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硕士学位论文

肉桂醛及其衍生物对酪氨酸酶抑制作用  
及生物学效应研究

The research on tyrosinase inhibition and biological  
activity of cinnamaldehyde and its derivatives

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## 目录

主要缩略词表 .....	1
摘要.....	2
Abstract.....	4
1. 前言.....	6
1.1 酪氨酸酶研究概况 .....	6
1.1.1 酪氨酸酶概述.....	6
1.1.2 酪氨酸酶的结构.....	7
1.1.3 酪氨酸酶的催化机制.....	8
1.1.4 酪氨酸酶与黑色素的生物合成.....	9
1.2 酪氨酸酶抑制剂概述 .....	11
1.3 酪氨酸酶抑制剂的应用 .....	12
1.3.1 在农业病害防治领域的应用.....	12
1.3.2 在抗菌领域的应用.....	13
1.3.3 在食品保鲜领域的应用.....	14
1.3.4 在医疗美容领域的应用.....	14
1.4 肉桂醛的研究现状 .....	15
1.4.1 肉桂醛的概述.....	15
1.4.2 肉桂醛的应用.....	15
1.5 肉桂醛的衍生物概述 .....	16
1.6 本课题的研究内容与意义 .....	17
2. 实验试剂与仪器 .....	18
2.1 实验材料 .....	18
2.2 实验试剂 .....	18
2.3 实验仪器 .....	19

<b>3. 实验方法</b> .....	<b>21</b>
<b>3.1 效应物对蘑菇酪氨酸酶活力的抑制作用</b> .....	<b>21</b>
3.1.1 效应物对酪氨酸酶单酚酶活力的影响.....	21
3.1.2 效应物对酪氨酸酶二酚酶活力的影响.....	21
3.1.3 效应物对酪氨酸酶二酚酶的抑制机理的判断.....	21
3.1.4 效应物对酪氨酸酶二酚酶的抑制类型的判断.....	21
3.1.5 效应物对酪氨酸酶二酚酶的抑制动力学常数的测定.....	22
3.1.6 Native-PAGE 研究效应物对酪氨酸酶活力的抑制作用 .....	22
3.1.7 模拟效应物与蘑菇酪氨酸酶 PPO3 的分子对接 .....	22
<b>3.2 光谱法分析效应物对蘑菇酪氨酸酶的作用</b> .....	<b>23</b>
3.2.1 效应物对酪氨酸酶二酚酶催化 L-DOPA 氧化反应光谱的影响 .....	23
3.2.2 效应物对高碘酸钠氧化 L-DOPA 反应光谱的影响 .....	23
3.2.3 效应物与铜离子的相互作用.....	23
3.2.4 荧光淬灭法研究效应物对蘑菇酪氨酸酶的作用机理.....	23
<b>3.3 效应物对其他来源酪氨酸酶活力的影响</b> .....	<b>23</b>
3.3.1 效应物对小鼠 B16 细胞中酪氨酸酶活力的影响 .....	23
3.3.2 效应物对棉铃虫粗提多酚氧化酶 (PPO) 活力的影响 .....	24
<b>3.4 效应物对棉铃虫杀虫毒力的测定</b> .....	<b>24</b>
<b>3.5 效应物对棉铃虫体内酶活力的影响</b> .....	<b>24</b>
3.5.1 效应物对棉铃虫体内保护酶系活力的影响.....	24
3.5.2 效应物对棉铃虫中肠消化酶系活力的影响.....	25
<b>3.6 效应物对细菌抗菌活性的测定</b> .....	<b>26</b>
3.6.1 琼脂扩散法测定效应物对细菌的抑制作用.....	26
3.6.2 效应物的最小抑菌浓度 (MIC) 的测定 .....	27
3.6.3 效应物的抑菌曲线的测定.....	27
<b>4 实验结果</b> .....	<b>28</b>
<b>4.1 肉桂醛及其衍生物对蘑菇酪氨酸酶活力的抑制效应</b> .....	<b>28</b>
4.1.1 肉桂醛及其衍生物对酪氨酸酶单酚酶活力的影响.....	28

4.1.2 肉桂醛及其衍生物对酪氨酸酶二酚酶活力的影响.....	30
4.1.3 肉桂醛及其衍生物对酪氨酸酶二酚酶的抑制机理.....	31
4.1.4 肉桂醛及其衍生物对酪氨酸酶二酚酶的抑制类型.....	32
4.1.5 $\alpha$ -溴代肉桂醛对酪氨酸酶二酚酶的抑制动力学.....	34
4.1.6 $\alpha$ -氯代肉桂醛对酪氨酸酶二酚酶的抑制动力学.....	37
4.1.7 Native-PAGE 法分析肉桂醛及其衍生物对酪氨酸酶的抑制作用.....	41
4.1.8 肉桂醛及其衍生物与酪氨酸酶 PPO3 模型的分子对接研究.....	42
<b>4.2 光谱法分析肉桂醛及其衍生物对蘑菇酪氨酸酶作用机理.....</b>	<b>44</b>
4.2.1 肉桂醛及其衍生物对酪氨酸酶催化 L-DOPA 氧化反应光谱的影响.....	44
4.2.2 肉桂醛及其衍生物对高碘酸钠氧化 L-DOPA 反应光谱的影响.....	45
4.2.3 肉桂醛及其衍生物与铜离子的相互作用.....	46
4.2.4 荧光淬灭法研究肉桂醛及其衍生物对蘑菇酪氨酸酶的作用机理.....	47
<b>4.3 肉桂醛及其衍生物对其他来源酪氨酸酶活力的抑制效果.....</b>	<b>50</b>
4.3.1 肉桂醛及其衍生物对小鼠 B16 细胞中酪氨酸酶活力的影响.....	50
4.3.2 肉桂醛及其衍生物对棉铃虫粗提多酚氧化酶 (PPO) 活力的影响.....	51
<b>4.4 平板涂布饲喂法测定肉桂醛及其衍生物的杀虫效果.....</b>	<b>52</b>
4.4.1 肉桂醛对棉铃虫的杀虫效果.....	52
4.4.2 $\alpha$ -溴代肉桂醛对棉铃虫的杀虫效果.....	54
4.4.3 $\alpha$ -氯代肉桂醛对棉铃虫的杀虫效果.....	57
4.4.4 $\alpha$ -甲基肉桂醛对棉铃虫的杀虫效果.....	60
<b>4.5 肉桂醛及其衍生物对棉铃虫体内酶活力的影响.....</b>	<b>62</b>
4.5.1 肉桂醛及其衍生物对棉铃虫体内保护酶活力的影响.....	62
4.5.2 肉桂醛及其衍生物对棉铃虫中肠消化酶活力的影响.....	64
<b>4.6 肉桂醛及其衍生物的抑菌效果的研究.....</b>	<b>67</b>
4.6.1 肉桂醛及其衍生物对枯草芽孢杆菌的抑制效果.....	67
4.6.2 肉桂醛及其衍生物对金黄色葡萄球菌的抑制效果.....	68
4.6.3 肉桂醛及其衍生物对大肠杆菌的抑制效果.....	69
4.6.4 肉桂醛及其衍生物对鼠伤寒沙门氏菌的抑制效果.....	70
4.6.5 $\alpha$ -溴代肉桂醛的最小抑菌浓度 (MIC) 的测定结果.....	71

4.6.6 $\alpha$ -溴代肉桂醛对细菌生长曲线的测定结果 .....	71
<b>5. 讨论.....</b>	<b>73</b>
5.1 肉桂醛及衍生物对蘑菇酪氨酸酶的抑制作用研究 .....	73
5.2 肉桂醛及衍生物对蘑菇酪氨酸酶的抑制机制研究 .....	74
5.3 肉桂醛及衍生物对棉铃虫影响研究 .....	77
5.4 肉桂醛及衍生物对细菌的抑制研究 .....	78
<b>6. 结论及展望 .....</b>	<b>79</b>
6.1 结论 .....	79
6.2 展望 .....	80
参考文献 .....	81
在学期间发表论文及所获荣誉 .....	89
致谢.....	91

## Contents

<b>Abbreviations .....</b>	<b>1</b>
<b>Chinese abstract.....</b>	<b>2</b>
<b>English abstract.....</b>	<b>4</b>
<b>1. Introduction.....</b>	<b>6</b>
<b>1.1 General introduction of tyrosinase .....</b>	<b>6</b>
1.1.1 The review of study on tyrosinase .....	6
1.1.2 The molecular structure of tyrosinase .....	7
1.1.3 The catalytic mechanism of tyrosinase .....	8
1.1.4 Tyrosinase and Melanogenesis.....	9
<b>1.2 General introduction of tyrosinase inhibitors.....</b>	<b>11</b>
<b>1.3 The application of tyrosinase inhibitors .....</b>	<b>12</b>
1.3.1 The application for insecticides .....	12
1.3.2 The application for bactericides .....	13
1.3.3 The application for food fresh keeping .....	14
1.3.4 The application for medicines and cosmetics .....	14
<b>1.4 The research status of cinnamaldehyde.....</b>	<b>15</b>
1.4.1 General introduction of cinnamaldehyde.....	15
1.4.2 The application of cinnamaldehyde .....	15
<b>1.5 General introduction of derivatives of cinnamaldehyde.....</b>	<b>16</b>
<b>1.6 Contents and significance of this study .....</b>	<b>17</b>
<b>2. Reagents and instruments .....</b>	<b>18</b>
<b>2.1 Materials.....</b>	<b>18</b>
<b>2.2 Reagents .....</b>	<b>18</b>
<b>2.3 Instruments .....</b>	<b>19</b>



<b>3. Methods.....</b>	<b>21</b>
<b>3.1 Inhibition effects of compounds on tyrosinase.....</b>	<b>21</b>
3.1.1 Effects of compounds on monophenolase activity .....	21
3.1.2 Effects of compounds on diphenolase activity .....	21
3.1.3 Inhibition mechanism of compounds on diphenolase activity.....	21
3.1.4 Inhibition types of compounds on diphenolase activity .....	21
3.1.5 Inhibition kinetics of compounds on diphenolase activity .....	22
3.1.6 Native-PAGE electrophoresis .....	22
3.1.7 Simulate molecular docking of compounds with mushroom PPO3 .....	22
<b>3.2 Enzyme inhibition mechanism study by spectrum.....</b>	<b>23</b>
3.2.1 L-DOPA oxidation by tyrosinase at presence of compounds .....	23
3.2.2 L-DOPA oxidation by NaIO <sub>4</sub> at presence of compounds .....	23
3.2.3 Copper interaction.....	23
3.2.4 Fluorescence quenching.....	23
<b>3.3 Effects of compounds on other tyrosinase.....</b>	<b>23</b>
3.3.1 Effects of compounds on tyrosinase from B16 murine melanoma.....	23
3.3.2 Effects of compounds on tyrosinase from <i>Helicoverpa armigera</i> .....	24
<b>3.4 The insecticidal activities of compounds on <i>Helicoverpa armigera</i> .....</b>	<b>24</b>
<b>3.5 Effects of compounds on enzyme activities of <i>Helicoverpa armigera</i>.....</b>	<b>24</b>
3.5.1 Effects of compounds on protective enzyme activities.....	24
3.5.2 Effects of compounds on midgut digestive enzyme activities.....	25
<b>3.6 The antibacterial activities of compounds.....</b>	<b>26</b>
3.6.1 The antibacterial effects study by agar diffusion method .....	26
3.6.2 Minimum inhibitory concentrations of compounds.....	27
3.6.3 The bacteriostatic curves of compounds .....	27
<b>4 Results .....</b>	<b>28</b>
<b>4.1 Effects of cinnamaldehyde and derivatives on mushroom tyrosinase.....</b>	<b>28</b>
4.1.1 Effects of cinnamaldehyde and derivatives on monophenolase activity .....	28

4.1.2 Effects of cinnamaldehyde and derivatives on diphenolase activity .....	30
4.1.3 Inhibition mechanism of cinnamaldehyde and derivatives on tyrosinase ...	31
4.1.4 Inhibition types of cinnamaldehyde and derivatives on tyrosinase .....	32
4.1.5 Inhibition kinetic of $\alpha$ -bromocinnamaldehyde on tyrosinase .....	34
4.1.6 Inhibition kinetic of $\alpha$ -chlorocinnamaldehyde on tyrosinase .....	37
4.1.7 Native-PAGE electrophoresis analysis .....	41
4.1.8 Molecular docking of cinnamaldehyde and derivatives with PPO3 .....	42
<b>4.2 The results of enzyme inhibition mechanism study by spectrum .....</b>	<b>44</b>
4.2.1 L-DOPA oxidation by enzyme with cinnamaldehyde and derivatives .....	44
4.2.2 L-DOPA oxidation by $\text{NaIO}_4$ with cinnamaldehyde and derivatives .....	45
4.2.3 The interaction between copper and cinnamaldehyde and derivatives.....	46
4.2.4 Enzyme inhibition mechanism study by fluorescence quenching .....	47
<b>4.3 Effects of cinnamaldehyde and derivatives on other tyrosinase .....</b>	<b>50</b>
4.3.1 Effects on activity of tyrosinase from B16 murine melanoma .....	50
4.3.2 Effects on activity of tyrosinase from <i>Helicoverpa armigera</i> .....	51
<b>4.4 The insectidal activities of cinnamaldehyde and derivatives.....</b>	<b>52</b>
4.4.1 The insectidal activities of cinnamaldehyde .....	52
4.4.2 The insectidal activities of $\alpha$ -bromocinnamaldehyde .....	54
4.4.3 The insectidal activities of $\alpha$ -chlorocinnamaldehyde .....	57
4.4.4 The insectidal activities of $\alpha$ -methylcinnamaldehyde.....	60
<b>4.5 Effects of cinnamaldehyde and derivatives on enzyme activities of</b> <b><i>Helicoverpa armigera</i> .....</b>	<b>62</b>
4.5.1 Effects on protective enzyme activities .....	62
4.5.2 Effects on midgut digestive enzyme activities.....	64
<b>4.6 The antimicrobial effects of cinnamaldehyde and derivatives .....</b>	<b>67</b>
4.6.1 The antimicrobial effects on <i>Bacillus subtilis</i> .....	67
4.6.2 The antimicrobial effects on <i>Staphylococcus aureus</i> .....	68
4.6.3 The antimicrobial effects on <i>Escherichia Coil</i> .....	69
4.6.4 The antimicrobial effects on <i>Salmonella typhimurium</i> .....	70
4.6.5 Minimum inhibitory concentrations of $\alpha$ -bromocinnamaldehyde .....	71

4.6.6 The bacteriostatic curves of $\alpha$ -bromocinnamaldehyde .....	71
<b>5. Discussion.....</b>	<b>73</b>
<b>5.1 Effects of cinnamaldehyde and derivatives on mushroom tyrosinase .....</b>	<b>73</b>
<b>5.2 Inhibition mechanism of cinnamaldehyde and derivatives on tyrosinase...</b>	<b>74</b>
<b>5.3 Effects of cinnamaldehyde and derivatives on <i>Helicoverpa armigera</i> .....</b>	<b>77</b>
<b>5.4 Antimicrobial effects of cinnamaldehyde and derivatives .....</b>	<b>78</b>
<b>6. Conclusion and prospect .....</b>	<b>79</b>
<b>6.1 Conclusion.....</b>	<b>79</b>
<b>6.2 Prospect .....</b>	<b>80</b>
<b>Reference.....</b>	<b>81</b>
<b>Publications .....</b>	<b>89</b>
<b>Acknowledgment.....</b>	<b>91</b>

## 主要缩略词表

英文简称	英文全称	中文全称
AMY	amylase	淀粉酶
CAT	catalase	过氧化氢酶
CPBS	citrate buffer	柠檬酸盐缓冲液
DMSO	dimethyl sulfoxide	二甲基亚砷
DNS	3,5-Dinitrosalicylic acid	3,5-二硝基水杨酸
FC	Folin-Ciocalteu	福林酚
$IC_{50}$	50% inhibiting concentration	半抑制浓度
$K_m$	Michaelis-Menten constant	米氏常数
L-DOPA	L-3,4-dihydroxyphenylalanine	L-3,4-二羟基苯丙氨酸
LIP	lipase	脂肪酶
L-Tyr	L-tyrosine	L-酪氨酸
MIC	minimum inhibitory concentration	最小抑菌浓度
MOE	molecular operating environment	药物研发可视化仿真环境
PBS	phosphate buffer	磷酸缓冲液
PPO	polyphenol oxidase	多酚氧化酶
SDS	sodium dodecyl sulfonate	十二烷基磺酸钠
TG	triglyceride	甘油三酯
T-PRO	total protease	总蛋白酶
$V_m$	maximum velocity	最大反应速率

## 摘要

酪氨酸酶 (EC 1.14.18.1) 是一种含铜的氧化还原酶, 广泛分布于微生物, 动物, 植物和人体中, 是生物体生命活动的关键酶。若人体中酪氨酸酶异常表达能引起雀斑和黑色素沉积等皮肤病, 且其在昆虫的蜕皮及果蔬褐变中起了关键的作用, 因此酪氨酸酶抑制剂的研究引起了广泛的关注。肉桂醛主要存在于天然樟科植物桂皮中, 在农业、医药上具有重要的作用。

本文首先以蘑菇酪氨酸酶为对象, 研究了肉桂醛及其  $\alpha$  位 C 上取代的三种衍生物:  $\alpha$ -溴代肉桂醛、 $\alpha$ -氯代肉桂醛和  $\alpha$ -甲基肉桂醛对酶的抑制作用, 结果表明四者抑制 50% 单酚酶活性的浓度 ( $IC_{50}$ ) 分别为 1.210, 0.075, 0.140, 0.440 mmol/L; 对二酚酶的  $IC_{50}$  分别为 0.160, 0.040, 0.110, 0.450 mmol/L。在对二酚酶的抑制类型上, 肉桂醛和  $\alpha$ -氯代肉桂醛为非竞争型抑制, 抑制常数分别为 0.163 和 0.116 mmol/L;  $\alpha$ -溴代肉桂醛为混合型抑制,  $K_I$  和  $K_{IS}$  分别为 0.044 和 0.063 mmol/L;  $\alpha$ -甲基肉桂醛为反竞争抑制类型, 抑制常数为 0.194 mmol/L。接着采用邹氏方法研究抑制效果较好的  $\alpha$ -溴代肉桂醛和  $\alpha$ -氯代肉桂醛对酪氨酸酶二酚酶的抑制作用动力学, 构建动力学模型, 测定抑制剂与游离酶 (E) 和酶-底物复合物 (ES) 结合的微观速度常数。

我们进一步采用紫外可见光谱、荧光猝灭、铜离子相互作用和计算机模拟分子对接技术探讨肉桂醛及其衍生物对蘑菇酪氨酸酶的抑制机理。结果发现四种化合物均能与酶活性中心重要的氨基酸残基结合, 改变酶活性中心的微环境, 虽不与双铜离子相互作用, 也能有效的降低酶的催化活力。同时, 还能够与 L-DOPA 氧化的产物结合, 形成无色复合物, 造成黑色素产量的降低, 从而表现出抑制酶活性的现象。

酪氨酸酶在昆虫体内参与表皮黑化, 骨针形成, 伤口愈合以及对外源病原体的包被吞噬等重要生命活动。本论文以棉铃虫为对象, 研究肉桂醛及其衍生物对棉铃虫生长发育及体内保护酶和消化酶的影响, 结果表明  $\alpha$ -溴代肉桂醛和  $\alpha$ -氯代肉桂醛对棉铃虫生长有良好的抑制作用, 且能抑制虫体内保护酶和消化酶的活力。而肉桂醛、 $\alpha$ -甲基肉桂醛对虫的生长发育没有影响, 两者能在一定程度上抑制虫体内的保护酶活力, 但对消化酶没有抑制。

黑色素能保护细菌的细胞的孢子免受紫外线的伤害,还能螯合重金属离子以消除重金属离子对细菌细胞的伤害。本论文以枯草芽孢杆菌、金黄色葡萄球菌、大肠杆菌和鼠伤寒沙门氏菌为研究对象,测定肉桂醛及其衍生物对这四种菌的抑制效应。结果表明, $\alpha$ -溴代肉桂醛对供试菌的抑制效果最优,且革兰氏阳性菌对其更为敏感;其次是 $\alpha$ -氯代肉桂醛;再次是肉桂醛;而 $\alpha$ -甲基肉桂醛几乎没有抑菌效果。

综上,通过本课题的研究,发现了 $\alpha$ -溴代肉桂醛同时具备良好的抑制酪氨酸酶效果和杀虫抑菌作用,在生物农药及果蔬保鲜剂的等方面具有潜在的应用价值。

**关键词:** 酪氨酸酶; 肉桂醛及其衍生物; 棉铃虫; 微生物

## Abstract

Tyrosinase (EC 1.14.18.1), a copper containing enzyme, is widely distributed in microorganisms, animals, plants and human beings. It is the key enzyme in the vital function of biology. In human, tyrosinase is responsible for skin pigmentation abnormalities, such as flecks and defects. Furthermore, tyrosinase causes browning in vegetables, fruits and mushrooms. Therefore, The inhibitors of tyrosinase have aroused the widespread interest.

The inhibitory effects of cinnamaldehyde,  $\alpha$ -bromocinnamaldehyde,  $\alpha$ -chlorocinnamaldehyde and  $\alpha$ -methylcinnamaldehyde on the activity of mushroom tyrosinase were investigated. For monophenolase activity, the inhibitor concentrations leading to 50% activity lost ( $IC_{50}$ ) of cinnamaldehyde,  $\alpha$ -bromocinnamaldehyde,  $\alpha$ -chlorocinnamaldehyde and  $\alpha$ -methylcinnamaldehyde were 1.210, 0.075, 0.140 and 0.440 mmol/L. For diphenolase activity, the  $IC_{50}$  of them were 0.160, 0.040, 0.110 and 0.450 mmol/L respectively. Cinnamaldehyde and  $\alpha$ -chlorocinnamaldehyde displayed a noncompetitive inhibitory type, the inhibition constant were determined to be 0.163 and 0.116 mmol/L, respectively.  $\alpha$ -bromocinnamaldehyde was mixed type inhibitor of tyrosinase, the value of  $K_I$  and  $K_{IS}$  were 0.044 and 0.063 mmol/L, respectively.  $\alpha$ -methylcinnamaldehyde displayed an uncompetitive mechanism, the inhibition constant was determined to be 0.194 mmol/L. The kinetic method of the substrate reaction described by Tsou was used to the inhibition of the enzyme by  $\alpha$ -bromocinnamaldehyde and  $\alpha$ -chlorocinnamaldehyde, the microscopic rate constants for the reaction of these inhibitors with free enzyme and the enzyme-substrate complex were determined.

The molecular inhibition mechanisms of tyrosinase by cinnamaldehyde and its derivatives were investigated by UV-scanning study, fluorescence quenching, copper interaction and molecular docking as well. The results implied that cinnamaldehyde and its derivatives could not form metal interactions with the copper ions of the enzyme, whereas could interact with the amino acid residues of active site center. Moreover, they could decreased the formation of *o*-quinones.

In insects, tyrosinase plays important roles in normal developmental processes, such as cuticular tanning, scleration, wound healing, production of opsonins, encapsulation and nodule formation for defense against foreign pathogens. In this study, we researched the insecticidal activity of cinnamaldehyde,  $\alpha$ -bromocinnamaldehyde,  $\alpha$ -chlorocinnamaldehyde and  $\alpha$ -methylcinnamaldehyde on *Helicoverpa armigera*, and determined activities of protective and digestive enzymes in insect. The results showed that  $\alpha$ -bromocinnamaldehyde and  $\alpha$ -chlorocinnamaldehyde could inhibit insectival growth and the activity of protective and digestive enzymes.

In the bacterium, melanins can protect the bacterial cells and spores against UV radiation. Meanwhile melanins can bind heavy metals that are toxic to the cells. In this study, we investigated the antibacterial effects of cinnamaldehyde,  $\alpha$ -bromocinnamaldehyde,  $\alpha$ -chlorocinnamaldehyde and  $\alpha$ -methylcinnamaldehyde on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia Coil* and *Salmonella typhimurium*. We found that  $\alpha$ -bromocinnamaldehyde was most effective against these bacteria.

In summary,  $\alpha$ -bromocinnamaldehyde had strong anti-tyrosinase activity to prevent browning of fruits and vegetables. Moreover, it had significant inhibition against all tested bacteria including  $G^+$  and  $G^-$  bacteria to avoiding the mildew. In conclusion,  $\alpha$ -bromocinnamaldehyde could be widely used in biopesticide.

**Key words:** Tyrosinase; cinnamaldehyde and derivatives; *Helicoverpa armigera*; microorganism.



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