CHEMISTRY OF HALOGENATED SECONDARY METABOLITES OF *Laurencia nipponica* (*RHODOPHYCEAE, CERAMIALES*)

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CHEMISTRY OF HALOGENATED SECONDARY METABOLITES OF *Laurencia nipponica* (*RHODOPHYCEAE, CERAMIALES*)

CHENG YEN SEE

THIS DISSERTATION IS SUBMITTED TO FULFILL THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

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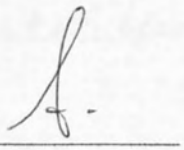
UNIVERSITY MALAYSIA SABAH

MARCH 2005

DECLARATION

I declare that this thesis contains my original research work. Sources of findings reviewed herein have been duly acknowledged.

30 MARCH 2005



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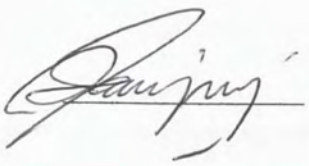

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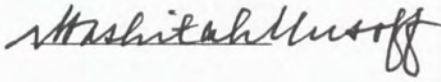

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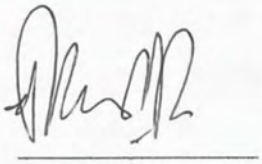

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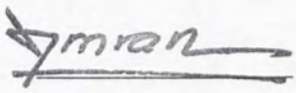

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ABSTRACT

Red algae genus *Laurencia*, particularly *Laurencia nipponica* is known as a profile producer of halogenated secondary metabolites. This investigation delves upon; 1) comparative chemistry of *Laurencia nipponica* collected from Japan and Russia, and 2) chemical correlation between the Japanese *Laurencia* with its epiphytes. In the first study, results indicate that the Japanese *Laurencia* contained three halogenated metabolites; pacifenol (**1**, **2**, **4**, **7**), prepacifenol (**3**, **6**) and 2,10-dibromo-3-chloro-α-chamigrene (**5**, **8**), are of the known prepacifenol race but Russian *Laurencia* does not contain prepacifenol. Therefore, the Russian *Laurencia* is suggested to be of a new race called pacifenol race. In the second investigation *Laurencia nipponica* growing in Muroran, Japan, was known to be infected by epiphytes known as *Janczewskia tokidae*. Authority, chemical comparison showed that similar halogenated metabolites; pacifenol (**1**, **2**, **4**, **7**), prepacifenol (**3**, **6**) and 2,10-dibromo-3-chloro-α-chamigrene (**5**, **8**), were present in the epiphytes as the host. Other members of *Janczewskia* are not known to contain halogenated metabolites. Therefore, this phenomenon could due to possible transfer of genomic materials that could have prompted the epiphytes to produce such secondary metabolites.

ABSTRAK

Alga merah genus *Laurencia*, khususnya *Laurencia nipponica* adalah dianggap sebagai penghasil profil sebatian sekunder berhalogen. Kajian ini selidiki ke atas; 1) perbandingan secara kimia bagi *Laurencia nipponica* yang dikumpul dari Jepun dan Rusia, dan 2) perhubungan kait secara kimia di antara *Laurencia nipponica* dari Jepun dengan epifitnya. Dalam kajian pertama, keputuasan telah menunjukkan bahawa *Laurencia* dari Jepun mengandungi tiga sebatian sekunder berhalogen; pacifenol (**1**, **2**, **4**, **7**), prepacifenol (**3**, **6**) dan 2,10-dibromo-3-chloro-α-chamigrene (**5**, **8**), yang dikategori dalam siri prepacifenol. Akan tetapi, *Laurencia* dari Rusia tidak mengandungi prepacifenol. Oleh sebab itu, *Laurencia* dari Rusia dicadangkan dikategori dalam satu siri yang baru iaitu siri pacifenol. Dalam analisi kedua pula, *Laurencia nipponica* yang tumbuh di Muroran, Japan telah dijangkiti oleh epifit yang dinamakan *Janczewskia tokidae*. Autoriti, perbandingan kimia menunjukkan bahawa terdapat sebatian berhalogen yang sama iaitu pacifenol (**1**, **2**, **4**, **7**), prepacifenol (**3**, **6**) dan 2,10-dibromo-3-chloro-α-chamigrene (**5**, **8**) di dalam epifit. Anggota *Janczewskia* yang lain adalah tidak mengandungi sebatian berhalogen tersebut. Oleh itu, fenomenon ini adalah mungkin disebabkan oleh pemindahan bahan genomic yang memcepatkan epifit menghasilkan sebatian sekunder berhalogen tersebut.

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LIST OF ABBREVIATION AND SYMBOLS

MeOH	Methanol
Na_2SO_4	Sodium sulphate
TLC	Thin layer chromatography
UV	Ultraviolet
R_f	Mobility relative to front
EtAc	Ethyl acetate
PTLC	Preparative thin layer chromatography
NMR	Nuclear magnetic resonance
$CDCl_3$	Chloroform-deuterated
TMS	Tetramethylsilane
OH	Hydroxide
Br	Bromine
Cl	Chlorine
mg	Milligram
L	Liter
°C	Degree Celsius
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid

CHAPTER 1

PERFACE

1.1 INTRODUCTION

The marine genus *Laurencia* (division Rhodophyta, class Rhodophyceae, subclass Florideophyceae, order Ceramiales, family Rhodomelaceae) was described by Lamouroux in 1813 (Erickson, K.L., 1983). The red algal genus *Laurencia* is unique due to its ability to produce a wide variety of halogenated secondary metabolites with diverse structural features depending on the species and localities (Vairappan *et al.*, 2001).

The red alga *Laurencia nipponica* is unique in the various biosynthesis of halogenated secondary metabolites. This species produces some 80 diverse, halogenated secondary metabolites (Fukuzawa *et al.*, 1990). Halogenated secondary metabolites are considered to be useful taxonomic markers at the species level.

It has been found that the constituents of *Laurencia nipponica* are greatly dependent upon the growth localities. The compounds mainly differentiate between the specimens which are collected either in the cold current regions or in the warm current

regions as major components. The populations of this alga growing in close proximity occasionally contain completely different compound as a major secondary metabolite (Fukuzawa and Kurosawa, 1980). This revealed that *Laurencia nipponica* consists of many kinds of sibling species which could be distinguished chemically but not taxonomically from the other populations (Fukuzawa *et al.*, 1990). When a specific species of *Laurencia* produces diverse varieties of halogenated metabolites due to its geographical location, it is referred to as chemical races.

There are seven types of chemical races in *Laurencia nipponica*: prepacifenol, halochamigrene epoxide, laurencin, laureatin, isoprelaurefucin, epilaurallene and kumausallene races. The prepacifenol and halochamigrene epoxide are grouped in the sesquiterpenoid group; whereas the laurencin, laureatin, isoprelaurefucin, epilaurallene and kumausallene races are grouped in the C_{15} bromoether group (Masuda *et al.*, 1997).

This investigation can be divided into two parts:

First, the experiments involve chemical analyses of *Laurencia nipponica* collected from Yablo and Tangi of Russia through Dr. Abe, a researcher in Japan. Results from this study can help to verify the chemical race of *Laurencia nipponica*.

Second, this investigation involves chemical correlation of *Laurencia nipponica* collected from Muroran Hokkaido with the epiphytes. These epiphytes will attach to its surface.

Some seaweed has been infected by epiphytes which grow on the host. Epiphytes are plants which by nature grow on other plants. Most epiphytes do not harm the host but use them merely as surfaces to attach themselves to.

Recently it has come to our attention that *Laurencia nipponica* prepacifenol race is being affected by the epiphytes, *Janczewskia tokidae* ("*Sozomakura*" in Japan), in Japan. The effects of these epiphytes, *Janczewskia tokidae*, on *Laurencia nipponica* may be beneficial or detrimental or parasitic.

The present investigation was initiated as a result. The sample of epiphytes, *Janczewskia tokidae*, which are grown on the *Laurencia nipponica* was collected to study the correlation between the host red alga, *Laurencia nipponica* especially the prepacifenol race, and the epiphytes.

Based on the unique characteristic of *Laurencia nipponica*, it would be interesting to study and identify chemical races in *Laurencia nipponica* from Russia and Japan. Besides the prepacifenol races, a new chemical race may be discovered.

1.2 RESEARCH OBJECTIVES

1.2.1 Investigation 1:

The objectives of the first part of the investigation are as follows,

1.2.1.1 To extract and study the chemical profile of halogenated secondary metabolites in *Laurencia nipponica.*

1.2.1.2 To identify the halogenated secondary metabolites by correlating the spectral data of ^{1}H-NMR spectra.

1.2.1.3 To calculate and quantity compounds in *Laurencia nipponica.*

1.2.2 Investigation 2:

The objectives of the second part of the investigation are as follows,

1.2.2.1 To extract and study the chemical profile of halogenated secondary metabolites in the epiphytic red alga, *Janczewskia tokidae.*

1.2.2.2 To identify the halogenated secondary metabolites by correlating the spectral data of ^{1}H-NMR spectra.

1.2.2.3 To deduce any possible correlation between host red alga with epiphytes *Janczewskia tokidae.*

CHAPTER 2

LITERATURE REVIEW

2.1 *Laurencia*

The marine genus *Laurencia* (division Rhodophyta, class Rhodophyceae, subclass Florideophyceae, order Ceramiales, family Rhodomelaceae) was described by Lamouroux in 1813 (Erickson, K.L., 1983). There are diverse halogenated or non-halogenated secondary metabolites were produced in species of the genus *Laurencia* (Rhodomelaceae, Rhodophyta) (Masuda *et al.*, 1997). The metabolites have been classified into four groups: sesquiterpenoid, diterpenoid, triterpenoid, and C_{15} bromoether (also called C_{15} nonterpenoid). Howard *et al.* (1980) had shown that the chemistries of several *Laurencia* species remained the same under varying field and culture conditions.

Laurencia producing diverse secondary metabolites may include several different species or varieties, and their taxonomic status demands reexamination at the morphological level (Masuda *et al.*, 1997). A "species" of *Laurencia* may produce diverse secondary metabolites. It is because of the differences of entity which were morphologically similar to but distinct from the identified "species", for example

Laurencia pacifica Kylin in the Gulf of California which was investigated by Fenical and Norris (1975). Besides, it may also be caused by the distribution of several sibling species that are morphologically indistinct along the same site of the "species". Sometimes, misidentification of a species may also be one of the reason to show that a species of *Laurencia* produce diverse secondary metabolites. The North American species, *Laurencia pacifica,* is morphologically identical but have chemically distinct populations (Horward *et al.*, 1980).

Irie *et al.* (1965, 1968) reported the isolation of a compound containing bromine from *Laurencia glandulifera*, Kützing, which was designated as laurencin.

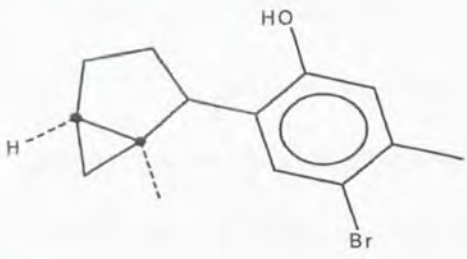
Fenical and Norris (1975) have shown that the three forms of *Laurencia pacifica* from the Gulf of California were in fact three separate species. They concluded that none of the Gulf of California species investigated by TLC with *Laurencia pacifica* Kylin which contains prepacifenol.

Sims *et al.* (1971) have reported that there were significant differences of the structures of secondary metabolites isolated from marine organisms compared to those isolated from terrestrial organisms. For further investigation of *Laurencia*, Sims *et al.* (1971) have isolated a known compound, laurinterol (**1**) from *Laurencia.*

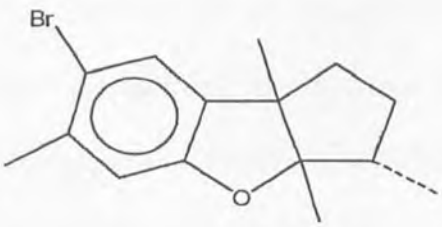
Sims *et al.* (1971) were interested to discover natural halogenated compounds in *Laurencia* and some sponges. They believed that the sea is full of natural metabolites

with mechanisms to detoxify toxic compounds. Halogenated compounds common or closely related to those of *Laurencia* have been isolated from sea hares, which were consistent with the observations that sea hares feed on *Laurencia* species producing such metabolites (Masuda *et al.*, 1997). Aplysin (**2**) was isolated from sea hares that ate *Laurencia* (Sims *et al.*, 1971). This compound can also be isolated from *Laurencia.* Furthermore, sea urchins also feed on *Laurencia nipponica* (Abe *et al.*, 1990).

The probable precursor of pacifenol, prepacifenol, was isolated from *Laurencia filiformis* by Sims *et al.* (1973). They found that the marine algae of the genus *Laurencia* were first noted for producing terpenoids containing bromine (Sims *et al.*, 1973). Besides, they also found that pacifenol (**3**) (Sims *et al.*, 1971) does not exist as a neutral product in *Laurencia tamasnica.* Pacifenol is the first natural compound isolated which contains bromine and chlorine (Sims *et al.*, 1971).



(**1**)



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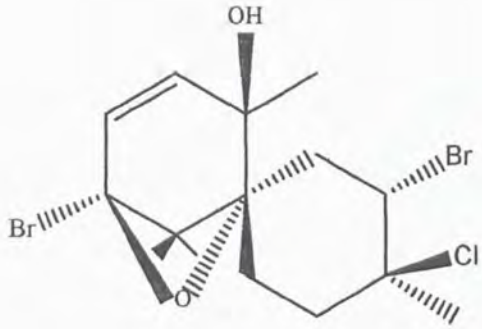


(**3**)

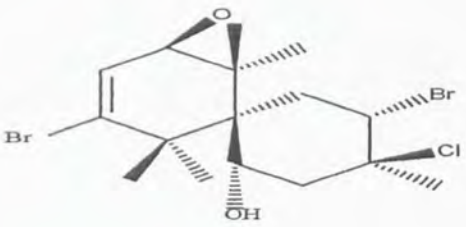
2.2 *Laurencia nipponica*

The marine red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales) have similar morphology, but is chemically distinct. This species produces some 80 diverse halogenated secondary metabolites (Fukuzawa *et al.*, 1990).

Halogenated C_{15} bromoethers, which have never been found in other species of *Laurencia* were isolated from *Laurencia nipponica* and the species characterized chemotaxonomically (Abe *et al.*, 1997). This special characteristic has allowed the analysis of a highly meaningful, dynamic picture of the population system to which the species belong to reflect the steps of biosynthetic diversification (Abe *et al.*, 1999). The differentiation of such races provides the opportunity for analysis of macromolecular based techniques (Abe *et al.*, 1999). Besides, similar to other species of *Laurencia*, *Laurencia nipponica* also produces a variety of sesquiterpenoids.

2.2.1 Chemical Races in *Laurencia nipponica*

The red alga *Laurencia nipponica* is known to contain several chemical races. Each of the chemical races is characterized in particular, by major halogenated secondary metabolites (Abe *et al.* 1997). There were seven types of chemical races in *Laurencia nipponica*: prepacifenol (**4**), halochamigrene epoxide (**5**), laurencin (**6**), laureatin (**7**), isoprelaurefucin (**8**), epilaurallene (**9**) and kumausallene (**10**) races. Two of these races, the prepacifenol and halochamigrene epoxide are grouped in the sesquiterpenoid group; whereas the laurencin, laureatin, isoprelaurefucin, epilaurallene and kumausallene races are grouped in the C_{15} bromoether group (Masuda *et al.*, 1997).



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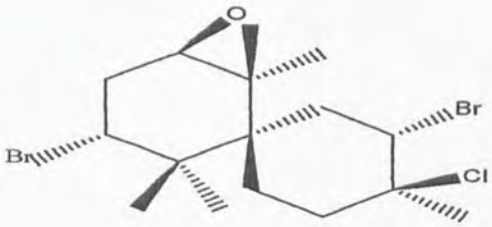
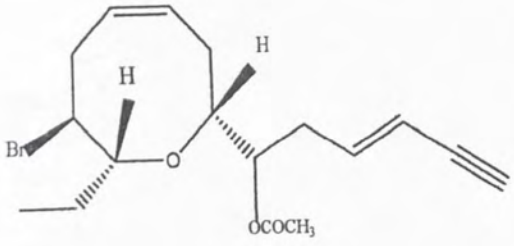


(**5**)



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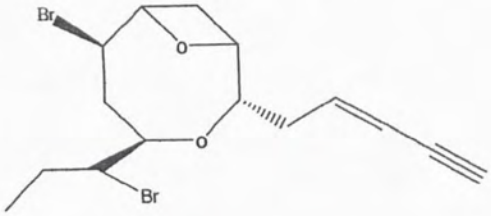

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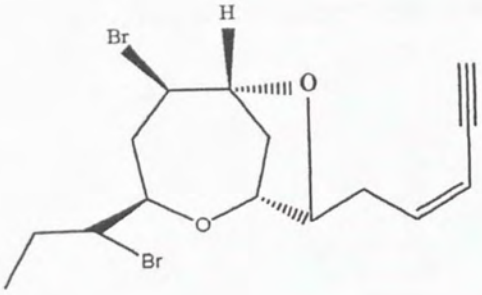

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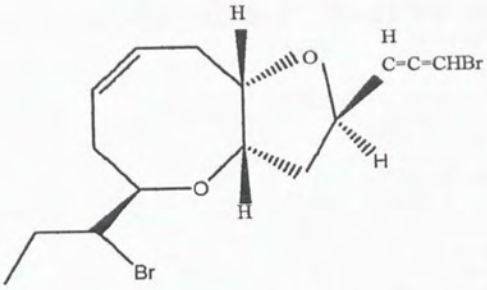

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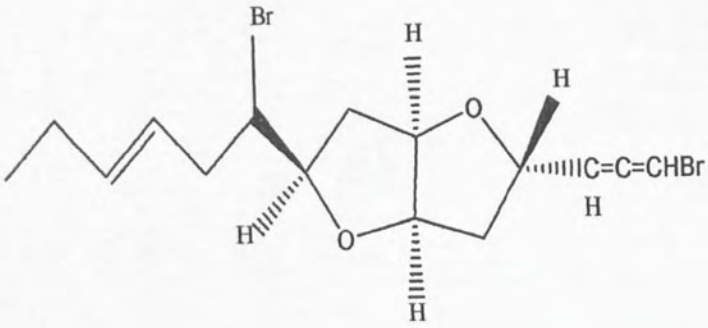

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