acid and 4-methoxy cinnamic acid were non-competitive inhibitors of diphenolase, and 4-hydroxy cinnamic acid was competitive inhibitors of diphenolase. And the results of the antimicrobials experiment showed, the minimum inhibitory concentration (MIC) of cinnamic acid, 4-hydroxy cinnamic acid and 4-methoxy cinnamic acid to *E. coli*, *B. subtilis*, *St. aureus* was 500 μg/mL, the minimum bactericidal concentration (MBC) was 1000 μg/mL. To *C. albicans*, the MIC of cinnamic acid and 4-hydroxy cinnamic acid was 500 μg/mL, the he minimum fungal concentration (MFC) was 1000 μg/mL, and the MIC of 4-methoxy cinnamic acid was 250 μg/mL, the MFC was 500 μg/mL.

The IC_{50} of cinnamaldehyde and 4-methoxy cinnamaldehyde for monophenolase were listed as: 0.64 and 0.39 mmol/L, for diphenolase were listed as 0.581and 0.700 mmol/L. And cinnamaldehyde and 4-methoxy cinnamaldehyde were non-competitive inhibitors of diphenolase, the inhibition constants (K_I) were determined to be 0.581

and 0.700 mmol/L. The results of the antimicrobials MIC and MBC to *E. coli*, *B. subtilis*, *St. aureus* showed: to cinnamaldehyde, they were 250 and 500 μg/mL, to 4-methoxy cinnamaldehyde, they were 125 and 250 μg/mL. The MIC and MFC which of cinnamaldehyde to *C. albicans* were 1000 and 2000 μg/mL, of 4-methoxy cinnamaldehyde were 62.5 and 125 μg/mL.

The effects of methyl cinnamate on mushroom tyrosinase were studied. The results showed, the IC_{50} of methyl cinnamate for monophenolase and diphenolase were 0.92 mmol/L and 1.65 mmol/L, and it was a non-competitive inhibitor. The MIC and MBC to *E. coli* were 500 μg/mL and 1000 μg/mL, to *B. subtilis* and *St. aureus* were 250 μg/mL and 500 μg/mL, The MIC and MFC to *C. albicans* were 1000 μg/mL and 2000 μg/mL.

The IC_{50} value of kojic for monophenolase and diphenolase were estimated as 32 μmol/L and 20 μmol/L. It was a mixed type inhibitor, and the inhibition constant K_I and K_{IS} were determined to be 13.0 and 100.0 μmol/L. The results of the antimicrobials experiment showed, the MIC to *E. coli*, *B. subtilis*, *St. aureus* was 1000 μg/mL, and it couldn't kill them when its concentration was 2000 μg/mL. The MIC and MFC to *C. albicans* were 1000 μg/mL and 2000 μg/mL.

The IC_{50} value of 4-cyanophenol and 3,4-dihydroxybenzonitrile on the monophenolase were determined to be 0.22 mmol/L and 9.2 μmol/L, on the diphenolase, which were determined to be 0.80 mmol/L and 13.5 μmol/L. Through the study of the inhibitory mechanism of them, the results showed 4-cyanophenol was a competitive inhibitor, 3,4-dihydroxybenzonitrile was a non-competitive inhibitor. The MIC and MBC of 4-cyanophenol to *E. coli*, *B. subtilis*, *St. aureus* were estimated as 500 and 1000 μg/mL, that of 3,4-dihydroxybenzonitrile were 1000 and 2000 μg/mL. The MIC and MFC to *C. albicans* of 4-cyanophenol were 500 μg/mL and 1000 μg/mL, and that of 3,4-dihydroxybenzonitrile were 1000 and 2000 μg/mL.

Key Word：mushroom tyrosinase; inhibitor; inhibitory effect; antimicrobial effect

1 前　言

1.1 酪氨酸酶抑制剂的研究进展

1.1.1 酪氨酸酶概述

酪氨酸酶(EC1.14.18.1,Tyrosinase)是一种含铜的金属酶，广泛分布于微生物、动植物及人体中[1-3]，是自然界中生物体内黑色素合成代谢的关键酶[4]。在植物中，酪氨酸酶一般称为多酚氧化酶；在昆虫中，则称为酚氧化酶[5-6]；在微生物和人体中，才称为酪氨酸酶。酪氨酸酶是含铜金属酶，其活性中心由两个含铜离子位点构成[7]。在催化过程中，由铜离子结合氧原子数的不同，酪氨酸酶可分为三种型态:氧化态(E_{oxy})、还原态(E_{met})和脱氧态(E_{deoxy})。这三种酶形态的存在和互相转化是酪氨酸酶独特双重催化功能的基础[8]。酪氨酸酶的双重催化活性为：羟基化单酚生成二酚（单酚酶活性）和氧化二酚生成醌（二酚酶活性），两个反应都需要氧分子的参与[9]。其反应机制如图 1.1 所示。

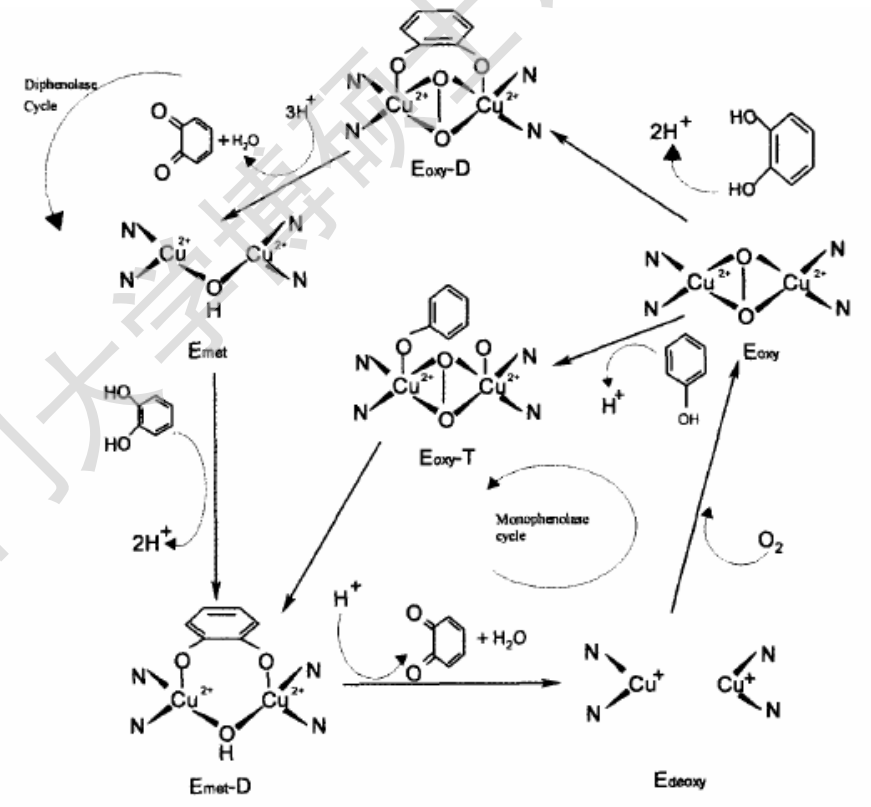

图 1.1 酪氨酸酶催化酚类化合物氧化的机理

Fig. 1.1 Mechanism of phenols oxidation with tyrosinase catalysis

酪氨酸酶作为黑色素合成的关键酶，催化 L-酪氨酸羟基化转变为 L-多巴和氧化 L-多巴形成多巴醌，多巴醌经一系列反应后，形成黑色素[10-11]。酪氨酸酶在生物体中具有重要的生理功能。在同种生物的不同器官中表现出不同的特征。鸟类的羽毛，昆虫的表皮，人类的眼睛、毛发，以及植物的果实、种子呈现出的黑色、褐色、黄色等表征，都与酪氨酸酶的存在与分布有着密切的关系。由于其存在的广泛性，因而在在医学[12-13]、美容[14-16]、果蔬保鲜[17-18]、昆虫防治[19-20]、环境监测与环境保护[21]等方面都有广泛应用。

1.1.2 酪氨酸酶抑制剂的作用机理及分类研究

酪氨酸酶抑制剂可以治疗目前常见的色素沉着皮肤病如雀斑、黄褐斑、老年斑。目前，市场上流行的美白化妆品中其增白剂均是酪氨酸酶抑制剂如熊果甙[22]、维生素 C 衍生物[23]、及一些中药提取物[24]等。酪氨酸酶抑制剂也被用作食品保鲜剂，如 4-已基间苯二酚已被用于虾的保鲜[25]。昆虫表皮的酪氨酸酶产生的黑色素可以保护昆虫免受紫外线的辐射，酪氨酸酶也与昆虫蜕皮过程中的鞣化作用有关[26]。因此，酪氨酸酶是昆虫赖于生存的一种重要的酶。对该酶抑制剂的研究将在新型的生物杀虫剂的设计中起指导作用。

鉴于酪氨酸酶抑制剂的广泛应用，国内外很多学者致力于寻找具有特异的、高效的酪氨酸酶抑制剂。研究抑制作用机理、抑制动力学、以及抑制剂的应用。

根据抑制剂与酶作用后是否能引起酶永久性失活，酶抑制剂可分为不可逆抑制与可逆抑制，而根据抑制剂与酶结合的方式，可逆抑制中又分为竞争性抑制、非竞争性抑制、混合型抑制和反竞争性抑制。而不可逆抑制剂对酪氨酸酶永久性灭活不具调控作用，所以一般没有研究意义。所以以下我们根据可逆抑制的几种分类对酪氨酸酶抑制剂的研究概况做个综述。

1.1.2.1 竞争性抑制剂

这类抑制剂是与底物竞争，从而阻止底物与酶的结合。因酶的活性中心不能同时既与抑制剂作用，又与底物作用。竞争性抑制剂具有与底物相类似的结构，与酶形成可逆的复合物，但这类复合物不能分解成产物。酶反应速度因此下降。可以通过增加底物浓度而解除这种抑制。酪氨酸酶的这类抑制剂主要包括酚类和黄酮类等一些衍生物。

羟基苯甲酸和羟基苯甲醛对酪氨酸酶均有明显得抑制作用，表 1.1 列出我们