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硕 士 学 位 论 文

天然防污活性物质的筛选及评价

Isolation and Evaluation of Natural Product Antifoulants

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摘要

随着国际社会对环境问题的日益关注及有机锡防污剂最终使用期限的日益接近, 高效环保型海洋防污涂料成为发展重点, 其中天然防污活性物质的筛选是研制高效环保型海洋防污涂料的基础。本研究主要结果如下:

1. 通过防污活性筛选, 对大戟科植物变叶木 (*Codiaeum variegatum*) 和一种南海海绵 (*Axinellidae sp.*) 进行分离纯化, 得到 6 个天然防污活性化合物, 分别是 5-十四碳烯酸-2,3 二羟基丙基酯、茄尼醇、十一异戊烯醇、十二异戊烯醇、2-吡啶羧酸-4-羟基甲基酯、2-hydroxynephtenol, 其中 2-吡啶羧酸-4-羟基甲基酯首次从天然产物中分离得到。除茄尼醇外, 其他 5 个化合物均为首次报道有防污活性。

2. 采用 3 种防污检测模型, 即沙筛贝防污模型、翡翠贻贝防污模型和希瓦氏菌属 (*Shewanella sp.*) 污损菌防污模型对变叶木和南海海绵 (*Axinellidae sp.*) 萃取组分进行防污活性检测及评价。检测结果如下。

2.1 变叶木粗提物石油醚层(A)、二氯甲烷层(B)、乙酸乙酯层(C)和水层(D)4 个萃取组分中, 石油醚层和二氯甲烷层防污活性最好。在沙筛贝防污模型中, EC_{50} 分别是 95.90ug/ml 和 16.30ug/ml; 在翡翠贻贝防污模型中, EC_{50} 分别是 83.00ug/ml 和 16.29ug/ml; 在 *Shewanella sp.* 菌防污模型中, 石油醚层 EC_{50} 是 16.30ug/ml, 二氯甲烷层无法测出。水层在三种防污模型中均未发现防污活性。

2.2 在南海海绵 (*Axinellidae sp.*) 3 个萃取组分中, 石油醚层 (E) 和二氯甲烷层 (F) 具有防污活性, 在沙筛贝防污模型中 EC_{50} 分别是 16.05ug/ml 和 108.58ug/ml; 翡翠贻贝防污模型中, 石油醚层 EC_{50} 小于 5ug/ml, 二氯甲烷层 EC_{50} 是 69.08ug/ml。水层在三种防污模型中均未发现防污活性。

3. 本研究中, 采用 3 种防污模型对分离纯化的化合物进行防污活性检测及评价。

3.1. 从变叶木植物中分离得到 5-十四烯酸-2,3-二羟基丙基酯、茄尼醇、十一异戊烯醇、十二异戊烯醇 4 个化合物。5-十四烯酸-2,3-二羟基丙基酯在沙筛贝模型没有防污活性, 在翡翠贻贝防污模型中 EC_{50} 为 40.12ug/ml, 在 *Shewanella*

*sp.*菌防污模型中 50ug/ml 浓度下有显著抑制作用；茄尼醇在沙筛贝模型和翡翠贻贝模型中未发现防污活性，在 *Shewanella sp.*菌防污模型中 50ug/ml 浓度下有显著抑制作用。十一异戊烯醇在沙筛贝防污模型中没有抑制活性，在翡翠贻贝防污模型和 *Shewanella sp.*菌防污模型中 50ug/ml 浓度下有显著性抑制作用；十二异戊烯醇在沙筛贝防污模型和翡翠贻贝防污模型中未发现防污活性，在 *Shewanella sp.*菌防污模型中 EC₅₀ 是 13.19ug/ml。4 种化合物死亡率均为 0。

3.2 从南海海绵 (*Axinellidae sp.*) 分离得到 2-吡啶羧酸-4-羟基甲基酯和 2-hydronephtenol。2-吡啶羧酸-4-羟基甲基酯在沙筛贝防污模型和翡翠贻贝防污模型中 EC₅₀ 分别是 19.54ug/ml 和 15.78ug/ml，在 *Shewanella sp.*菌防污模型中未发现防污活性；2-hydronephtenol 在沙筛贝防污模型和翡翠贻贝防污模型中 EC₅₀ 分别是 9.62ug/ml 和 7.49ug/ml，在 *Shewanella sp.*菌防污模型中未发现防污活性。死亡率统计，2 个化合物在翡翠贻贝防污模型 50ug/ml 浓度下死亡 1 只，该浓度实验组死亡率为 10%。

4.初步分析了化合物构效关系。多聚戊烯醇类化合物对 *Shewanella sp.*菌防污活性更好，提示其可能对抗微型污损方面更有效。在翡翠贻贝防污模型中多聚戊烯醇类化合物防污活性差异性提示异戊二烯聚合的个数会影响防污活性。

综上所述，通过防污活性筛选，从大戟科植物变叶木和从南海海绵 (*Axinellidae sp.*) 中分离纯化得到 6 个天然防污活性化合物，这些化合物具有开发成安全环保型防污物质的潜力。

关键词：天然防污产物；变叶木；南海海绵

Abstract

Along with the increasing concern about environment from the international society and the deadline of the ban on the usage of organotin compounds as antifoulants being increasingly close, the efficient and environmentally friendly antifouling coating has become important to be developed. The development of the efficient and environmentally friendly antifouling coating is based on the researches of natural product antifoulants. In the present study, The main results were shown as followed.

1. Through screening of antifouling activity, six natural product antifoulants were isolated by *Codiaeum variegatum* (*Euphorbiaceae*) and China South Sea marine sponge *Axinellidae sp.*, namely 5-Tetradecenoic acid, 2,3-dihydroxypropyl ester, solanesol, decaprenyl alcohol, undecaprenyl alcohol, 2-Pyridinecarboxylic acid, 4-hydroxy-, methyl ester, 2-hydroxynephtenol. 2-Pyridinecarboxylic acid, 4-hydroxy-, methyl ester was first isolated from natural products. In addition to solanesol, other five compounds were found to have antifouling activity for the first time.

2. By using three kinds of antifouling detection models, namely *Mytilopsis sallei* *Reeluz* antifouling detection model, *Perna viridis* antifouling detection model and *Shewanella sp.* antifouling detection model, components extracted from *Codiaeum variegatum* and China South Sea sponge *Axinellidae sp.* were to be tested and evaluated the antifouling activity.

2.1 Of four extracts from *Codiaeum variegatum*, Petroleum ether extract and Dichloromethane extract showed highest antifouling activity with the values of EC_{50} respectively 95.90ug/ml and 16.30ug/ml in *Mytilopsis sallei* *Reeluz* antifouling detection model; with the values of EC_{50} respectively 83.00ug/ml and 16.29ug/ml in *Perna viridis* antifouling detection model. In *Shewanella sp.* antifouling detection model, petroleum ether extract EC_{50} was 16.30ug/ml, dichloromethane extract EC_{50}

was not measured. The aqueous extract of three models was not found the antifouling activity.

2.2 Of three extracts from China South Sea sponge *Axinellidae sp.*, Petroleum ether extract and dichloromethane extract showed antifouling activity with the values of EC_{50} respectively 16.05ug/ml and 108.58ug/ml in *Mytilopsis sallei Reeluz* antifouling detection model; with the values of EC_{50} respectively lower than 5ug/ml and 69.08ug/ml in *Perna viridis* antifouling detection model. The aqueous extract of three models was not found the antifouling activity.

3. In the present study, three antifouling detection models were to test and evaluate the antifouling activity of natural compounds isolated and purified from *Codiaeum variegatum* and China South Sea sponge *Axinellidae sp.*

3.1 5-Tetradecenoic acid, 2,3-dihydroxypropyl ester, solanesol, decaprenyl alcohol, undecaprenyl alcohol were isolated and purified from *Codiaeum variegatum*. 5-Tetradecenoic acid, 2,3-dihydroxypropyl ester showed no antifouling activity in *Mytilopsis sallei Reeluz* antifouling detection model; showed antifouling activity with the value of EC_{50} 40.12ug/ml in *Perna viridis* antifouling detection model; under 50ug/ml concentrations significantly inhibited fouling in *Shewanella sp.* antifouling detection model. Solanesol show no antifouling activity in *Mytilopsis sallei Reeluz* antifouling detection model; under 50ug/ml concentrations significantly inhibited fouling in *Shewanella sp.* antifouling detection model. decaprenyl alcohol showed no antifouling activity in *Mytilopsis sallei Reeluz* antifouling detection model; under 50ug/ml concentrations significantly inhibited fouling in *Perna viridis* antifouling detection model and *Shewanella sp.* antifouling detection model. undecaprenyl alcohol showed no antifouling activity in *Mytilopsis sallei Reeluz* antifouling detection model and *Perna viridis* antifouling detection model; show antifouling with the value of EC_{50} 13.19ug/ml. The mortality of four compounds was 0.

3.2 2-Pyridinecarboxylic acid, 4-hydroxy-, methyl ester, 2-hydroxynephtenol were isolated and purified from China South Sea sponge *Axinellidae sp.* 2-Pyridinecarboxylic acid, 4-hydroxy-, methyl ester showed antifouling activity with the values of EC_{50} 19.54ug/ml and 15.78ug/ml in *Mytilopsis sallei Reeluz* antifouling

detection model and *Perna viridis* antifouling detection model; showed no antifouling activity in *Shewanella sp.* antifouling detection model. 2-hydroxynephtenol showed antifouling activity with the values of EC₅₀ 9.62ug/ml and 7.49ug/ml, showed no antifouling activity in *Shewanella sp.* antifouling detection model. The mortalities of them were 10% under 50ug/ml concentrations in *Perna viridis* antifouling detection model.

4. Preliminary analysis of the compound structure-activity relationships had been taken. MultiPolyprenols compounds show higher antifouling activity in *Shewanella sp.* antifouling detection model, suggesting that they may more effective against micro-fouling. The differences of antifouling activity in *Perna viridis* antifouling detection model recommended that the number of isoprene may affect antifouling activity.

In summary, through the screening of antifouling activity, six natural product antifoulants were isolated and purified from *Codiaeum variegatum* and China South Sea sponge *Axinellidae sp.* These compounds may developed into safe environmentally friendly antifouling material.

keywords: Natural Product Antifoulant; *Codiaeum Variegatum*; China South Sea Sponge

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厦门大学博硕士学位论文摘要库

第一章 绪论

1.1 海洋生物污损的危害及防污技术研究

1.1.1 海洋污损生物

我们生活在一个美丽的地球，它色彩斑斓，其中约四分之三的蓝色代表了海洋。海洋孕育了人类，人类在诞生之日起就与海洋相依相成。随着人类海洋活动的增加，海洋污损生物的危害越来越受到人类的重视。海洋污损生物指附着生殖在船底、水下管道、石油平台、渔业的网具及其他一切海中人为设施表面的有害生物，是海洋生物的特殊生态类群。目前，世界上已报道的海洋污损生物超过 4000 余种^[1]，其中中国海域已有记录的污损生物就有 2000 多种。随着相关研究的深入，海洋污损生物的种类会进一步增加。目前常见的海洋污损生物类群有海洋底栖细菌、海洋底栖硅藻、海洋大型藻类、龙介虫、双壳类、藤壶、海鞘以及海绵动物、腔肠动物、苔藓动物等^[2]。

本文中防污模型采用了污损生物沙筛贝、翡翠贻贝和希瓦氏菌属 (*Shewanella sp.*)。沙筛贝学名 *Mytilopsis sallei* Reeluz，属帘蛤目 *Beneroidae*，饰贝科 *Dreissenidae*，中文异名萨氏仿贻贝，英文名 *False Mussel, Zebra Mussel*。在厦门，沙筛贝在 20 世纪 90 年代初就已是污损生物群落的优势种^[3]。沙筛贝生命力和繁殖力极强且生长迅速，常发现于浅海水域（如码头、海湾和海堤）和养殖水域。在浅海水域成群落附着在船底，码头岸边；在养殖水域，会与养殖的贝类争夺附着基和饲料以及生活空间，导致养殖贝类减产。同时沙筛贝的大量繁殖，其排泄物增加了有机物污染^[4, 5]，也会导致水体缺氧，是一种需引起高度重视的污损生物。

翡翠贻贝学名 *Perna viridis* (Linnaeus)，属贻贝目 *Mytiloida*，壳菜蛤科 *Mytilidae*，是海洋污损生物中的主要优势物种，其特有的足丝可以牢固地胶黏在各种固体表面，如渔网、航标以及地下管道等，具有分布广、生长快和繁殖能力强的特点，是一种极难控制又造成极大危害的污损生物。翡翠贻贝能与本地贝类争夺附着基，携带的病毒和寄生虫会对本地物种造成一定的危

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