องค์ประกอบทางเคมีของรากก้านเหลือง *Nauclea orientalis* (L.) L.

นายจีราพัสฐ์ สีแจ่ม

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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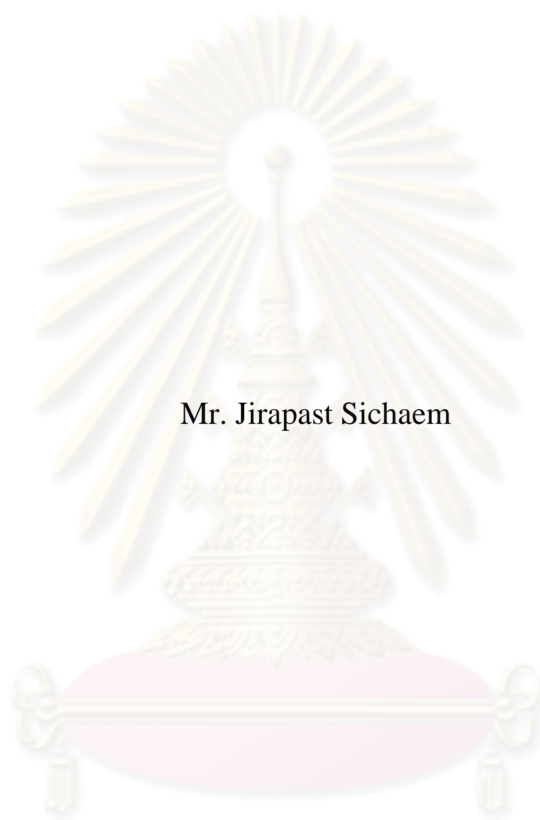
ปีการศึกษา 2552

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Nauclea orientalis* (L.) L.



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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry

Department of Chemistry
Faculty of Science
Chulalongkorn University

Academic Year 2009

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Thesis Title	CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Nauclea orientalis* (L.) L.
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จีราพัสฐ์ สีแจ่ม : องค์ประกอบทางเคมีของรากก้านเหลือง *Nauclea orientalis* (L.) L. (CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Nauclea orientalis* (L.) L.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร. สันติ ทิพยางค์, 56 หน้า.

การศึกษาองค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพจากสิ่งสกัดไดคลอโรมีเทน เอทิลอะซิเตต และเมทานอลของรากก้านเหลือง *Nauclea orientalis* (L.) L. สามารถแยกสารในกลุ่มอินโดลอัลคาลอยด์ชนิดใหม่ได้ 3 ชนิด คือ naucleaorien (3), naucleaoral A (5) และ B (6) พร้อมกับสารที่มีรายงานแล้ว 9 ชนิด ได้แก่ nauclefícine (1), naucleactonin A (2), vanillic acid (4), naucleidinal (7), 19-*epi*-naucleidinal (8), strictosamide (9), alpigenoside (10), sweroside (11) และ pumiloside (12) การพิสูจน์โครงสร้างของสารทั้งหมดที่แยกได้นี้ อาศัยวิธีทางกายภาพและวิธีทางสเปกโทรสโกปี ร่วมกับการเปรียบเทียบกับข้อมูลที่มีรายงานแล้ว จากการทดสอบฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด HeLa และ KB พบว่า สาร 1, 3, 6 และ 7 มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด HeLa และ KB ได้ปานกลางที่ค่า IC_{50} เท่ากับ 6.8 และ 6.8, 9.5 และ 5.0, 7.8 และ 9.5 และ 5.5 และ 4.8 μg/mL ตามลำดับ นอกจากนี้ สาร 5 ยังมีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด HeLa ได้ดีที่ค่า IC_{50} เท่ากับ 4.0 μg/mL และสาร 8 มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB ได้ปานกลางที่ค่า IC_{50} เท่ากับ 6.8 μg/mL ส่วนสาร 2, 9 และ 11-12 ไม่มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด HeLa และ KB (IC_{50} >30.0 μg/mL) ในการทดสอบฤทธิ์ต้านเชื้อมาลาเรียของสารในกลุ่มอินโดลอัลคาลอยด์ที่ไม่ใช่ไกลโคไซด์ (สาร 1-2 และ 5-8) พบว่า ไม่มีฤทธิ์ต้านเชื้อมาลาเรีย (IC_{50} >10.00 μg/mL)

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ปีการศึกษา 2552

5172247023: MAJOR CHEMISTRY
KEYWORDS: *Nauclea orientalis* (L.) L./INDOLE ALKALOIDS/
CYTOTOXICITY/ANTIMARALIAL ACTIVITY

JIRAPAST SICHAEM: CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Nauclea orientalis* (L.) L. THESIS ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, Ph.D., 56 pp.

The investigation for chemical constituents and their biological activities from the CH_2Cl_2, EtOAc and MeOH crude extracts of the roots of *Nauclea orientalis* (L.) L. led to the isolation of three new indole alkaloids, naucleaorien (**3**), naucleaoral A (**5**) and B (**6**) along with nine known compounds, naucleficine (**1**), naucleactonin A (**2**), vanillic acid (**4**), naucleidinal (**7**), 19-*epi*-naucleidinal (**8**), strictosamide (**9**), alpigenoside (**10**), sweroside (**11**) and pumiloside (**12**). The structures of all isolated compounds were elucidated by physical properties and spectroscopic methods as well as comparison with previous literature data. Most of isolated compounds were tested for cytotoxicity on HeLa and KB cell lines. Compounds **1**, **3**, **6** and **7** showed very modest cytotoxicity against both HeLa and KB cell lines (IC_{50} values of 6.8 and 6.8, 9.5 and 5.0, 7.8 and 9.5 and 5.5 and 4.8 μg/mL, respectively). Compound **5** exhibited significant cytotoxic activity against only HeLa cells (IC_{50} value of 4.0 μg/mL), while compound **8** showed only very modest cytotoxic activity against KB cells (IC_{50} value of 6.8 μg/mL). On the contrary, compounds **2**, **9** and **11-12** were inactive (IC_{50} >30.0 μg/mL). The results of testing for antimalarial activity of non-glycosidic indole alkaloids (compounds **1-2** and **5-8**) showed that these compounds could be regarded as inactive (IC_{50} >10.00 μg/mL).

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ACKNOWLEDGEMENTS

I would like to express my faithful gratitude to my advisor, Associate Professor Dr. Santi Tip-pyang for his assistance and encouragement in conducting this research.

I also gratefully acknowledge the members of my thesis committee, Assistant Professor Dr. Warinthorn Chavasiri, Assistant Professor Dr. Worawan Bhanthumnavin and Dr. Withawat Mingvanish for their discussion and guidance. The special thank goes to Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University for plant identification and making the voucher specimen of plant material as well as providing the roots of *Nauclea orientalis* (L.) L. in this study.

My appreciation is also expressed to thank Dr. Pongpan Siripong and the staffs of Natural Products Research Section, Research Division, National Cancer Institute for their testing on cytotoxicity against KB and HeLa cell lines experiment, Dr. Jonkolnee Jong-aramruang from Department of Chemistry, Faculty of Science, Burapha University, for her kind help with the NMR measurements and Mr. Serm Surapinit for his advice.

I would like to express my gratitude to the Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University for providing the chemicals and facilities throughout the course of study. A deep affectionate gratitude is acknowledged to my family for their understanding, encouragement and support throughout the education course and I specially thank Ms. Wisuttaya Worawalai, Mr. Thanatip Ruksilp and Ms. Suwannee Saisin for their technical assistance. On the other hand, I would like to thank all of my friends in the laboratory for their friendships and help during the course of my graduate research.

Finally, I would like to thank the Center of Excellence for Petroleum, Petrochemicals and Advanced Materials and CU. Graduate school thesis grant for the financial supports, which is highly appreciated.

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LIST OF ABBREVIATIONS

^{13}C NMR	carbon 13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
br s	broad singlet (NMR)
c	concentration
COSY	correlated spectroscopy
d	doublet (NMR)
dd	doublet of doublet (NMR)
ESIMS	electrospray ionization mass spectrometry
g	gram (s)
HMBC	heteronuclear multiple bond correlation
HRESIMS	high resolution electrospray ionization mass spectrometry
HSQC	heteronuclear single quantum correlation
Hz	hertz
IC_{50}	concentration that is required for 50% inhibition in vitro
J	coupling constant
m	multiplet (NMR)
M	molar
MeOH	methanol
mg	milligram (s)
MHz	megahertz
min	minute
mL	milliliter (s)
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser enhancement spectroscopy
q	quartet (NMR)
s	singlet (NMR)
t	triplet (NMR)
UV	ultraviolet

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VLC	vacuum liquid chromatography
δ	chemical shift
δ_C	chemical shift of carbon
δ_H	chemical shift of proton
μ	micro
ε	molar extinction coefficient
λ_{max}	maximum wavelength
2D NMR	two dimentional nuclear magnetic resonance
$[\alpha]_D^{20}$	specific optical rotation

CHAPTER I

INTRODUCTION

The most common conventional causes of death in Thailand are accident, cardiovascular disease, and cancer. Other causes of Thai death are diabetes mellitus, HIV, malarial, and Alzheimer's diseases.

In recent years, the rates of different kinds of cancers have increased and became leading cause of deaths. Moreover, the incidence of many cancers, including cancers of the skin, prostate, breast and kidney, continue to increase [1]. “Cancer” is, in fact, a general term that refers to over 100 distinct diseases affecting many different tissues and cell types. However, all forms of cancer are characterized by abnormal cell growth resulting from a relatively small number of inherited or environmentally-induced genetic mutations [2]. There are many risk factors directly influencing cancer in human body such as too much alcohol, sun, food, certain hormones and smoking**,** which have fuelled a massive rise in some forms of tumor that linked with a number of cancers.

Malaria is a parasitic disease that is responsible for many million deaths each year. This is caused by protozoan parasites of the genus *Plasmodium.* There are four species of *Plasmodium* that infect humans, the most deadly of these being *P. falciparum*. The parasite requires two hosts, a female *Anopheles* mosquito and a human. Almost one-half of the world’s population lives under the constant threat of malaria, and a large percentage of the fatalities occur in Africa. However, malaria is endemic throughout most of Southeast Asia, the Indian subcontinent, the South Pacific region and Latin America.

Natural products are secondary metabolites produced by plants, fungi, bacteria, protozoans, and animals in response to external stimuli such as nutritional changes, infection and competition [3]. Reports of the use of medicinal plants go back to ancient times. Written records dated back at least 5000 years to the Sumerians, and archeological records suggest even earlier use of medicinal plants [4].

Medicinal plants have many curative properties and have been one of the major sources of medicines that are also basic needs for human since the dawn of

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human civilization. They have especially pharmacological activities and they are used as therapeutic drugs or herbal medicines. Some tropical plants have many bioactive activities such as potent antioxidant, antiinflammatory, antimutagenic and antimicrobial activities. In addition, some of them also have a potential anticancer activity in the prevention of cancer and the management of infectious and chronic diseases. In modern times, the contribution of plant-derived drugs is also significant and much interest has been investigated on wide diversity of medicinal plants to modern drug development.

Plants in genus *Nauclea* (Rubiaceae) are known to produce many kinds of indole alkaloids having some significant biological activities. Their extracts have been reported to exhibit antimicrobial, antiparasitic [5,6], antimalarial [7] and cytotoxic activities [8]. *Nauclea orientalis* (L.) L. is locally known as “Kan Luang”, a large fruit-bearing tree with large glossy leaves often found in the Northern, Northeastern and central of Thailand. It has been used as a traditional medicine and was chosen as the subject of the present investigation due to its significant cytotoxicity which has been observed in the crude extracts against KB and HeLa cells.

1.1 Indole alkaloids: distribution and their biosynthesis pathway

Several indole alkaloids have been isolated from *Nauclea* (Rubiaceae), which are the characteristic metabolites of plants in this genus.

1.1.1 Biosynthesis and Phytochemistry of Rubiaceae

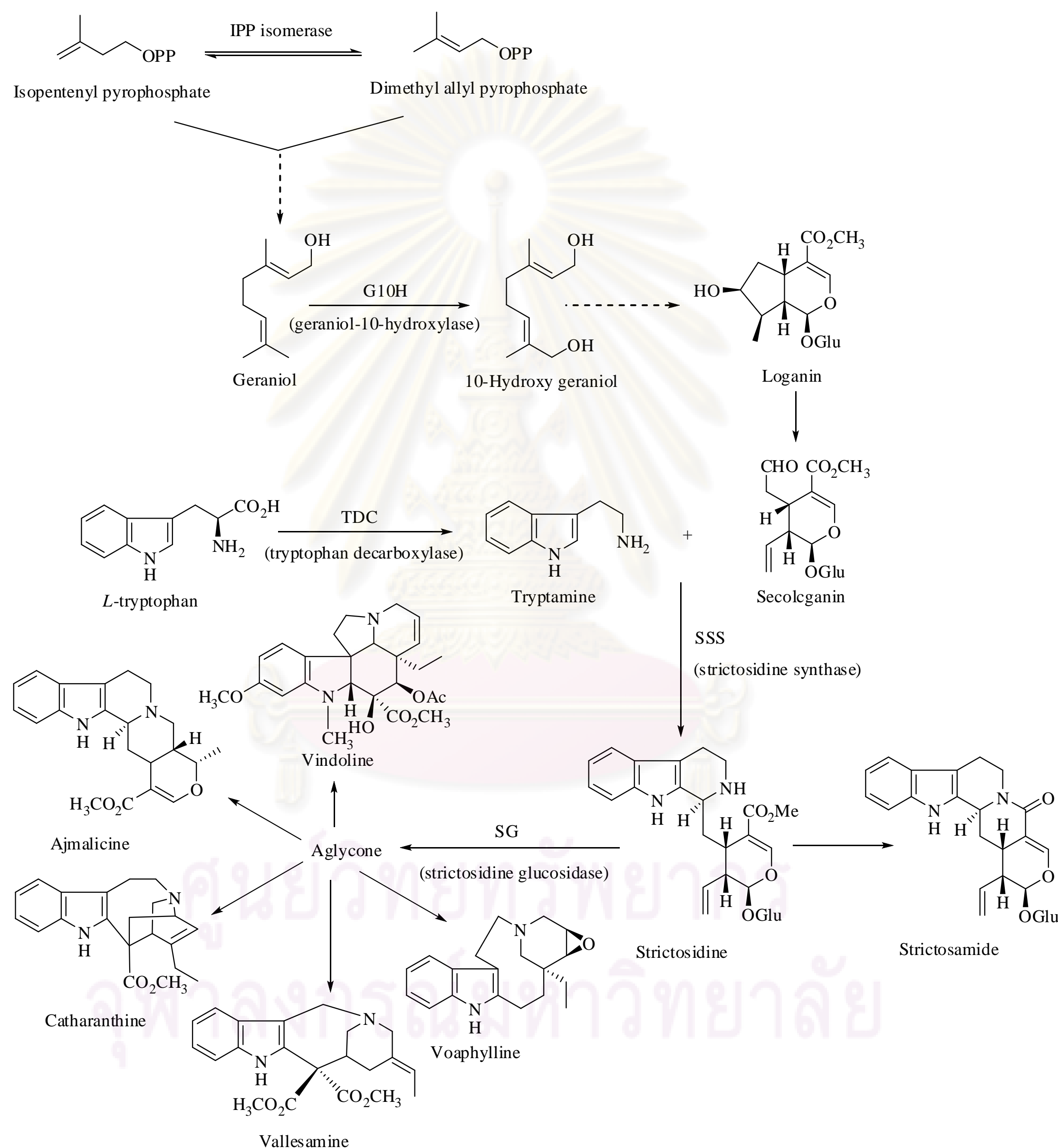

Figure 1.1 Biosynthetic pathways of strictosidine and its derivatives.

The biosynthesis of monoterpene indole alkaloids is known to involve tryptamine and secologanin in higher plant species. This pathway consists of five enzymes: IPP isomerase: isopentenyl pyrophosphate isomerase; GIOH: geraniol-10-

hydroxylase; TDC: tryptophan decarboxylase; SSS: strictosidine synthase; SG: strictosidine glucosidase. The primary metabolite, isopentenyl pyrophosphate could be isomerized to dimethyl allyl pyrophosphate by the first enzyme, IPP isomerase. The second enzyme, G10H (geraniol-10-hydroxylase) transforms geraniol into the secondary monoterpene metabolites such as 10-hydroxygeraniol/nerol, 7-deoxyloganin, and secologanin. The third enzyme, TDC (tryptophan decarboxylase) converts *L*-tryptophan to tryptamine as a precursor. In the biosynthesis, strictosidine and other indole alkaloids could be derived biosynthetically from the condensation of tryptamine and secologanin by the fourth enzyme, SSS (strictosidine synthase) to give strictosidine whose intramolecular cyclization yields strictosamide. On the other hand, strictosidine was hydrolyzed glucose by the last enzyme, SG (strictosidine glucosidase) to afford aglycone part which was shown to be involved in the biosynthetic pathway leading to many derivatives such as ajmalicine, catharanthine, vindoline, vallesamine and voaphylline (Figure 1.1) [9,10].

1.2 Chemical constituents from *Nauclea* species and their biological activities

There are many chemical and biological investigations which have not been carried out on *Nauclea* genus. The major indole alkaloid components of this genus were previously reported. In addition, many of these compounds have demonstrated a number of interesting biological activities such as antiproliferative, antimalarial, antitumor, antiparasitic and antileishmanial activities.

N. diderrichii (De Wild) Merr. (*Sarcocephahs diderrichii* De Wild) is a large evergreen tree abundant in the rain forests of West Africa. The bark finds some local use in the treatment of gonorrhoea, stomach pains, fever and sometimes diarrhoea. Its strength as a timber and resistance to termites make it valuable in construction work. Previous work on the stem bark of *N. diderrichii* has yielded quinovic acid, 3-oxoquinovic acid and 3-*O*-glucosylquinovic acid [11]. A terpenoid glycoside, marounoside also was isolated from the bark of this plant [12] (Figure 1.2).

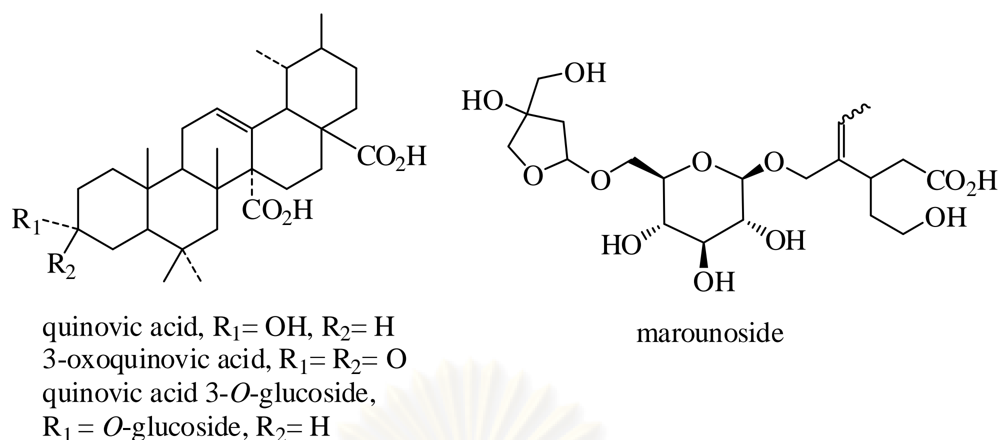

Figure 1.2 Isolated compounds from *N. diderrichii* (De Wild) Merr.

N. pobeguinii is used in DR Congo for the treatment of fever, including fever caused by malaria, and against intestinal worms, abdominal pains, sexual asthenia and gonorrhea [13]. It has been reported that the alkaloid strictosamide was the main constituent of *N. pobeguinii.* Other alkaloids identified from the same plant including angustine, naufoline, angustoline, nauclefine, *O*-acetyl-angustoline and 3,14-dihydroangustine. In addition, two quinovic acid glycosides were reported [14].

N. latifolia also named *N. esculentus,* is one of the medicinal plants used in Africa. The stem bark, the leaves, the roots and the fruits of this plant are used to treat various types of diseases [15-17]. In Cameroon, *N. latifolia* is used in the treatment of fever, yellow fever, malaria and diseases of the central nervous system like epilepsy [15]. According to Cameroonian traditional healers, the plant is also used in the treatment of anxiety and agitation. Previous experiments have shown that if the aqueous extract of *N. latifolia* is administered intraperitoneally, it lowers the rectal temperature of guinea pigs and also exhibits analgesic, antidiabetic and hepatoprotective properties [18-19]. Sourabies *et al.* [20] and Benoit-Vical *et al.* [21] found that the extracts of the leaves and bark possess antibacterial and antiplasmodial activities. Several indoloquinolizidine alkaloids such as angustine, angustoline, nauclefine, naucietine and naucleamides A-E were isolated from the roots of this plant. Naucleamide E is a unique monoterpene indole alkaloid possessing a pentacyclic ring system with an amino acetal bridge [22] (Figure 1.3).

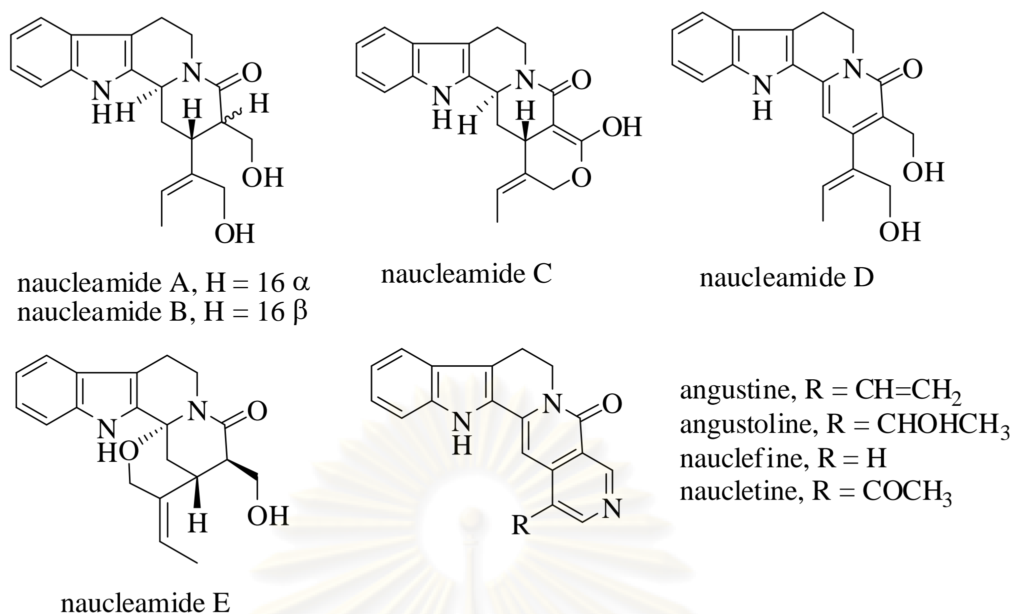

Figure 1.3 Isolated compounds from *N. latifolia.*

N. officinalis (Pierre ex Pitard) merr. & Chem., a traditional Chinese Herb, is widely used to cure colds, pink eye and other ailments [23]. Several indole alkaloids (Figure 1.4) were isolated from this species. Naucleofficines A-E, naucleidinal and angustoline were isolated from the stems (with bark) of this plant. *In vitro* activity screening of the above seven compounds showed weak to moderate inhibitory activity against *P. falciparum* [8].

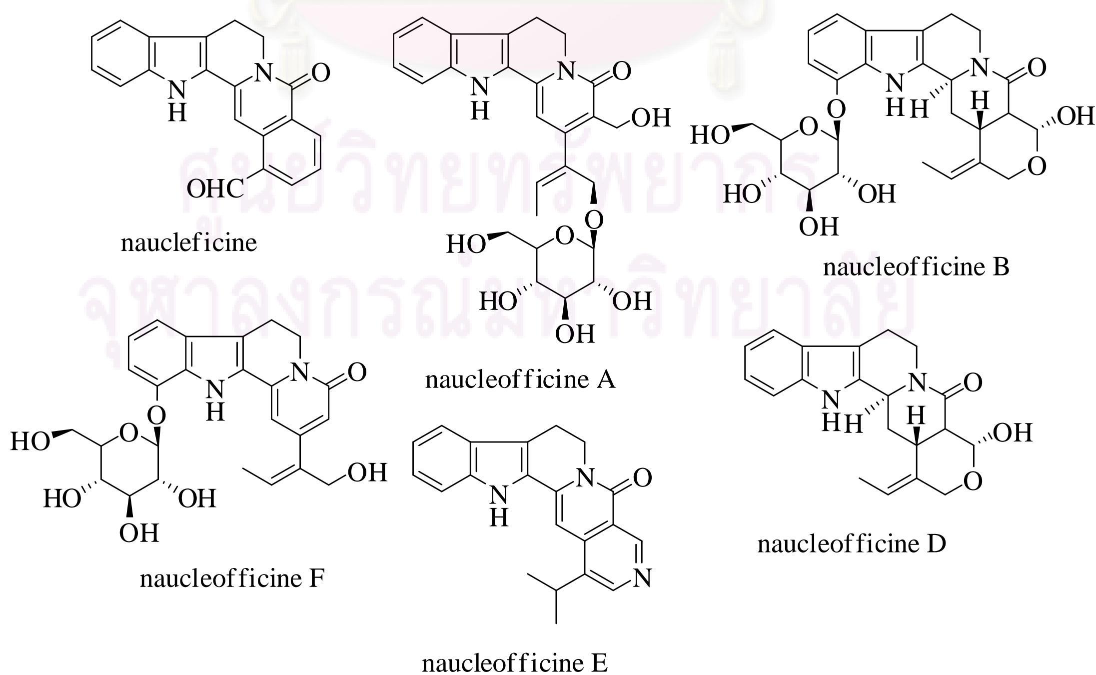

Figure 1.4 Isolated compounds from *N. officinalis*.

Many compounds such as angustine, 18,19-dihydroangustine, nauclefine, angustoline, 10-hydroxyangustine, 3,14-dihydroangustine, diastereoisomer of 3,14-dihydroangustoline, nauclealines A-B, naucleosides A-B, strictosamide, vincosamide, pumiloside, 3,4,18,19-tetrahydroangustine, kelampayoside A, pumiloside, sitosterol and sitosteryl β-*D*-glucoside were also isolated from *N. orientalis* (L.) L. [24-26] (Figure 1.5).

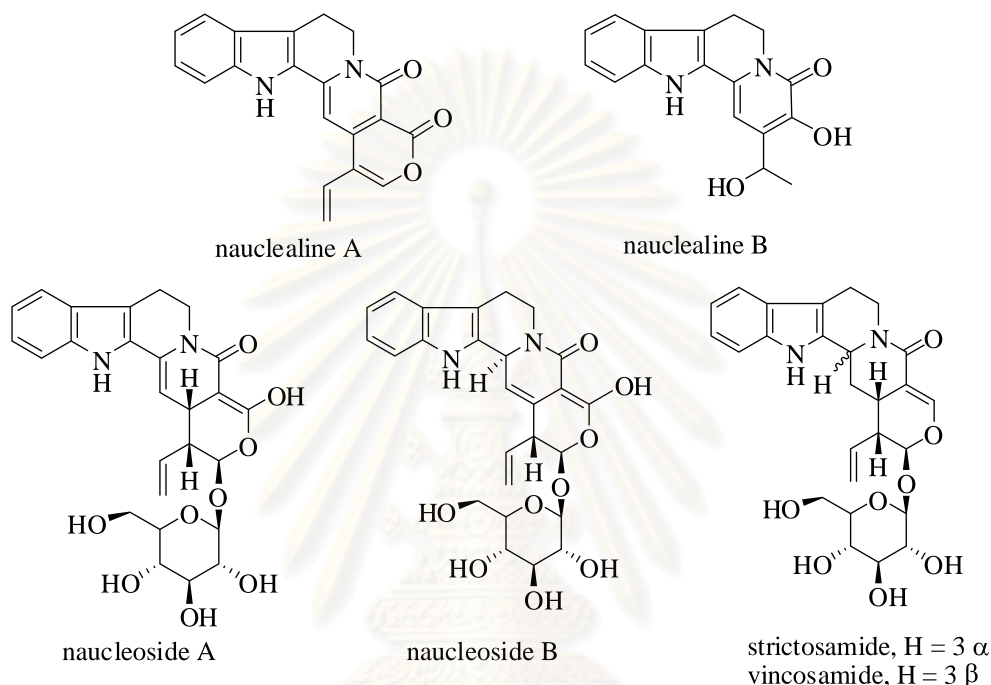

Figure 1.5 Isolated compounds from *N. orientalis* (L.) L.

1.3 Botanical aspect and distribution

Rubiaceae is a family of flowering plants and variously called the madder family, bedstraw family or coffee family. Other common plants included here are *gardenia*, *cinchona*, *sweet woodruff*, *partridgeberry*, *gambier*, *ixora* and *noni*. A number of traditionally accepted families (*Dialypetalanthaceae*, *Henriqueziaceae*, *Naucleaceae* and *Theligonaceae*) are now incorporated within the Rubiaceae following genetic research by the Angiosperm Phylogeny Group. As now circumscribed, there are about 650 genera and more than 13,000 species in Rubiaceae [27], and most genus are herbaceous.

Nauclea is a genus of tropical evergreen trees and shrubs in the Rubiaceae family. Medium-sized to large trees; tips of branches typically with strongly flattened buds. Leaves opposite, petiolate, blades chartaceous to coriaceous; stipules

interpetiolar, large, ovate, elliptic, or obovate, flattened to strongly keeled, adpressed, deciduous or semi-persistent. Inflorescences terminal and axillary, consisting of stalked, many-flowered flowering heads in groups of 2-5, or of solitary flowering heads; young heads not surrounded by involucre-like stipules or bracts. Flowers of a head united by their ovaries, 4- or 5-merous. Calyx persistent, with triangular, obtuse to oblong lobes or clavate to (sub) spathulate lobes with a distinct narrow shaft. Corolla hypocrateriform to infundibular, lobes imbricate in bud, ascending in open flowers. Stamens inserted in the upper part of the tube, filaments short, anthers partially exserted. Ovary 2-celled, each locule with numerous ovules; placenta attached to the upper third of the septum, Y-shaped with 2 short ascending arms and a long descending foot; style with spindle-shaped stigma exserted; ovaries of adjoining flowers of a head fused with each other. Fruitlets of a head united into an indehiscent syncarp; fruitlets crowned by the persistent calyx lobes. Seeds numerous, very small, ovoidal to ellipsoidal, sometimes slightly bilaterally compressed, not winged. There is about 40 species distributed in Tropical Asia and 7 species are native to Thailand, for example:

N. jung hunii Miq. Merr.

N. pallida Reinw. Ex Havil.

N. latifolia

N. orientalis (L.) L.

N. officinalis (Pierre ex Pitard) merr. & Chem.

N. cordifolia Roxb.

N. cadamba Roxb.

N. orientalis (L.) L. is a tall tree, but often smaller; bark dark brown to blackish, slightly fissured and flaky; young twigs often lenticellate. Leaves coriaceous, broadly ovate to orbicular, (10)15-30 by 7-15 cm, base rounded to cordate, rarely cuneate, glabrous above, glabrous to pubescent below; 5-8 pairs of lateral veins; petiole c. 10-40 mm long; stipules broadly ovate to orbicular or obovate, 2-4 x 1-2 cm, keeled, glabrous or slightly pubescent on the keel. Inflorescence axillary and terminal, usually a single flowering head (rarely up to 3), peduncles 2-5 cm long. Flowering heads c. 10 mm in diam. across calyces and 15-25(30) mm across corollas. Flowers 4- or 5-merous. Calyx lobes 3 mm long, clavate to (sub) spathulate, with a distinct narrow shaft, pubescent. Corolla (pale) orange to greenish-yellow, glabrous,

tube 6-9 mm long, lobes ovate, 4-5 mm long. Stamens subsessile, anthers c. 1 mm long. Ovaries of a flowering head fused; style with spindle-shaped stigma 12-15 mm long, white. Syncarp woody, brown, globose to slightly ovoid, 25-30 mm in diam (Figure 1.6).

Thailand - Northern: Mae Hong Son, Chiang Mai, Phrae, Phitsanulok, Kamphaeng Phet, Phichit; North Eastern: Khon Kaen; Eastern: Chaiyaphum, Ubon Ratchathani; South Weastern: Uthai Thani, Kanchanaburi, Ratchaburi, Phetchaburi; Central: Bangkok [cultivated]; South Eastern: Sa Kaeo, Chanthaburi; Peninsular: Satun.

Distribution - Sri Lanka; continental SE. Asia (Myanmar, Laos, Cambodia, Vietnam); throughout Mal esia to N. & NE. Australia.

Ecology - Scattered in mixed dipterocarp forest, mixed evergreen and deciduous forest, dry evergreen forest or in pine-dipterocarp forest with patches of dry evergreen forest; occasionally in lowland evergreen forest; often near streams (but not rheophytic); sometimes over limestone; also in disturbed, secondary forest. Altitude: 50–850 m. Flowers, May - June; fruits, June - November (old fruits often persisting until the following flowering period).

Vernacular: Kan Luang (ก้านเหลือง), Kra Toom Nam (กระทุ่มน้ำ) and tagoo (ตะกู).

Note - The most common and widely distributed *Nauclea* species in Thailand [28].

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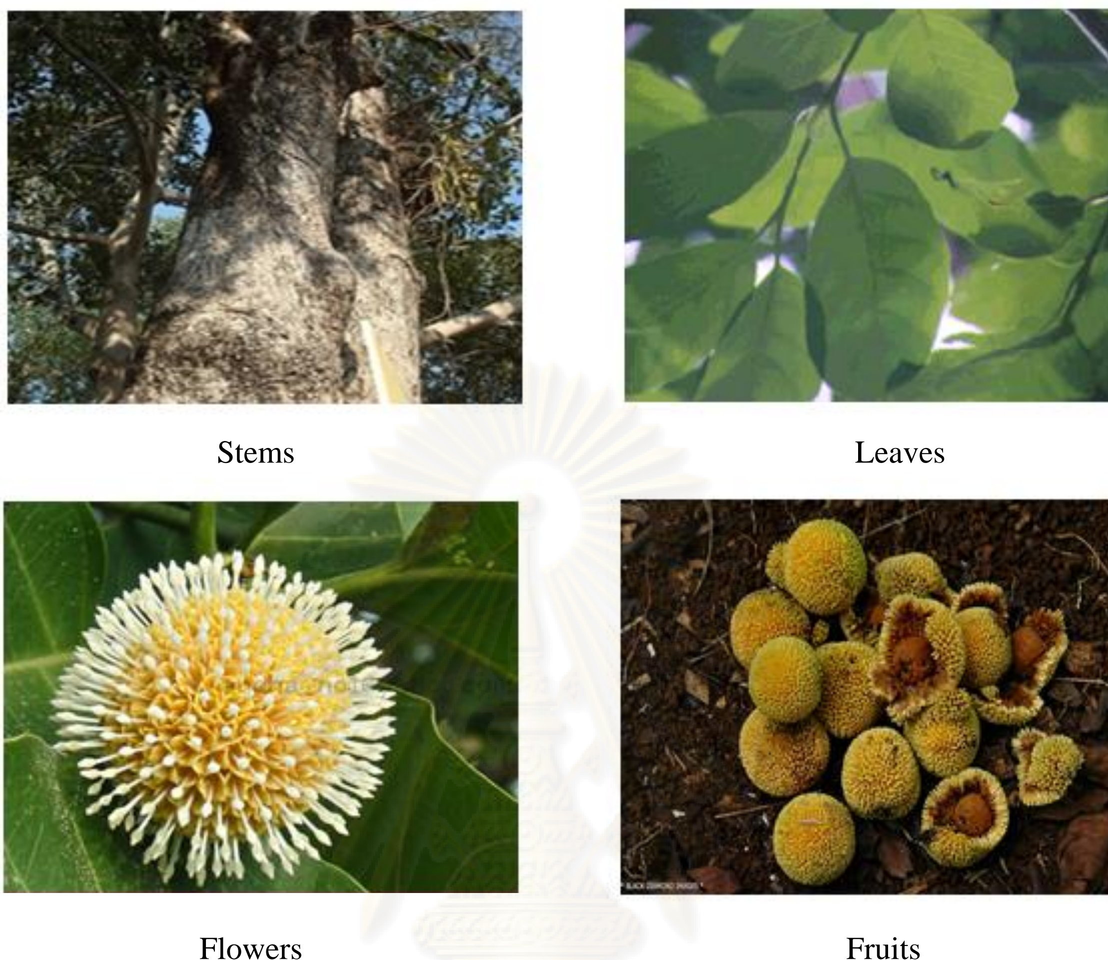

Figure 1.6 The stems, leaves, flowers and fruits of *N. orientalis* (L.) L.

1.4 Biological activities

1.4.1 Cytotoxicity against KB and HeLa cell lines

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds, if they are interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or they can screen "hits" from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical [29]. Cytotoxicity can also be monitored using the MTT or MTS assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Viable cells will reduce the MTS reagent to a colored formazan product. A similar redox-based assay has also been developed using the fluorescent dye, resazurin. In addition to using dyes to indicate the redox potential of cells in

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order to monitor their viability, researchers have developed assays that use ATP content as a marker of viability [30]. Such ATP-based assays include bioluminescent assays in which ATP is the limiting reagent for the luciferase reaction [31]. Cytotoxicity can also be measured by the sulforhodamine B (SRB) assay, WST assay and clonogenic assay. A label-free approach to follow the cytotoxic response of adherent animal cells in real-time is based on electric impedance measurements when the cells are grown on gold-film electrodes. This technology is referred to as electric cell-substrate impedance sensing (ECIS). Label-free real-time techniques provide the kinetics of the cytotoxic response rather than just a snapshot like many colorimetric endpoint assays.

1.4.2 Antimalarial activity

Antimalarial drugs are designed to prevent or cure malaria. Some antimalarial agents, particularly chloroquine and hydroxychloroquine, are also used in the treatment of rheumatoid arthritis and lupus associated arthritis. However, strategies for combating malaria change rapidly, and when drugs are administered in combination, it can be impractical to identify whether agents are prophylactic or therapeutic. Another approach for classifying antimalarials is to group them by mechanism and by chemical structure [32].

The literature review on the chemical constituents, cytotoxic and antimalarial activities from *N. orientalis* (L.) L. and the attractive results of primary screening test are based on cytotoxicity. Thereby, this plant was selected for further investigation and could be a promising source for chemotherapeutic agents.

The objectives of this research:

The main objectives in this investigation are as follows:

1. To isolate and purify compounds from the roots of *N. orientalis* (L.) L.
2. To identify the chemical structures of all isolated compounds.
3. To evaluate the cytotoxicity against HeLa and KB cell lines of the isolated compounds.
4. To evaluate the antimalarial activity of non-glycosidic indole alkaloids.

CHAPTER II

EXPERIMENTAL

2.1 Plant material

The roots of *Nauclea orientalis* (L.) L. were collected from Mahasarakham Province of Thailand in April, 2008 and identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, where a voucher specimen (Khumkratok no. 84-08) is deposited.

2.2 General experimental procedures

NMR spectra were recorded with a Varian model Mercury$^+$ 400 spectrometer operated at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR and a Bruker 400 AVANCE spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The chemical shift in δ (ppm) was assigned with reference to the signal from the residual protons in deuterated solvent and using TMS as an internal standard in some cases. Most solvents used in this research were commercial grade and were distilled prior to use. Adsorbents such as Dianion HP-20, Sephadex LH-20 and silica gel (60 Merck cat. No. 7730, 7734 and 7749 were used for quick column chromatography, preparative TLC, open column chromatography and centrifugal thin layer chromatograph (chromatotron), respectively). Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F_{254} plates (0.25 mm thick layer). ESIMS data were obtained from a mass spectrometer model VG TRIO 2000. High resolution mass spectra were recorded by Micromass LCT and Bruker MICROTOF models. UV-visible adsorption spectra were recorded on UV-2552PC UV-Vis spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were measured on a Jasco P-1010 polarimeter. Melting points were determined with Fisher-Johns Melting Point Apparatus. IR data were obtained from a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation, Madison, WI, USA) equipped with a mercury-cadmium-telluride (MCT) detector.

2.3 Extraction and purification

The air-dried roots of *N. orientalis* (L.) L. (4.5 kg) were collected in April 2008 from Mahasarakham Province, Thailand, and successively extracted in a Soxhlet apparatus with CH_2Cl_2, EtOAc and MeOH (each 500 mL, 16 h). The solvents were evaporated under vacuum to yield CH_2Cl_2 (150.7 g), EtOAc (256.4 g) and MeOH (148.3 g) crude extracts, respectively. The CH_2Cl_2 extract was subjected to vacuum liquid chromatography (VLC) over silica gel (Merck Art 7730) using hexane, CH_2Cl_2, EtOAc and MeOH with increasing polarity to afford seven fractions (D1-D7). The VLC fraction D2 was chromatographed on silica gel column using a stepwise gradient elution of hexane, CH_2Cl_2 and EtOAc, and further purified by centrifugal thin layer chromatograph (chromatotron) using a stepwise gradient elution of hexane and EtOAc to yield naucleficine (**1**, 590.5 mg) and naucleactonin A (**2**, 89.5 mg). The VLC fraction D3 was chromatographed on silica gel column using a stepwise gradient elution of EtOAc in CH_2Cl_2 to yield three fractions (D3-1-D3-3). The fraction D3-2 was subjected to the preparative TLC developed by the eluent of EtOAc and hexane (40:60) to give naucleaorien (**3**, 10.6 mg), vanillic acid (**4**, 4.8 mg), naucleaoral A (**5**, 5.3 mg) and B (**6**, 10.6 mg). The VLC fraction D5 was subjected to column chromatography over silica gel with the eluent of EtOAc and hexane to afford naucleidinal (**7**, 194.8 mg) and 19-*epi*-naucleidinal (**8**, 74.5 mg). The EtOAc crude extract was chromatographed on silica gel column using a gradient elution system of CH_2Cl_2 and MeOH to obtain five fractions (E1-E5). The fraction E3 was chromatographed over silica gel using column using a stepwise elution system of MeOH and CH_2Cl_2 to give three fractions (E3-1-E3-3). The fraction E3-1 was purified by chromatotron using 10% MeOH in EtOAc to obtain strictosamide (**9**, 25.4 g). The fraction E3-3 was subjected to the preparative TLC using a gradient elution of MeOH and EtOAc (20:80) to afford alpigenoside (**10**, 4.6 mg) and sweroside (**11**, 5.4 mg). Finally, the MeOH crude extract was cyclic loaded onto a Dianion HP-20 column with increasing amount of water. Water was washed through the column to remove any sugar and salt, and the organic material was eluted using 100% MeOH. This material was subject to silica gel column chromatography using MeOH/EtOAc (1:9), EtOAc/MeOH/H_2O (4:1:0.1) and EtOAc/MeOH/H_2O (7:3:0.3) elution systems to yield fractions M1, M2 and M3, respectively. The fraction M2 was purified by Sephadex LH-20 using 50% MeOH in EtOAc as an eluent and crystallized in 30%

MeOH in EtOAc to obtain pumiloside (**12**, 118 mg). The identification of all isolated compounds was determined by means of various spectroscopic methods including IR, MS, 1D and 2D NMR techniques as well as comparison with the literature data.

The extraction and purification of all isolated compounds from the CH_2Cl_2, EtOAc and MeOH extracts of the roots of *N. orientalis* (L.) L. were briefly summarized in Schemes 2.1- 2.4.

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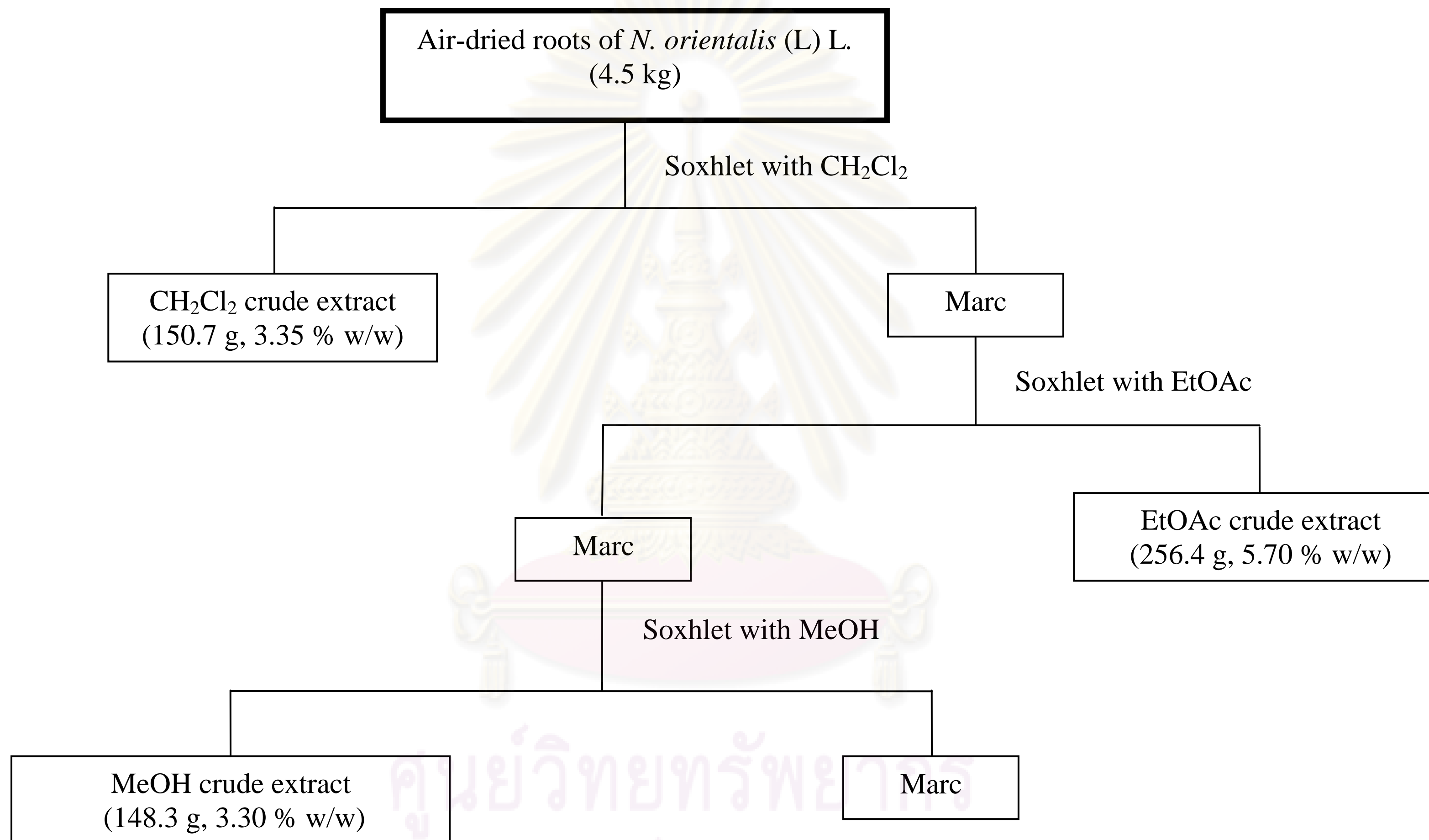
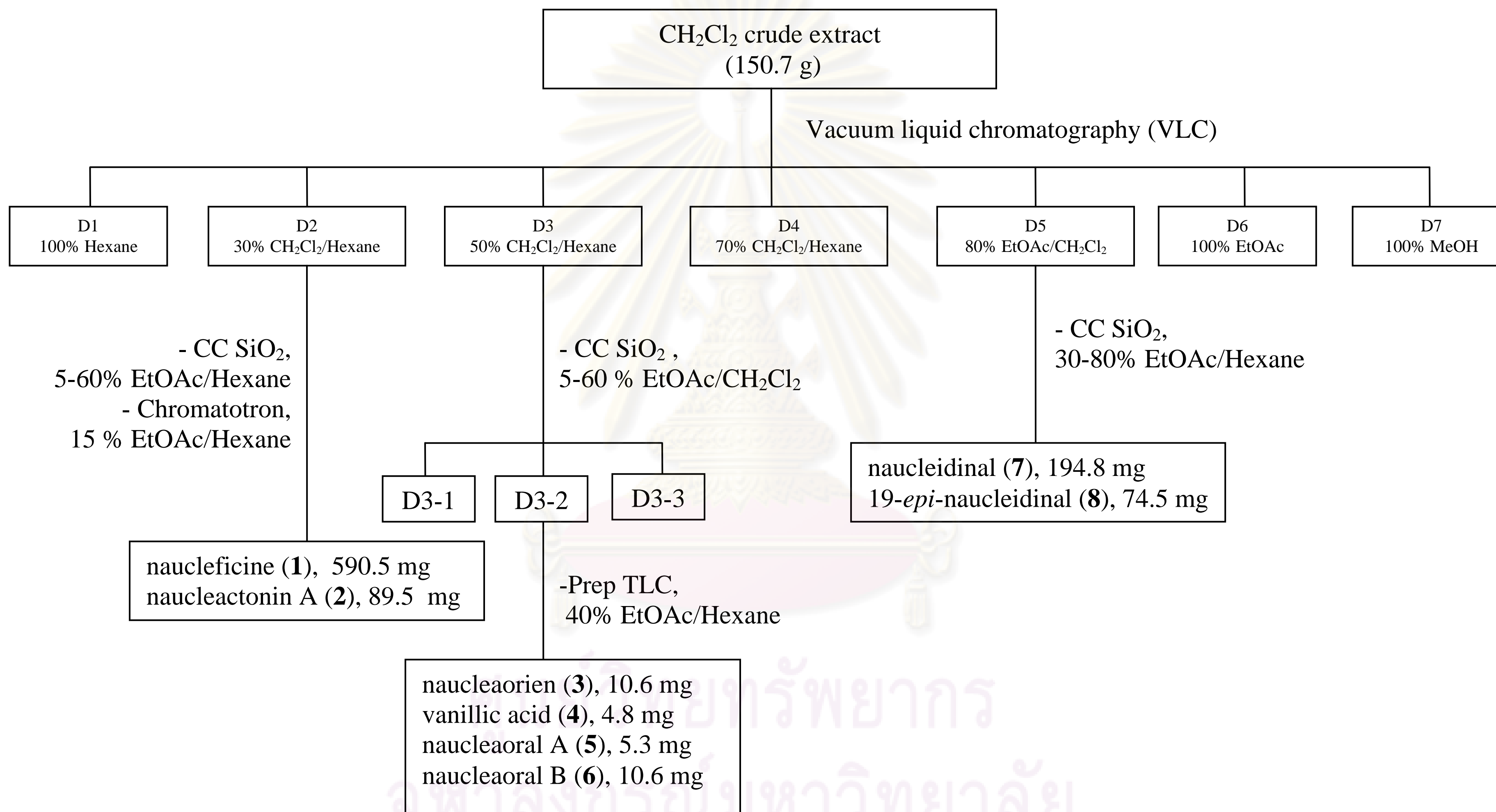
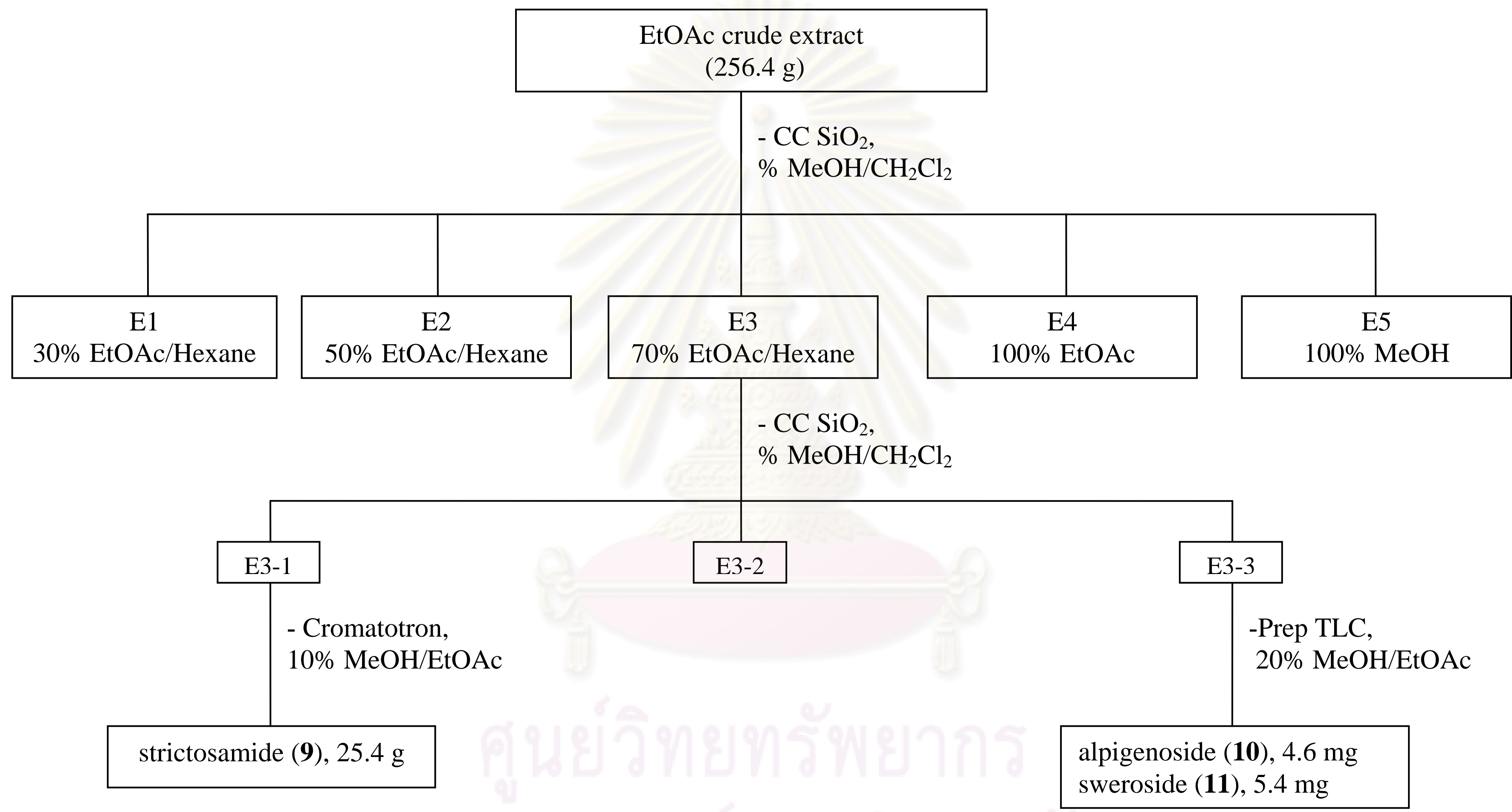


Scheme 2.1 Extraction procedure of *N. orientalis* (L.) L. roots.



Scheme 2.2 Isolation procedure of the CH_2Cl_2 crude extract.



Scheme 2.3 Isolation procedure of the EtOAc crude extract.

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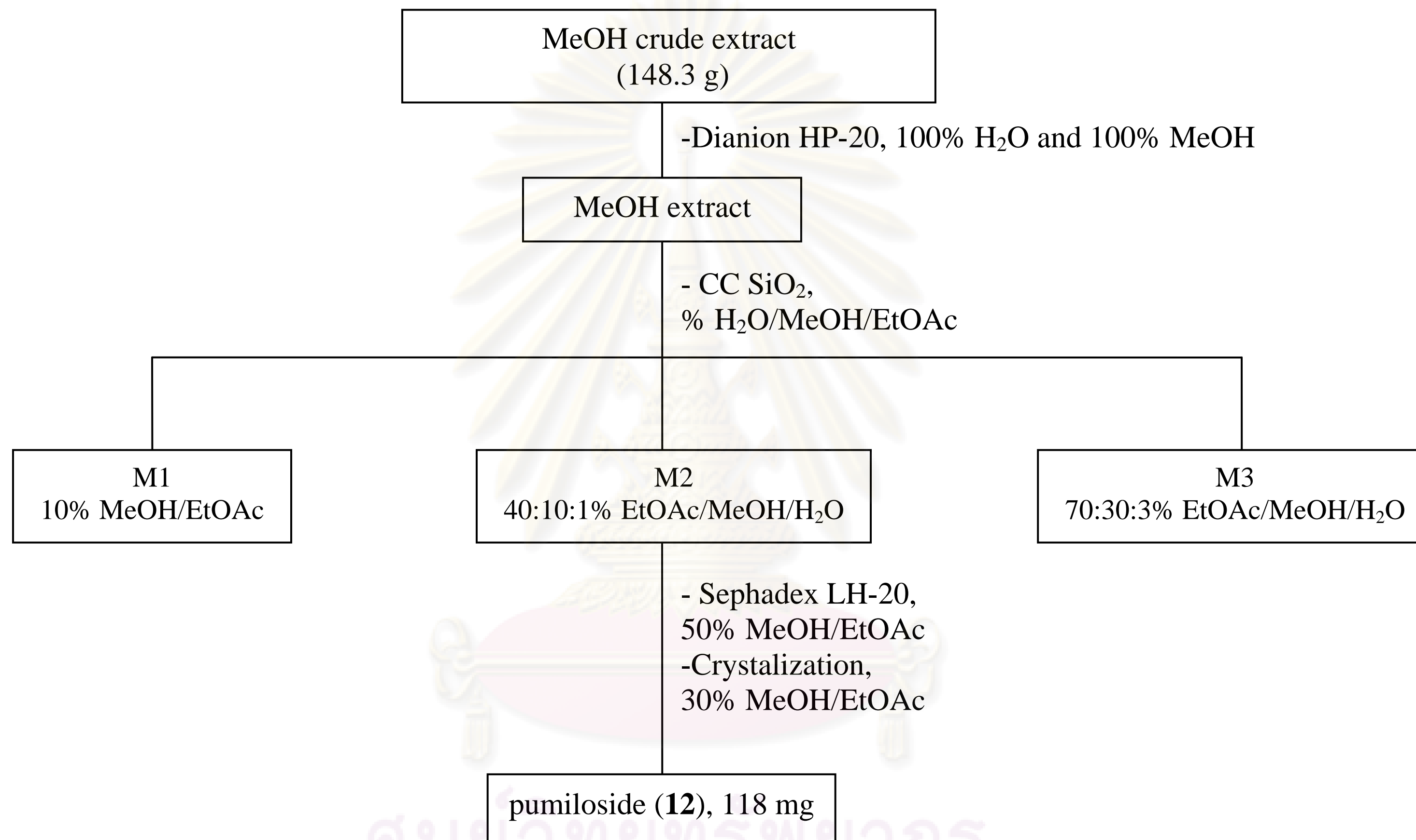

Scheme 2.4 Isolation procedure of the MeOH crude extract.

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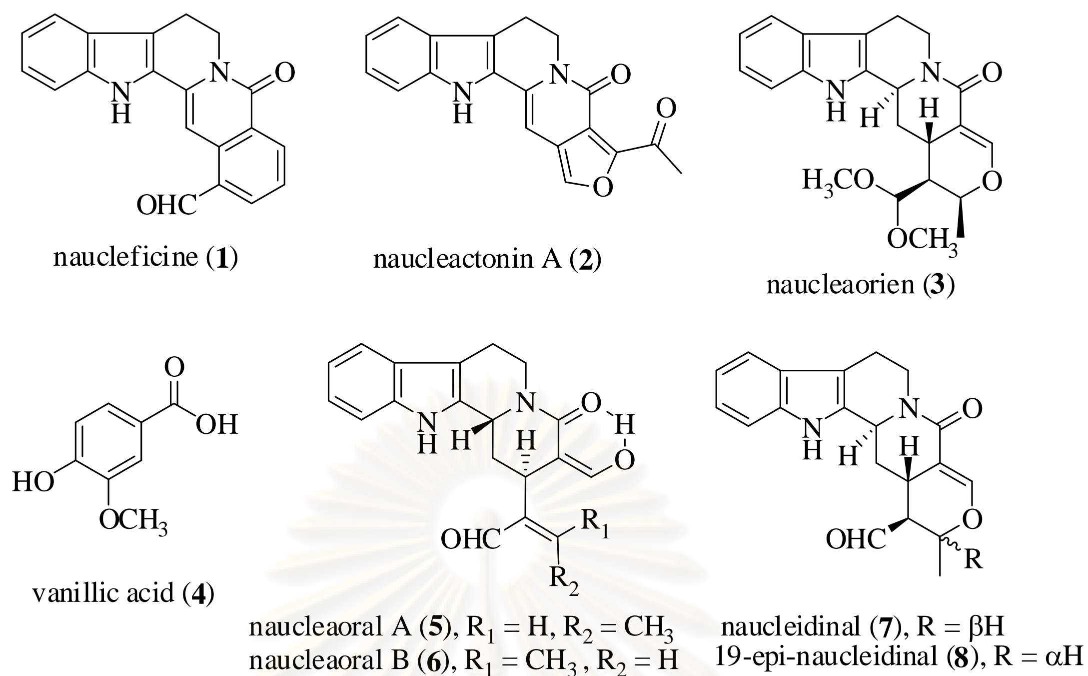


Figure 2.1 Isolated compounds from the CH_2Cl_2 crude extract of *N. orientalis* (L.) L. roots.

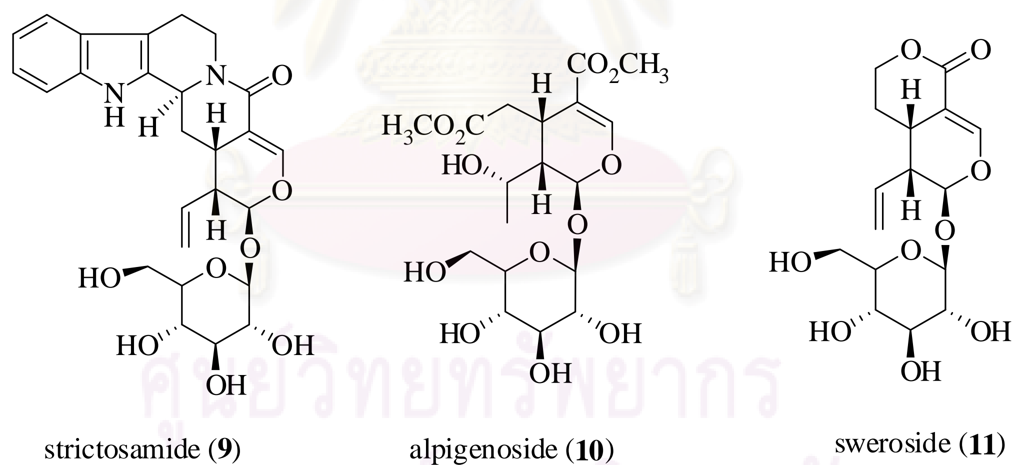
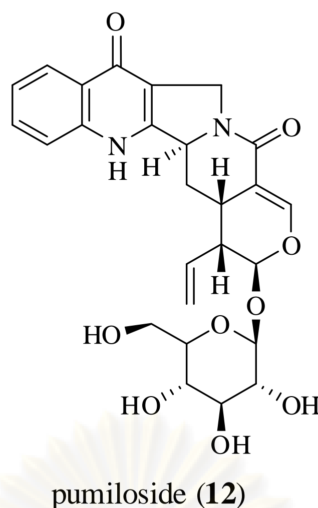


Figure 2.2 Isolated compounds from the EtOAc crude extract of *N. orientalis* (L.) L. roots.



pumiloside (**12**)

Figure 2.3 Isolated compound from the MeOH crude extract of *N. orientalis* (L.) L. roots.

2.4 Bioassay procedure

2.4.1 The cytotoxicity against HeLa and KB cell lines by MTT colorimetric assay

All tested compounds (1 mg each) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the MTT colorimetric assay. Adriamycin was used as standard antibiotic antitumor agent which exhibits activity against KB and HeLa cell lines according to the method of Kongkathip *et al.* [34]. This assay was kindly performed by Natural Products Research Section, Research Division, National Cancer Institute, Thailand.

2.4.2 Antimalarial assay

Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), using the method of Trager and Jensen [35]. Quantitative assessment of *in vitro* activity was determined by means of the microculture radioisotope technique based upon the method described by Desjardins *et al.* [36]. The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. The standard compound was artemisinin. This assay was performed by the Bioassay Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA, Thailand.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Primary bioassay screening results of crude extracts

3.1.1 Cytotoxicity of crude extracts

The crude extracts of *N. orientalis* (L.) L. roots were preliminarily evaluated using cytotoxicity on human tumor cells assay. The dichloromethane crude extract showed significant cytotoxicity. The cytotoxic result of all crude extracts are shown in Table 3.1.

Table 3.1 Cytotoxicity against KB and HeLa cell lines of crude extracts.

Crude extracts	**IC_{50} (μg/mL) at λ 550 nm**	
	KB	**HeLa**
CH_2Cl_2	23	22
EtOAc	32	12
MeOH	90	62
Adriamycin (standard agent)	0.018	0.018

Pure compound ≤ 4 μg/mL

Crude extract ≤ 30 μg/mL

KB cell line: Human epidermoid carcinoma

HeLa cell line: Human cervical carcinoma

3.2 Properties and structural elucidation of isolated compounds

3.2.1 Naucleaorein (3)

Naucleaorein (**3**) was obtained as a yellowish amorphous solid. The molecular formula of $C_{22}H_{26}N_2O_4$ was deduced from the HRESI-MS ion at *m/z* 383.1960 $[M+H]^+$ (calcd for $C_{22}H_{27}N_2O_4$, 383.1972) and NMR data.

The ^1H NMR spectrum of compound **3** displayed aromatic proton signals in the downfield region for two triplets at δ_H 7.13 (1H, t, *J*=7.6 HZ, H-10) and 7.19 (1H, t, *J*=7.6 HZ, H-11), two doublets at δ_H 7.36 (1H, d, *J*=7.6 HZ, H-12) and 7.49 (1H,

d, *J*=7.6 HZ, H-9) and the singlet at δ_H 7.94 (1H, s, NH-1), which indicated that it was an unsubstituted in ring A of indole nucleus. The ^{1}H, ^{13}C and 2D NMR data of **3** (Table 3.2) revealed the presence of the methyl group signal at δ_H 1.02 (3H, d, *J*=6.4 HZ, H-21) and δ_C 14.5 (C-21) substituted on C-19 at δ_C 71.4, which was confirmed by the HMBC correlation of C-19 with methyl protons, while a singlet at δ_H 7.43 was assigned to H-17 (1H, s). These evidences were compared with ^{1}H, ^{13}C and 2D-NMR data of **8**, 19-*epi*-naucleidinal. It could be confirmed that these two compounds had the same basic skeleton. In addition, the presence of a -CH(OCH_3)$_2$ group at C-20 at δ_C 40.7 was indicated by the cross peaks of the methine proton at δ_H 4.22 (H-23) to two methoxy groups at δ_H 3.41 (3H, s, OCH_3-24) and 3.36 (3H, s, OCH_3-24´), which was confirmed by the detection of HMBC and HSQC correlations (Figure 3.2). The relative configuration of the four chiral carbons, C-3, C-15, C-19 and C-20 was confirmed by NOESY spectrum. The complete assignment of ^{1}H and ^{13}C NMR data of **3** are shown in Table 3.2. Thus, this new indole alkaloid was given the name as naucleaorein (Figure 3.1).

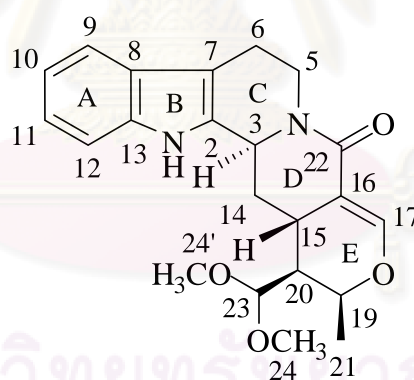


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Figure 3.1 Structure of naucleaorein (**3**, new compound).

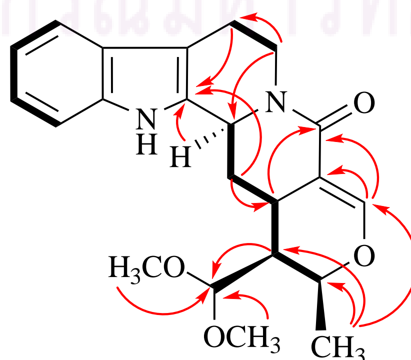

Figure 3.2 Selected HMBC (arrow curves) and COSY (bold lines) correlations of **3**.

Table 3.2 NMR data of **3** in $CDCl_3$ (400 MHz for ^1H, 100 MHz for ^{13}C).

Position	δ_C	δ_H (mult, J in Hz)	HMBC
1-NH	-	7.94 (1H, s)	C-8, C-12
2	132.0	-	-
3	53.2	4.93 (1H, m)	C-2, C-14
5a	42.9	3.05 (1H, m)[a]	-
5b	-	5.10 (1H, m)	C-3, C-6
6a	21.2	2.69 (1H, m)	C-2
6b	-	3.02 (1H, m)[b]	-
7	108.2	-	-
8	127.5	-	-
9	118.3	7.49 (1H, d, J=7.6 Hz)	C-11,13
10	119.9	7.13 (1H, t, J=7.6 Hz)	C-8,12
11	122.0	7.19 (1H, t, J=7.6 Hz)	C-9,13
12	111.1	7.36 (1H, d, J=7.6 Hz)	C-8,10
13	136.0	-	-
14a	29.6	1.90 (1H, ddd, J=5.6,13.4, 13.6 Hz)*	C-2, C-3, C-15, C-16
14b	-	2.90 (1H, ddd, J=5.6, 13.4, 13.6 Hz)	-
15	25.8	2.00 (1H, m)*	C-22
16	108.0	-	-
17	149.3	7.43 (1H, s)	C-15, C-16, C-19, C-22
19	71.4	4.42 (1H, m)	C-15, C-17
20	40.7	2.10 (1H, m)	C-23
21	14.5	1.02 (3H, d, J=6.4 Hz)	C-19, C-20
22	165.0	-	-
23	105.0	4.22 (1H, d, J=7.2 Hz)	C-24, C-24´
24-OCH_3	51.9	3.41 (3H, s)	C-23
24´-OCH_3	53.8	3.36 (3H, s)	C-23

[a,b] Signals were overlapped

* Data can be interchanged

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3.2.2 Naucleaoral A (5)

Naucleaoral A (**5**) was isolated as a yellowish amorphous solid, its molecular formula was determined as $C_{20}H_{20}N_2O_3$ on the basis of HRESIMS spectrum at *m/z* 359.1367 $[M+Na]^+$.

The ^1H NMR spectrum of compound **5** displayed signals for an aromatic proton of an unsubstituted indole nucleus (ring A) [two doublet of doublets at δ_H 7.05 (1H, dd, J=7.2, 7.6 Hz, H-10) and 7.12 (1H, dd, J=7.2, 7.6 Hz, H-11), two doublets at δ_H 7.24 (1H, d, J=7.6 Hz, H-12) and 7.43 (1H, d, J=7.2 Hz, H-9) and the singlet at

δ_H 7.71 (1H, s, 1-NH)]. Besides indole ring signals, compound **5** also showed the appearance of a δ-lactam ring (ring D) with a hydroxymethylene at C-16 (δ_C 101.1) that demonstrated the signal of hydrogen-bonded hydroxyl proton at δ 13.85 (s, 1-OH), which was elucidated by HMBC correlations of H-17 at δ_H 6.90 (1H, d, J=10.1 Hz) to C-16 (δ_C 101.1) and C-18 (δ_C 169.1). The interpretation of HMBC correlations of H-5b at δ_H 5.06 (1H, m) to C-3 (δ_C 49.1) and C-18 (δ_C 169.1), H-6a at δ_H 2.82 (1H, m) to C-2 (δ_C 132.0) and C-7 (δ_C 110.0) and H-14a at δ_H 1.83 (1H, m) to C-2 (δ_C 132.0) confirmed that ring B was fused to an indole nucleus at C-2 and C-7 and a δ-lactam ring (ring D) at C-3. In addition, the occurrence of a -(CHO)C=CCH_3 group was substituted on C-15 (δ_C 32.6), which inferred from the reveal of the methyl group signal at δ_H 2.16 (3H, d, J=7.2 Hz, H-19 and δ_C 13.2) allotted to C-20 (δ_C 147.9) while a singlet at δ_H 10.23 (1H, s, H-22) was assigned to an aldehyde proton also connected at C-22, which was deduced by HMQC and HMBC correlations (Figure 3.4). The NOESY interaction between H-22 (-CHO) and H-19 ($-CH_3$) revealed the *Z* configuration of this group as found in vallesiachotamine [33]. On the other hand, the ambiguously relative configurations of the two chiral carbons at C-3 and C-15 were also confirmed by NOESY spectrum (Figure 3.5). The complete assignment of ^{1}H and ^{13}C NMR data of **5** are shown in Table 3.3. On the basis of the above evidence, we proposed the structure of **5** as a new indole alkaloid, named naucleaoral A (Figure 3.3).

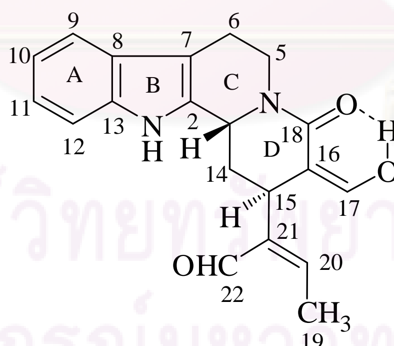

Figure 3.3 Structure of naucleaoral A (**5**, new compound).

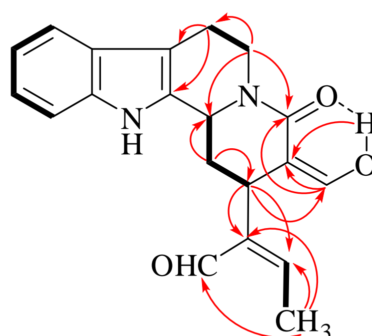

Figure 3.4 Selected HMBC (arrow curves) and COSY (bold lines) correlations of **5**.

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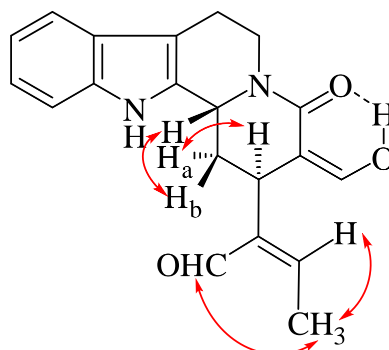

Figure 3.5 Key NOESY correlations for **5**.

Table 3.3 NMR data of **5** in $CDCl_3$ (400 MHz for ^{1}H, 100 MHz for ^{13}C).

Position	δ_C	δ_H	HMBC
1-NH	-	7.71 (1H, s)	C-7, C-8
2	132.0	-	-
3	49.1	4.43 (1H, d, *J*=11.6 Hz)	C-2, C-14
5a	39.0	2.79 (1H, m)*	C-6, C-18
5b	-	5.06 (1H, m)	C-3, C-6, C-18
6a	21.0	2.82 (1H, m)	C-2, C-5, C-7
6b	-	2.78 (1H, m)*	-
7	110.0	-	-
8	127.0	-	-
9	118.3	7.43 (1H, d, *J*=7.2 Hz)	C-7, C-8, C-12
10	119.9	7.05 (1H, dd, *J*=7.2, 7.6 Hz)	C-8, C-12
11	122.3	7.12 (1H, dd, *J*=7.2, 7.6 Hz)	C-10, C-12
12	111.0	7.24 (1H, d, *J*=7.6 Hz)	C-9, C-8
13	136.0	-	-
14a	33.1	1.83 (1H, dt, *J*=3.2, 13.6 Hz)	C-2,
14b	-	2.43 (1H, dt, *J*=3.2, 13.6 Hz)	C-16, C-17, C-18
15	32.6	3.65 (1H, br s)	C-17, C-18, C-21, C-22
16	101.1	-	-
17	161.6	6.90 (1H, d, *J*=10.1 Hz)	C-16, C-18
18	169.1	-	-
19	13.2	2.16 (3H, d, *J*=7.6 Hz)	C-20, C-22
20	147.9	6.50 (1H, q, *J*=7.6 Hz)	C-15, C-19, C-21
21	142.0	-	-
22	190.4	10.23 (1H, s)	C-15, C-20, C-21
-OH	-	13.85 (1H, d, *J*=10.1 Hz)	C-16

* Data can be interchanged

3.2.3 Naucleaoral B (6)

Naucleaoral B (**6**) was afforded as a yellowish amorphous solid and the molecular formula was established as $C_{20}H_{20}N_2O_3$ by means of HRESIMS spectrum at *m/z* 359.1377 $[M+Na]^+$, which was identical to that of **5**.

The ^1H NMR spectrum of compound **6** displayed aromatic proton signals in downfield for two doublet of doublets at δ_H 7.12 (1H, dd, *J*=7.2, 7.6 Hz, H-10) and 7.18 (1H, dd, *J*=7.2, 7.6 Hz, H-11), two doublets at δ_H 7.31 (1H, d, *J*=7.6 Hz, H9) and 7.51 (1H, d, *J*=7.2 Hz, H-12) and the singlet at δ_H 7.95 (1H, s, 1-NH), which suggested that this compound contained an unsubstituted indole nucleus (ring A). The ^1H and ^{13}C NMR spectra of **6** were very similar to those of **5** except for the signals of the -(CHO)C=CCH$_3$ group that connected at C-15 (δ_C 30.7), which was confirmed by HMQC and HMBC correlations (Figure 3.7). The downfield shift for the methyl protons at δ_H 2.03 (3H, d, *J*=7.2 Hz) and aldehyde proton at δ_H 10.23 (1H, s), and the highfield shift for methine proton at δ_H 6.78 (1H, q, *J*=7.2 Hz) of **6** was observed. After assigning the constitution of the whole planar structure of **6**, we turned our attention to the stereochemistry of the -(CHO)C=CCH$_3$ group. The NOESY spectrum of **6** (Figure 3.8) showed NOSEY interaction between H-22 (-CHO) and H-20, which supported a *E*-type of double bond of C-20 and C-21 for **6**. On the other hand, two absolute configurations of C-3 and C-15 were also indicated by means of NOESY spectrum. The complete assignments of **6** are shown in Table 3.4. Thereby, this new indole alkaloid was given the name as naucleaoral B (Figure 3.6).

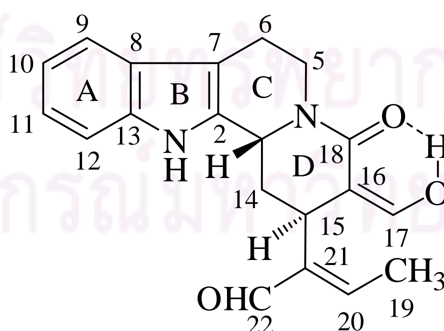

Figure 3.6 Structure of naucleaorein (**6**, new compound).

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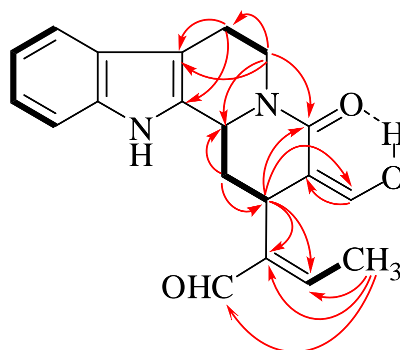

Figure 3.7 Selected HMBC (arrow curves) and COSY (bold lines) correlations of **6**.

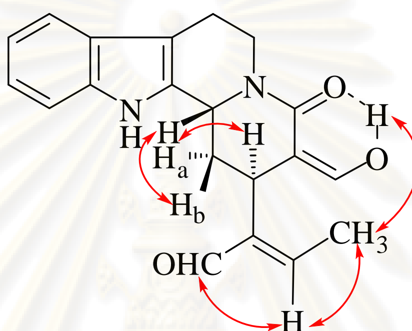

Figure 3.8 Key NOESY correlations for **6**.

Table 3.4 NMR data of **6** in $CDCl_3$ (400 MHz for ^1H, 100 MHz for ^{13}C).

Position	δ_C	δ_H (mult, J in Hz)	HMBC
1-NH	-	7.95 (1H, s)	C-2, C-7, C-8, C-13
2	132.0	-	-
3	50.3	4.58 (1H, d, J=10.0 Hz)	C-2, C-7, C-14
5a	39.2	2.91 (1H, m)*	C-6, C-18
5b	-	5.17 (1H, m)	C-3, C-6, C-18
6a	21.0	2.84 (1H, m)	C-2, C-5, C-7
6b	-	2.90 (1H, m)*	-
7	110.0	-	-
8	126.7	-	-
9	118.3	7.51 (1H, d, J=7.2 Hz)	C-7, C-8, C-12
10	119.9	7.12 (1H, dd, J=7.2, 7.6 Hz)	C-8, C-12
11	122.3	7.18 (1H, dd, J=7.2, 7.6 Hz)	C-10, C-12
12	111.0	7.31 (1H, d, J=7.6 Hz)	C-9, C-8
13	136.2	-	-
14a	33.6	1.87 (1H, dt, J=3.0, 13.2 Hz)	C-2, C-3
14b	-	2.34 (1H, dt, J=3.0, 13.2 Hz)	C-16, C-17, C-18
15	30.7	3.83(1H, br s)	C-17, C-18, C-21, C-22
16	101.1	-	-

Table 3.4 NMR data of **6** in $CDCl_3$ (400 MHz for ^1H, 100 MHz for ^{13}C) (Cont.).

Position	δ_C	δ_H (mult, J in Hz)	HMBC
17	160.3	6.97 (1H, d, J=10.4 Hz)	C-16, C-17, C-18
18	169.5	-	-
19	15.0	2.03 (3H, d, J=7.2 Hz)	C-20, C-22
20	153.2	6.78 (1H, q, J=7.2 Hz)	C-15, C-19, C-21
21	145.6	-	-
22	195.2	9.39 (1H, s)	C-15, C-20, C-21
-OH	-	13.80 (1H, d, J=10.4 Hz)	-

* Data can be interchanged

Naucleficine (1): orange amorphous solid; ^1H NMR (DMSO, 400 MHz): δ_H 11.82 (1H, s, NH-1), 10.41 (1H, s, H-21), 8.52 (1H, d, J = 7.6 Hz, H-17), 8.25 (1H, d, J = 7.2 Hz, H-19), 8.12 (1H, s, H-14), 7.62 (1H, d, J = 8.0 Hz, H-9), 7.60 (1H, dd, J =7.2, 7.6Hz, H-18), 7.46 (1H, d, J = 8.4 Hz, H-12), 7.24 (1H, t, J = 7.2, 8.4 Hz, H-11), 7.08 (1H, dd, J = 7.2, 8.0 Hz, H-10), 4.41 (2H, dd, J = 6.8, 6.4 Hz, H-5), 3.11 (2H, dd, J = 6.8, 6.4 Hz, H-6). ^{13}C NMR (DMSO, 100 MHz): δ 193.2 (C-21), 161.2 (C-22), 139.1 (C-19), 138.9 (C-3), 136.0 (C-15), 134.2 (C-17), 132.0 (C-20), 129.9 (C-2), 128.3 (C-8), 125.9 (C-18), 125.0 (C-7), 124.5 (C-11), 120.1 (C-10), 119.8 (C-9), 114.5 (C-16), 112.4 (C-12), 95.3 (C-14), 40.9 (C-5), 20.0 (C-6).

Naucleactonin A (2): yellowish powder; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 8.49 (1H, s, H-1), 8.23 (H, s, H-17), 7.53 (1H, d, J = 8 Hz, H-9), 7.37 (1H, d, J = 8 Hz, H-12), 7.25 (1H, t, J = 8 Hz, H-11), 7.10 (1H, t, J = 8 Hz, H-10), 7.00 (1H, s, H-14), 4.36 (2H, t, J = 6.4 Hz, H-5), 3.05 (2H, t, J = 6.4 Hz, H-6).

Naucleaorien (3): orange amorphous powder; mp 206-208 °C; $[\alpha]^{20}_D$ = -12° (c 0.005, MeOH); UV (MeOH) λ_{max} (log ε): 225 (2.45), 251 (2.24) nm; ; IR bands (KBr): 3458, 2925, 1721, 1659, 1622, 1382, 1059, 673 cm^{-1}; positive ion ESIMS m/z: 383.1960 $[M+H]^+$ (calcd for $C_{22}H_{27}N_2O_4$, 383.1972); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) are shown in Table 3.2.

Vanillic acid (4): pale yellow oil; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 7.45 (1H, d, J = 1.6 Hz, H-2), 7.45 (1H, dd, J = 8.8, 1.6 Hz, H-5), 6.74 (1H, d, J = 8.8 Hz, H-6), 3.71 (3H, s, OCH_3-8).

Naucleaoral A (5): orange amorphous powder; mp 165-167 °C; $[\alpha]^{20}_{D}$ = -74° (*c* 0.005, MeOH); UV (MeOH) λ_{max} (log ε): 264 (2.65), 272 (2.69), 278 (2.66) nm; IR bands (KBr): 3427, 2925, 2856, 1719, 1659, 1630, 1444, 1381 1264, 1164, 1111, 752, 674 cm^{-1}; positive ion HRESIMS *m/z*: $[M+Na]^+$ 359.1367 (calcd for $C_{20}H_{20}N_2O_3Na$, 359.1367); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) are shown in Table 3.3.

Naucleaoral B (6): orange amorphous powder; mp 177-179 °C; $[\alpha]^{20}_{D}$ = -81° (*c* 0.010, MeOH); UV (MeOH) λ_{max} (log ε): 263 (2.71), 272 (2.69), 278 (2.70) nm; IR bands (KBr): 3413, 2927, 2850, 1707, 1657, 1631, 1442, 1382, 1198, 1116, 752, 672 cm^{-1}; positive ion HRESIMS *m/z*: $[M+Na]^+$ 359.1377 (calcd for $C_{20}H_{20}N_2O_3Na$, 359.1367); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) are shown in Table 3.4.

Naucleidinal (7): orange amorphous powder; ^1H NMR (CD_3COCD_3, 400 MHz): δ_H 10.18 (1H, br s, NH-1), 9.60 (1H, d, *J* = 3.2 Hz, H-21), 7.29 (1H, d, *J* = 8.0 Hz, H-9), 7.26 (1H, s, H-17), 7.20 (1H, d, *J* = 8.0 Hz, H-12), 6.95 (1H, t, *J* = 8.0 Hz, H-11), 6.88 (1H, t, *J* = 8.0 Hz, H-11), 4.95 (1H, m, H-5), 4.40 (1H, dd, *J* = 5.4, 2.0 Hz, H-3), 4.03 (1H, m, H-19), 2.5-2.9 (2H, m, H-5, H-6), 2.60 (1H, m, H-14), 2.60 (1H, m, H-20), 1.80 (1H, ddd, *J* = 13.7, 13.6, 5.3 Hz, H-14), 1.30 (3H, d, *J* = 6.0 Hz, H-18). ^{13}C NMR (CD_3 $COCD_3$, 100 MHz): δ 201.5 (C-21), 163.7 (C-22), 149.9 (C-17), 136.3 (C-13), 133.8 (C-2), 127.5 (C-8), 121.3 (C-11), 118.9 (C-10), 117.7 (C-9), 111.8 (C-12), 110.0 (C-16), 107.9 (C-7), 71.0 (C-19), 56.5 (C-20), 53.3 (C-3), 42.9 (C-5), 29.0 (C-14), 28.0 (C-15), 20.7 (C-6), 18.6 (C-18).

19-*Epi*-naucleidinal (8): orange amorphous powder; ^1H NMR (CD_3COCD_3, 400 MHz): δ_H 10.19 (1H, br s, NH-1), 9.72 (1H, d, *J* = 3.2 Hz, H-21), 7.30 (1H, d, *J* = 8.0 Hz, H-9), 7.24 (1H, d, *J* = 8.0 Hz, H-12), 7.19 (1H, s, H-17), 6.96 (1H, t, *J* = 8.0 Hz, H-11), 6.88 (1H, t, *J* = 8.0 Hz, H-11), 4.95 (1H, m, H-5), 4.84 (1H, dd, *J* = 5.4, 2.0 Hz, H-3), 4.17 (1H, m, H-19), 2.5-2.9 (2H, m, H-5, H-6), 2.62 (1H, m, H-14), 2.60 (1H, m, H-20), 1.82 (1H, ddd, *J* = 13.8, 13.5, 5.5 Hz, H-14), 2.09 (3H, d, *J* = 6.8 Hz, H-18). ^{13}C NMR (CD_3 $COCD_3$, 100 MHz): δ 189.9 (C-21), 163.5 (C-22), 148.8 (C-17), 136.0 (C-13), 134.8 (C-2), 128.0 (C-8), 121.2 (C-11), 118.9 (C-10), 117.7 (C-9),

111.2 (C-12), 110.0 (C-16), 107.5 (C-7), 69.6 (C-19), 53.4 (C-20), 53.3 (C-3), 42.7 (C-5), 32.5 (C-14), 23.2 (C-15), 20.8 (C-6), 14.9 (C-18).

Strictosamide (9): yellow amorphous powder; ^{1}H NMR (CD_3OD, 400 MHz): δ_H 7.28 (1H, s, H-17), 7.27 (1H, d, *J* = 7.2 Hz, H-9), 7.22 (1H, d, *J* = 7.6 Hz, H-12), 6.98 (1H, dd, *J* = 7.2, 7.6 Hz, H-11), 6.89 (1H, dd, *J* = 7.2, 7.6 Hz, H-10), 5.53 (1H, m, H-19), 5.30 (1H, s, H-21), 5.28 (1H, m, H-18a), 5.23 (1H, m, H-18b), 4.93 (1H, m, H-3), 4.85 (1H, t, *J* = 4 Hz, H-5b), 4.51 (1H, s, H-2'), 4.47 (1H, d, *J* = 8 Hz, H-1'), 3.77 (1H, d, *J* =12 Hz, H-6'a), 3.51 (1H, m, H-6'b), 3.38 (1H, m, H-5'), 3.15-3.30 (1H, m, H-4'), 2.96 (1H, m, H-5a), 2.85 (1H, m, H-3'), 2.93-2.98 (1H, m, H-6b), 2.77 (1H, m, H-15), 2.66-2.69 (1H, m, H-6a), 2.62-2.69 (1H, m, H-20), 2.33 (1H, m, H-14b), 1.91 (1H, m, H-14a). ^{13}C NMR (CD_3OD, 100 MHz): δ 165.6 (C-22), 147.8 (C-17), 136.3 (C-13), 133.3 (C-2), 132.9 (C-19), 127.3 (C-8), 121.1 (C-11), 119.2 (C-18), 118.7 (C-10), 117.3 (C-9), 110.9 (C-12), 108.9 (C-7), 107.7 (C-16), 99.0 (C-1'), 96.6 (C-21), 76.8 (C-3'), 76.5 (C-5'), 72.9 (C-2'), 69.9 (C-4'), 61.2 (C-6'), 53.7 (C-3), 43.4 (C-5), 43.3 (C-20), 25.9 (C-14), 23.5 (C-15), 20.7 (C-6).

Alpigenoside (**10**)**:** white crystal; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 7.54 (6H, s, H-3), 5.23 (1H, m, H-1), 3.65 and 3.67 (2 x 3H, 2s, 2 x $COOCH_3$), 4.43 (1H, d, *J* = 7.6 Hz, H-1'), 3.89 (1H, m, H-6'a), 3.81 (1H, m, H-6'b), 3.79 (1H, m, H-4'), 3.76 (1H, m, H-3'), 3.57 (1H, m, H-5’), 3.51 (1H, m, H-2'), 3.07 (1H, m, H-8), 2.63 (2H, m, H-16), 2.34 (1H, m, H-5), 2.09 (1H, m, H-9), 1.36 (3H, d, *J* = 6.4 Hz, H-10).

Sweroside (11): yellow amorphous powder; ^{1}H NMR (CD_3OD, 400 MHz): δ_H 7.59 (1H, d, *J* = 2.0 Hz, H-3), 5.55 (1H, s, H-1), 5.55 (1H, dt, *J* = 9.6, 17.2 Hz, H-8), 5.31 (1H, d, *J* = 17.6 Hz, H-10b), 5.26 (1H, d, *J* = 10.4 Hz, H-10a), 4.68 (1H, d, *J* = 7.6 Hz, H-1'), 4.44 (1H, m, H-7b), 4.37 (1H, d, *J* =2.0, 11.7 Hz, H-7a), 3.89 (1H, t, *J* = 10.4 Hz, H-6'a), 3.67 (1H, t, *J* = 6.4 Hz, H-6'b), 3.44 (1H, m, H-4'), 3.38 (1H, m, H-3'), 3.34 (1H, m, H-5'), 3.19 (1H, t, *J* = 8.3 Hz, H-2'), 3.19 (1H, m, H-5), 2.76 (1H, dd, *J* = 1.1, 5.6 Hz, H-9), 1.79 (1H, m, H-6a), 1.68 (1H, dd, *J* = 4.4, 14.0 Hz, H-6b). ^{13}C NMR (DMSO, 100 MHz): δ 167.13 (C-11), 152.56 (C-3), 131.89 (C-8), 119.44 (C-10), 104.59 (C-4), 98.23 (C-1'), 96.54 (C-1), 76.94 (C-3'), 76.77 (C-5'), 73.26 (C-2'), 70.08 (C-4'), 68.31 (C-7), 61.93 (C-6'), 42.38 (C-9), 27.00 (C-5), 24.49 (C-6).

Pumiloside (12): white amorphous poder; ^{1}H NMR (DMSO, 400 MHz): δ_H 8.10 (1H, d, *J* = 8.0 Hz, H-9), 7.64 (1H, t, *J* = 8.0 Hz, H-11), 7.59 (1H, d, *J* = 8.0 Hz, H-12), 7.32 (1H, t, *J* = 8.0 Hz, H-10), 7.02 (1H, d, *J* = 2.4 Hz, H-17), 5.76 (1H, m, H-19), 5.44 (1H, d, *J* = 17.2 Hz, H-18b), 5.36 (1H, s, H-21), 5.31 (1H, d, *J* = 10.4 Hz, H-18a), 4.72 (1H, d, *J* =11.6 Hz, H-3), 4.52 (1H, d, *J* = 8.0 Hz, H-1'), 4.46 (1H, d, *J* = 14.8 Hz, H-5b), 4.31 (1H, d, *J* = 14.8 Hz, H-5a), 3.69 (1H, m, H-6'b), 3.42 (1H, m, H-6'a), 3.23 (1H, m, H-15), 3.14 (1H, m, H-5'), 3.13 (1H, m, H-3'), 3.00 (1H, m, H-4'), 2.98 (1H, m, H-2'), 2.60 (1H, m, H-20), 2.50 (1H, overlapped, H-14b), 1.96 (1H, q, *J* = 11.2 Hz, H-14a). ^{13}C NMR (DMSO, 100 MHz): δ 173.0 (C-7), 164.6 (C-22), 150.6 (C-2), 145.6 (C-17), 141.0 (C-13), 132.3 (C-19), 132.1 (C-11), 125.6 (C-8), 125.1 (C-9), 123.8 (C-10), 121.1 (C-18), 119.1 (C-12), 113.3 (C-6), 109.3 (C-6), 98.2 (C-1'), 95.3 (C-21), 77.7 (C-3'), 76.8 (C-5'), 73.6 (C-2'), 70.5 (C-4'), 61.5 (C-6'), 59.9 (C-3), 47.9 (C-5), 44.0 (C-20), 28.6 (C-14), 24.1 (C-15).

3.3 Bioassay activity of isolated compounds

3.3.1 Cytotoxicity against KB and HeLa cell lines of isolated compounds

The cytotoxicity against HeLa and KB cell lines of all isolated compounds were determined using MTT colorimetric assay and the results were shown in Table 3.5.

Table 3.5 *In vitro* cytotoxicity of compounds **1**–**12** against HeLa and KB cells.

Isolated compounds	IC_{50} (μg/mL)	
	HeLa cell	**KB cell**
naucleficine (**1**)	6.8	6.8
naucleactonin A (**2**)	NA[a]	42.0
naucleaorien (**3**)	9.5	5.0
vanillic acid (**4**)	NT[b]	NT[b]
naucleaoral A (**5**)	4.0	38.9
naucleaoral B (**6**)	7.8	9.5
naucleidinal (**7**)	5.5	4.8
19-*epi*-naucleidinal (**8**)	18.0	6.8
strictosamide (**9**)	NA[a]	80.0
alpigenoside (**10**)	NT[b]	NT[b]
sweroside (**11**)	38.0	50.0
pumiloside (**12**)	NA[a]	NA[a]
Adriamycin[c]	0.018	0.018

[a] No activity

[b] Not test

[c] Standard Agent

As seen in Table 3.5, compounds **1**, **3**, **6** and **7** showed very modest cytotoxicity against both HeLa and KB cell lines with IC_{50} values of 6.8 and 6.8, 9.5 and 5.0, 7.8 and 9.5 and 5.5 and 4.8 μg/mL, respectively. Compound **5** exhibited significant cytotoxic activity against only HeLa cells (IC_{50} = 4.0 μg/mL), while compound **8** showed only very modest cytotoxic activity against KB cells (IC_{50} = 6.8

μg/mL). On the other hand, compounds **2**, **9** and **11-12** could be regarded as inactive (IC_{50} >30.0 μg/mL).

3.3.2 Antimalarial activity of non-glycosidic indole alkaloids

The antimalarial activity of non-glycosidic indole alkaloids (compounds **1-2** and **5-8**) were determined using antimalarial assay and the results showed that these compounds could be regarded as inactive (IC_{50} >10.00 μg/mL).

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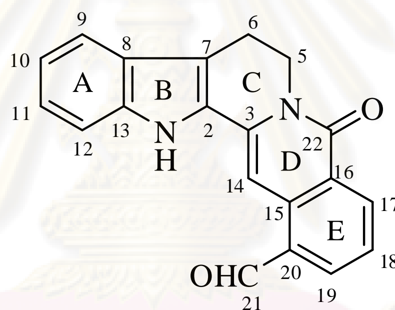
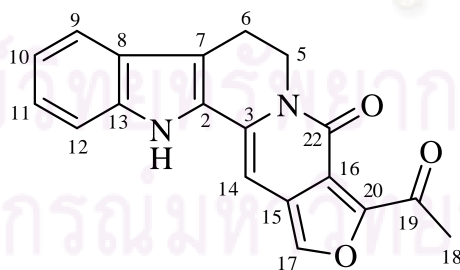
CHAPTER IV

CONCLUSION

In conclusion, the isolation and purification of the CH_2Cl_2, EtOAc and MeOH crude extracts from the roots of *N. orientalis* (L.) L. afforded three new indole alkaloids, naucleaorien (**3**), naucleaoral A (**5**) and B (**6**) along with nine known compounds, naucleficine (**1**), naucleactonin A (**2**), vanillic acid (**4**), naucleidinal (**7**), 19-*epi*-naucleidinal (**8**), strictosamide (**9**), alpigenoside (**10**), sweroside (**11**) and pumiloside (**12**). The chemical structures of all isolated compounds were characterized according to means of spectral analysis as well as comparison with the previous literature data.



naucleficine (**1**)



naucleactonin A (**2**)

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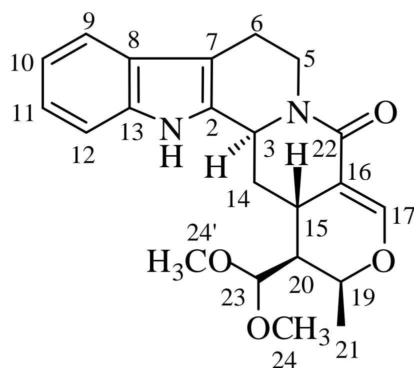
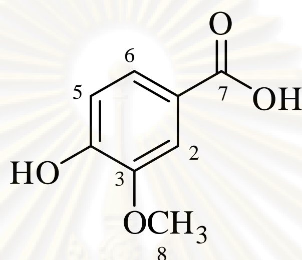
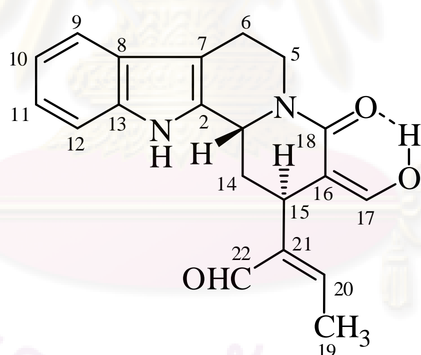


naucleaorein (**3**, new compound)



vanillic acid (**4**)



naucleaoral A (**5**, new compound)

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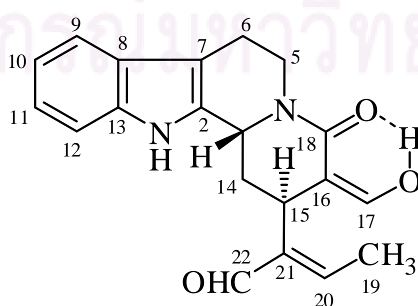
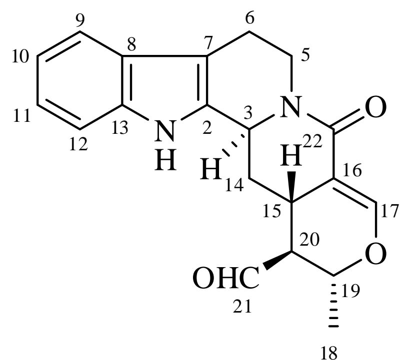
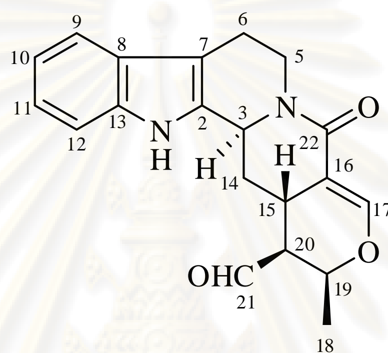


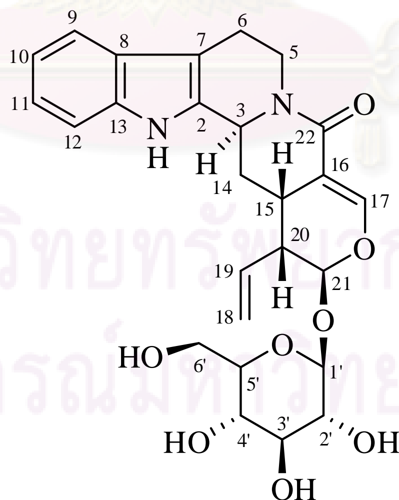
naucleaoral B (**6**, new compound)



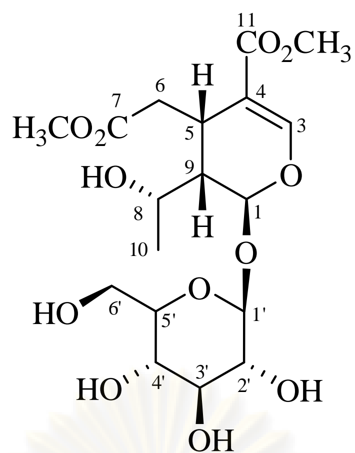
naucleidinal (**7**)



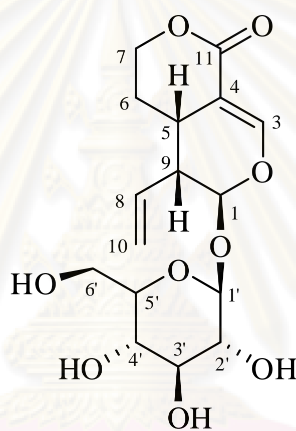
19-*epi*-naucleidinal (**8**)



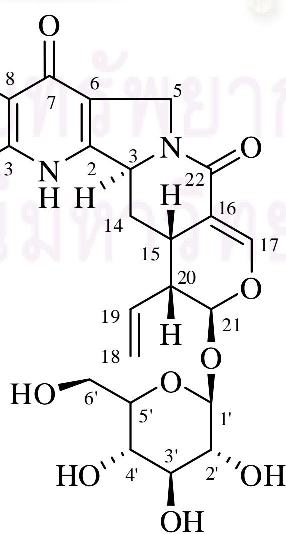
strictosamide (**9**)



alpigenoside (**10**)



sweroside (**11**)



pumiloside (**12**)

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All of the isolated compounds were examined for cytotoxicity on HeLa and KB cell lines and non-glycosidic indole alkaloids were also evaluated for antimalarial activity. Compounds **1**, **3**, **6** and **7** showed very modest cytotoxicity against both HeLa and KB cell lines (IC_{50} = 6.8 and 6.8, 9.5 and 5.0, 7.8 and 9.5 and 5.5 and 4.8 µg/mL, respectively). Compound **5** exhibited significant cytotoxic activity against only HeLa cells (IC_{50} = 4.0 µg/mL), while compound **8** showed only very modest cytotoxic activity against KB cells (IC_{50} = 6.8 µg/mL). On the other hand, compounds **2**, **9** and **11-12** were inactive (IC_{50} >30.0 µg/mL). Theses results led us to conclude that the presence of aldehyde groups on indole alkaloids could respond a significant cytotoxicity. It is worth noting that compounds **1**, **3** and **5-7** might be a lead compounds as antitumor agents.

The results of testing for antimalarial activity of non-glycosidic indole alkaloids (compounds **1-2** and **5-8**) showed that these compounds could be regarded as inactive (IC_{50} >10.00 μg/mL).

The future work may involve the synthesis of isolated compounds for increasing quantity and biological activity that could be developed into new drugs. Novel active compounds will afford the target for future synthesis and structure-activity relationship studies as well. This will lead to better understanding on the interaction between active compounds and diseases.

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APPENDIX

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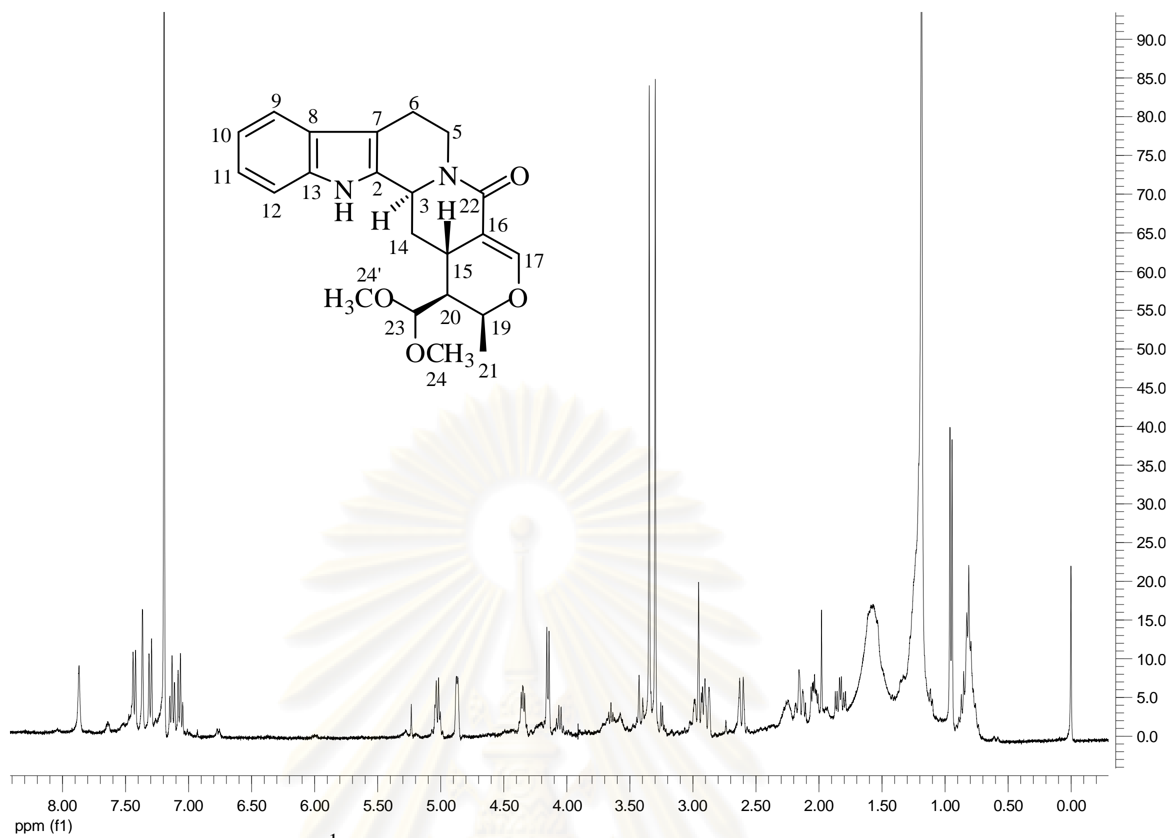

Figure A-1.1 ^{1}H NMR spectrum ($CDCl_3$-d_1) of naucleaorien (**3**).

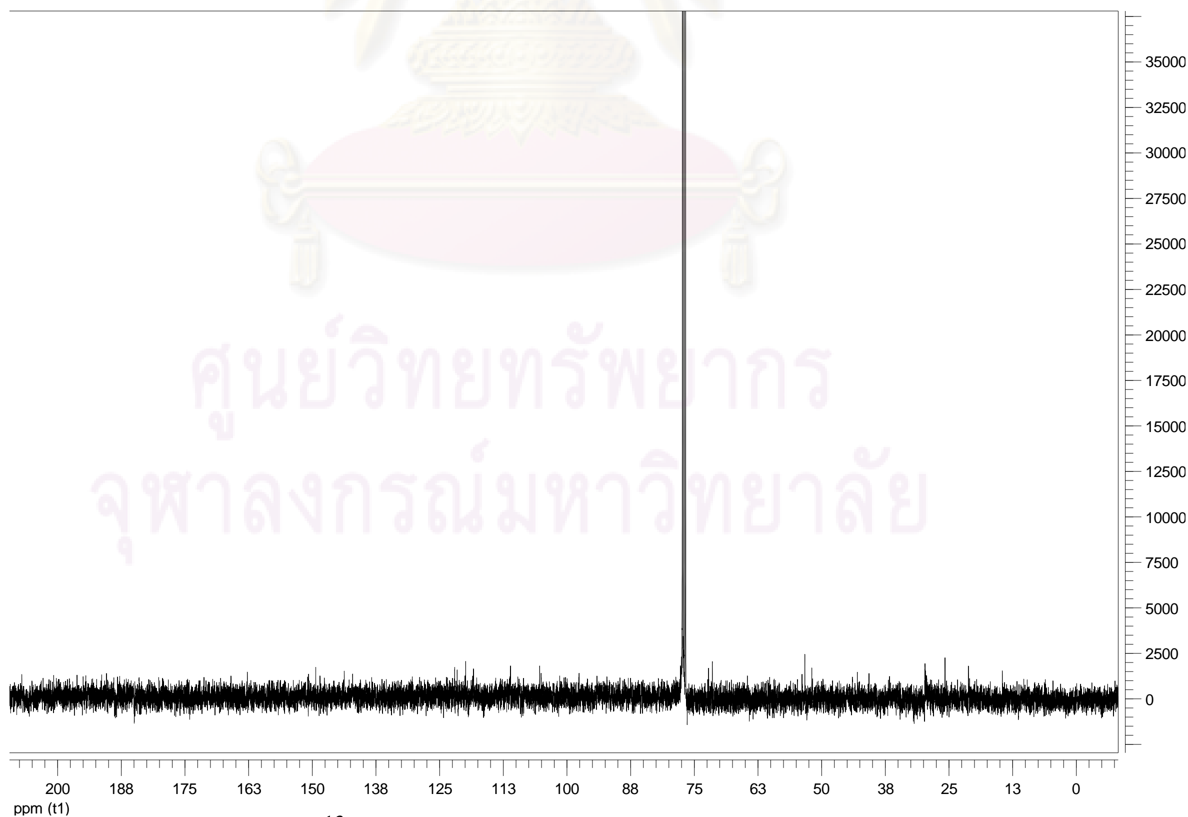

Figure A-1.2 ^{13}C NMR spectrum ($CDCl_3$-d_1) of naucleaorien (**3**).

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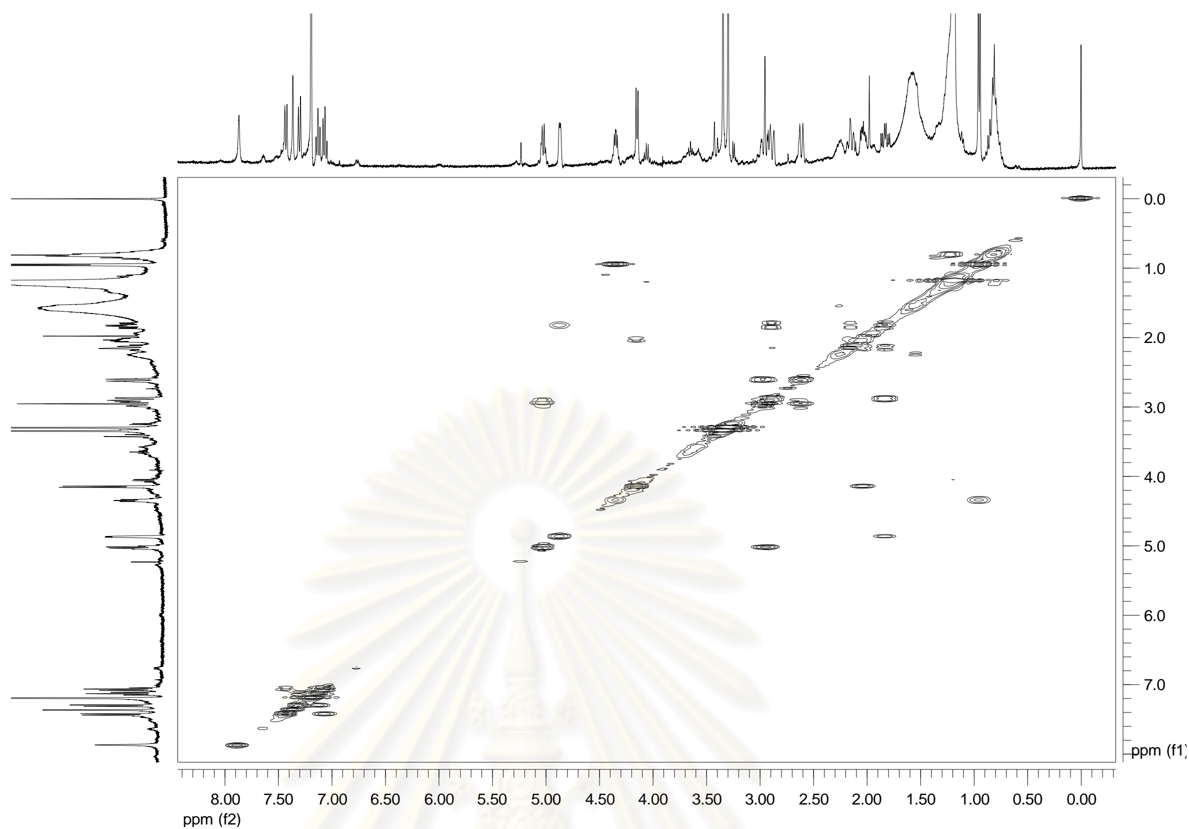
จุฬาลงกรณ์มหาวิทยาลัย



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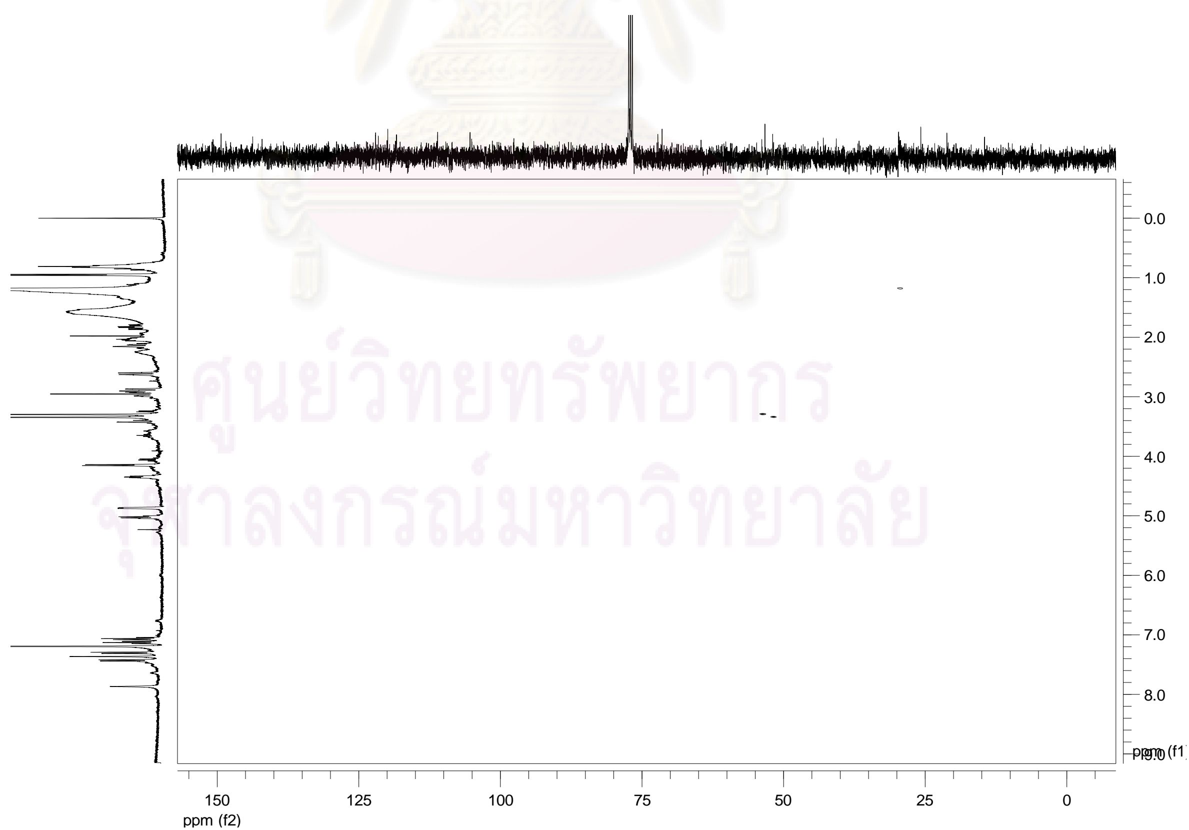

Figure A-1.4 HSQC spectrum ($CDCl_3$-d_1) of naucleaorien (**3**).

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จุฬาลงกรณ์มหาวิทยาลัย

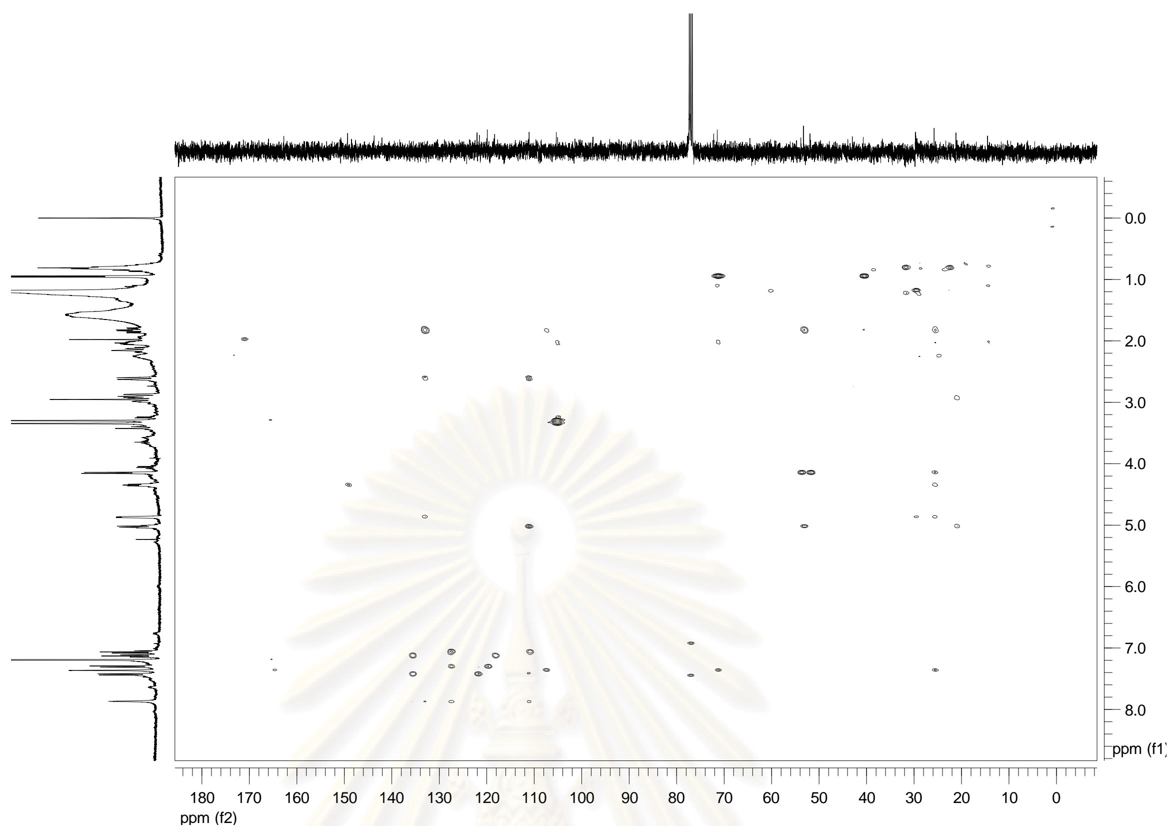

Figure A-1.5 HMBC spectrum ($CDCl_3$-d_1) of naucleaorien (**3**).

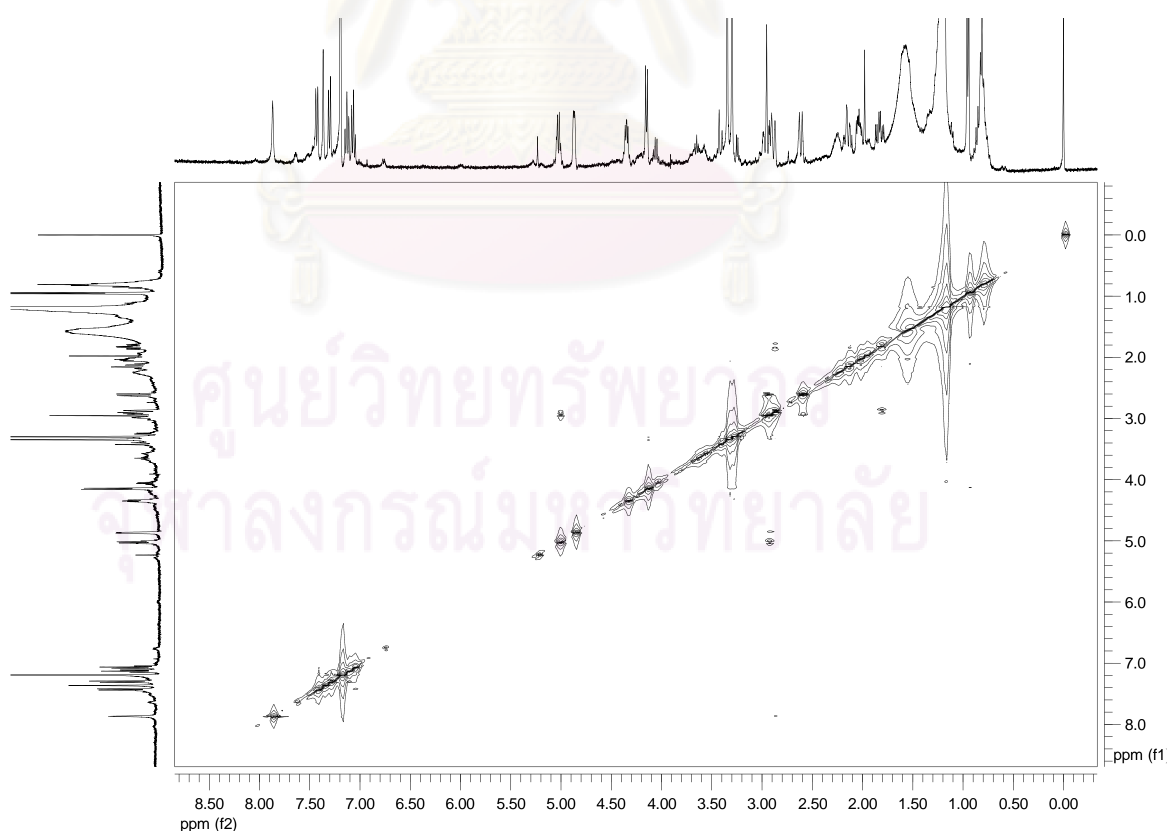

Figure A-1.6 NOESYspectrum ($CDCl_3$-d_1) of naucleaorien (**3**).

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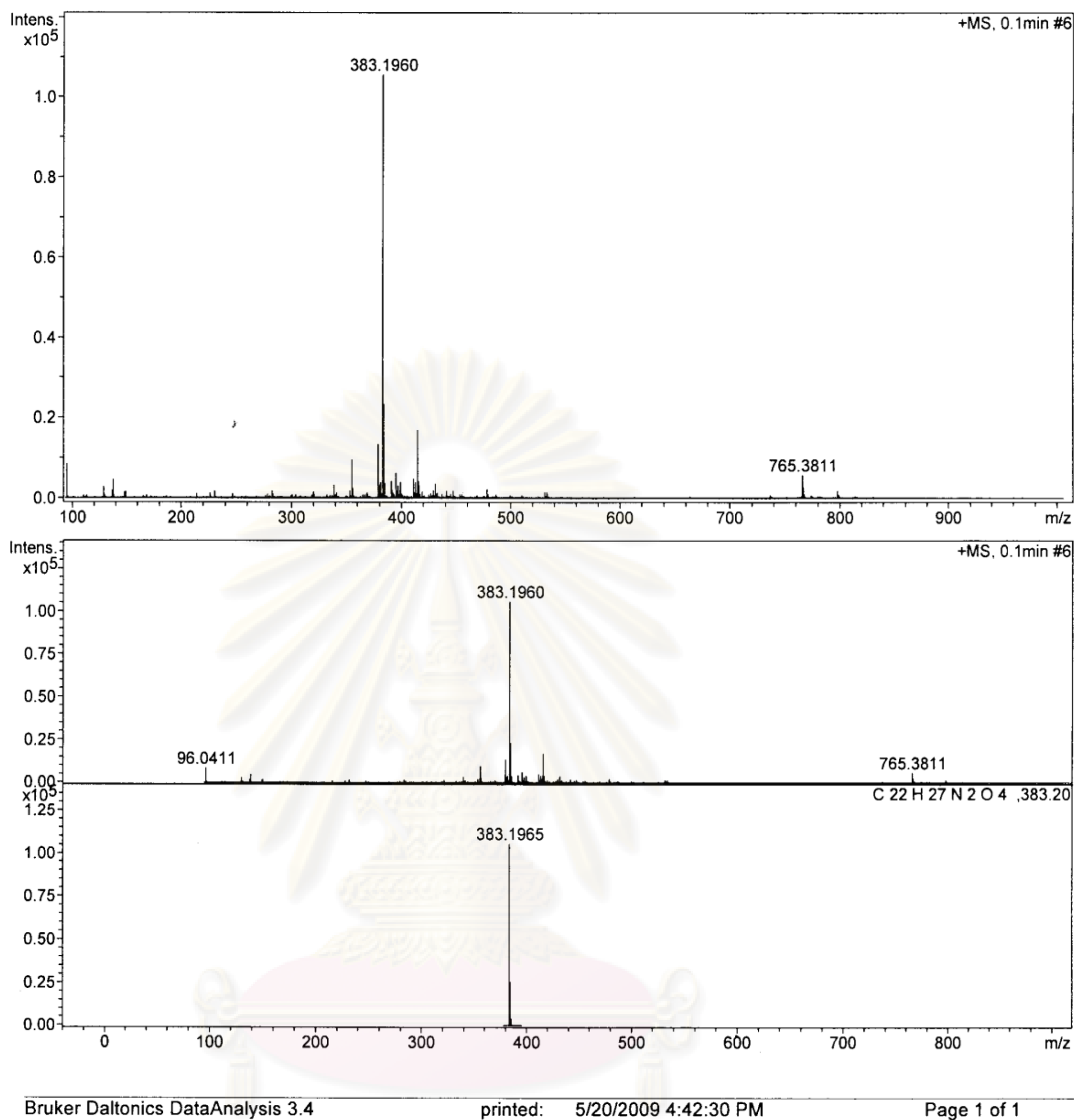

Figure A-1.7 High resolution mass spectrum of naucleaorien (**3**).

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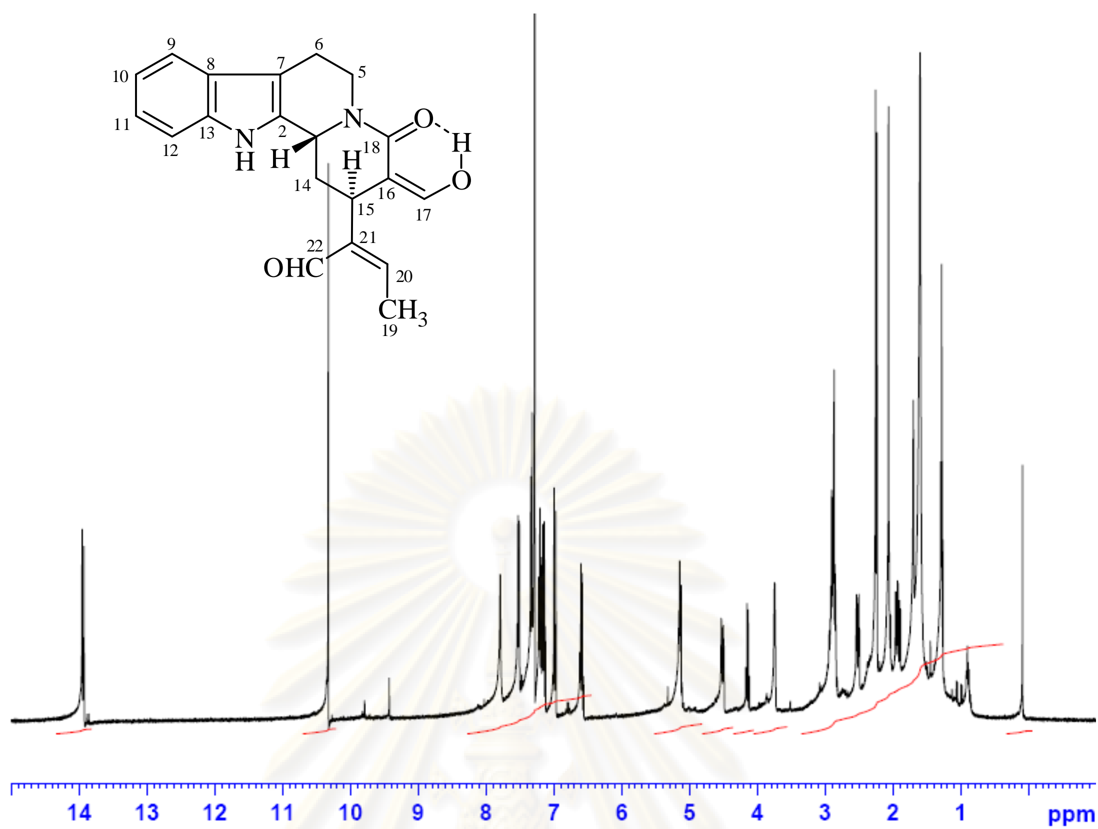

Figure B-1.1 ^{1}H NMR spectrum ($CDCl_3$-d_1) of naucleaoral A (**5**).

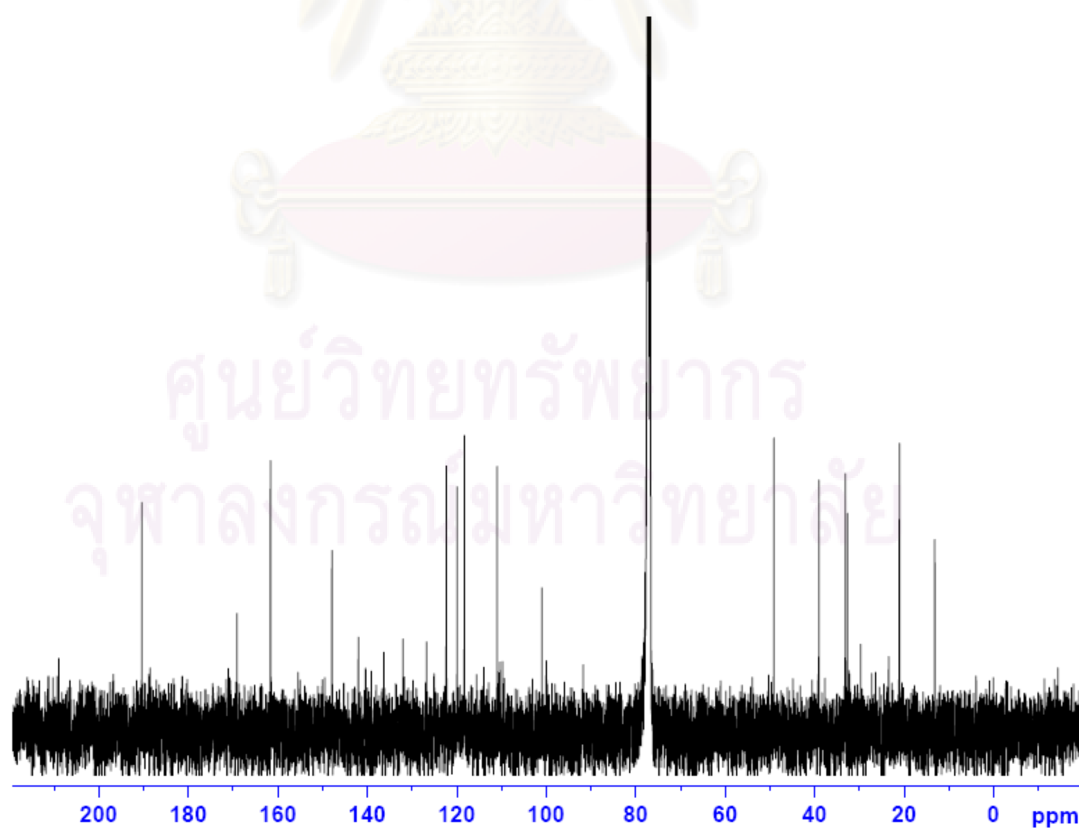

Figure B-1.2 ^{13}C NMR spectrum ($CDCl_3$-d_1) of naucleaoral A (**5**).

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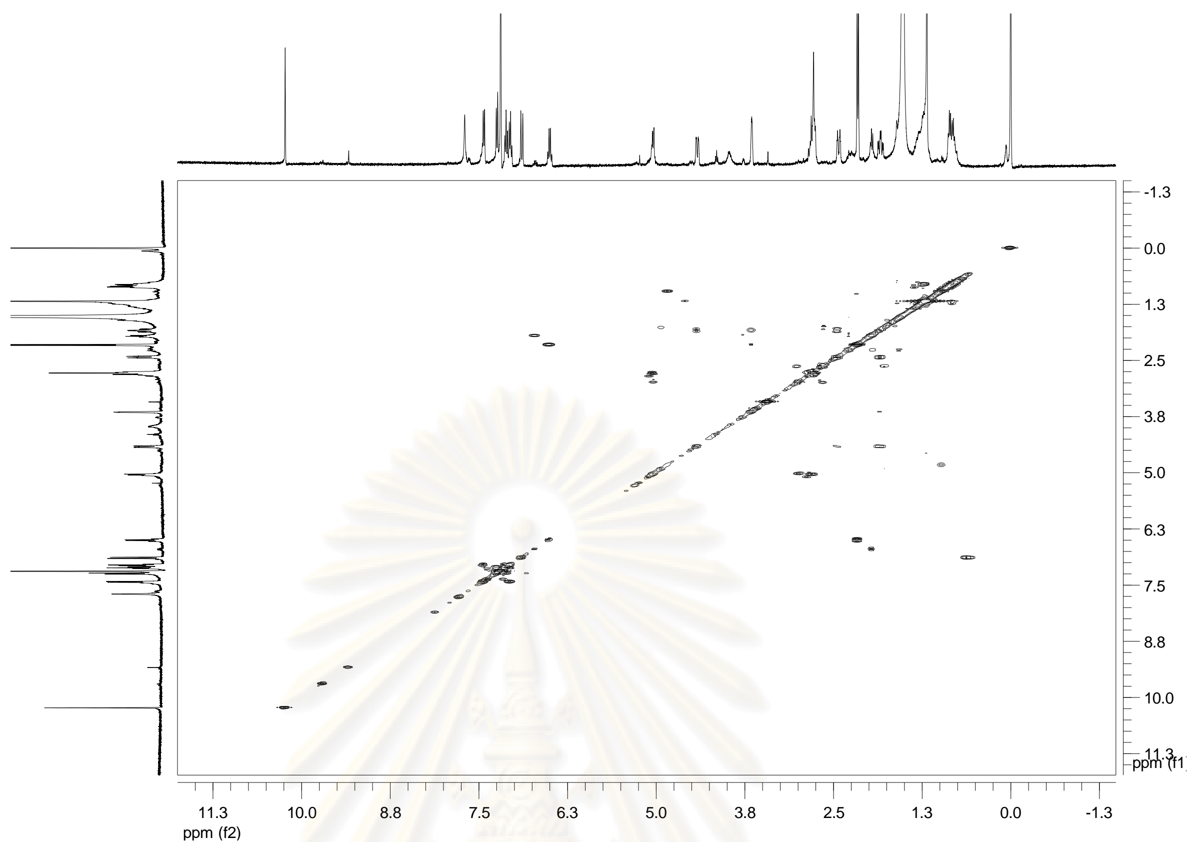

Figure B-1.3 COSY spectrum ($CDCl_3$-d_1) of naucleaoral A (**5**).

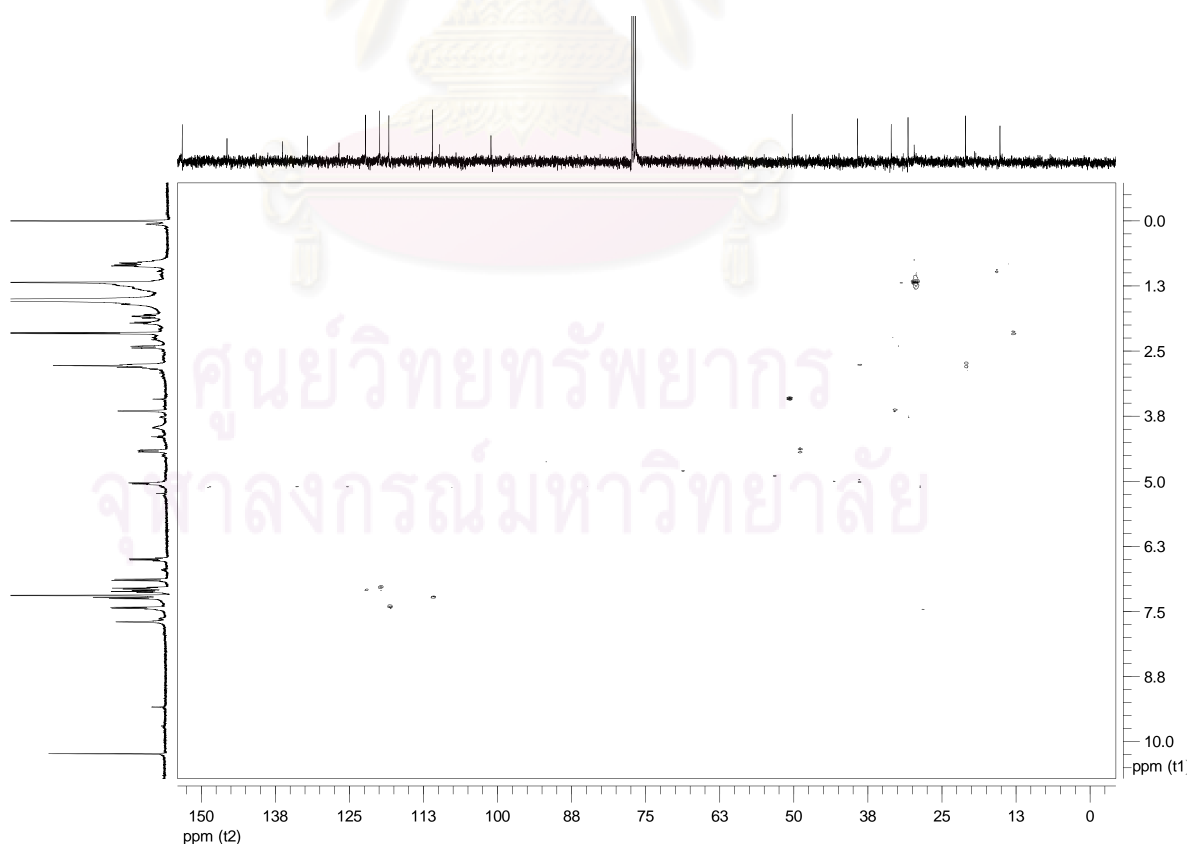

Figure B-1.4 HSQC spectrum ($CDCl_3$-d_1) of naucleaoral A (**5**).

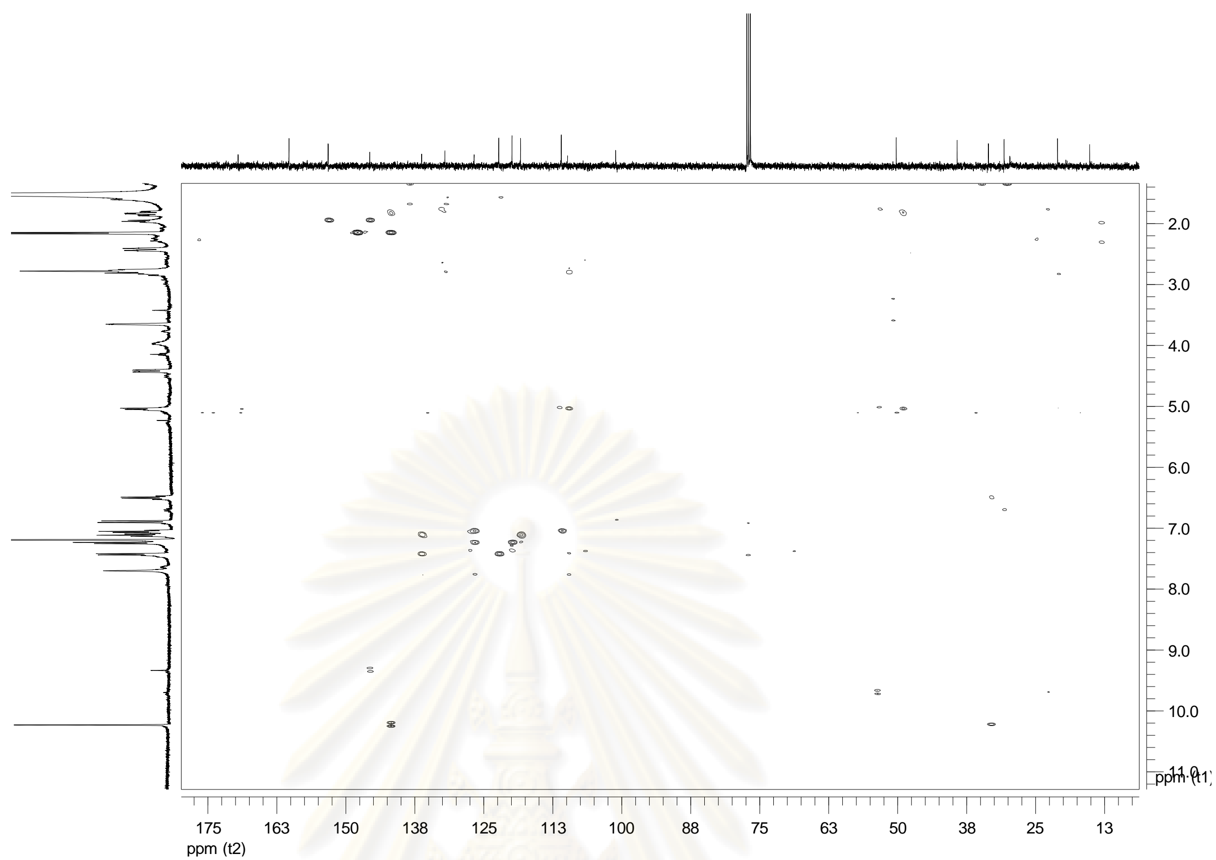

Figure B-1.5 HMBC spectrum ($CDCl_3$-d_1) of naucleaoral A (**5**).

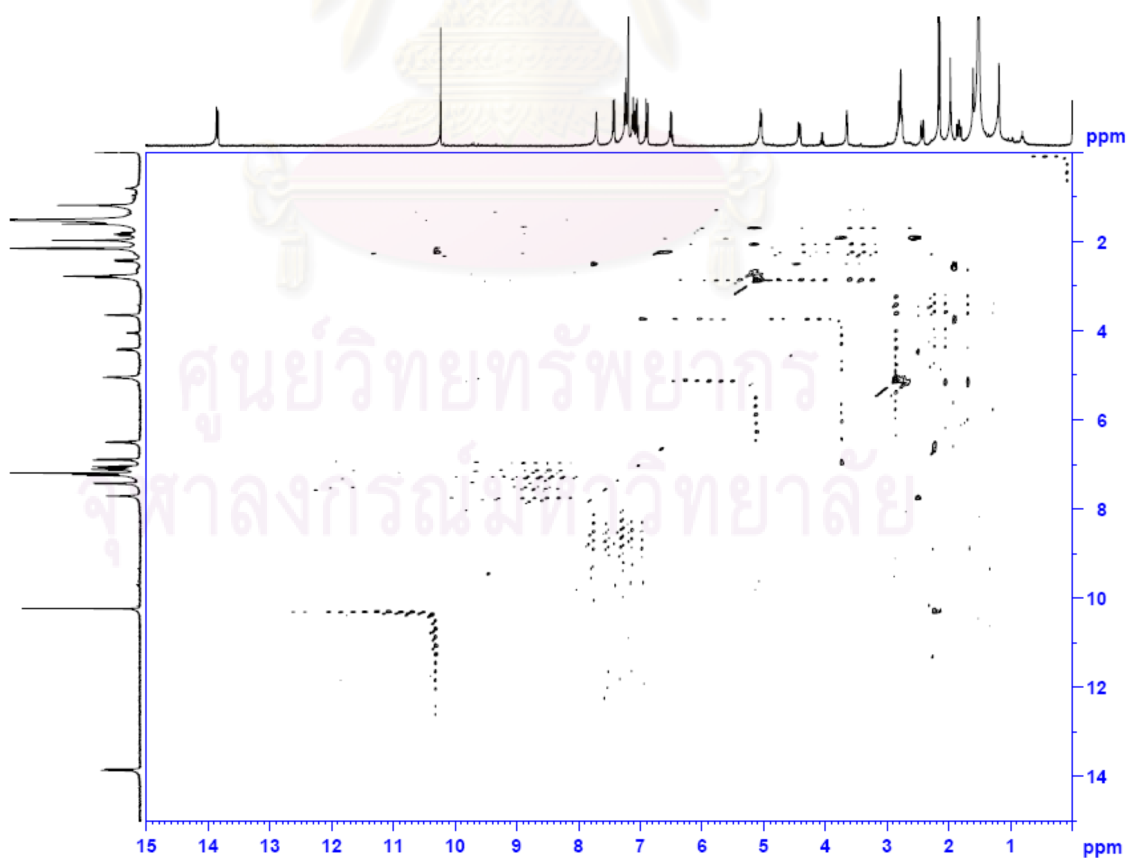

Figure B-1.6 NOESYspectrum ($CDCl_3$-d_1) of naucleaoral A (**5**).

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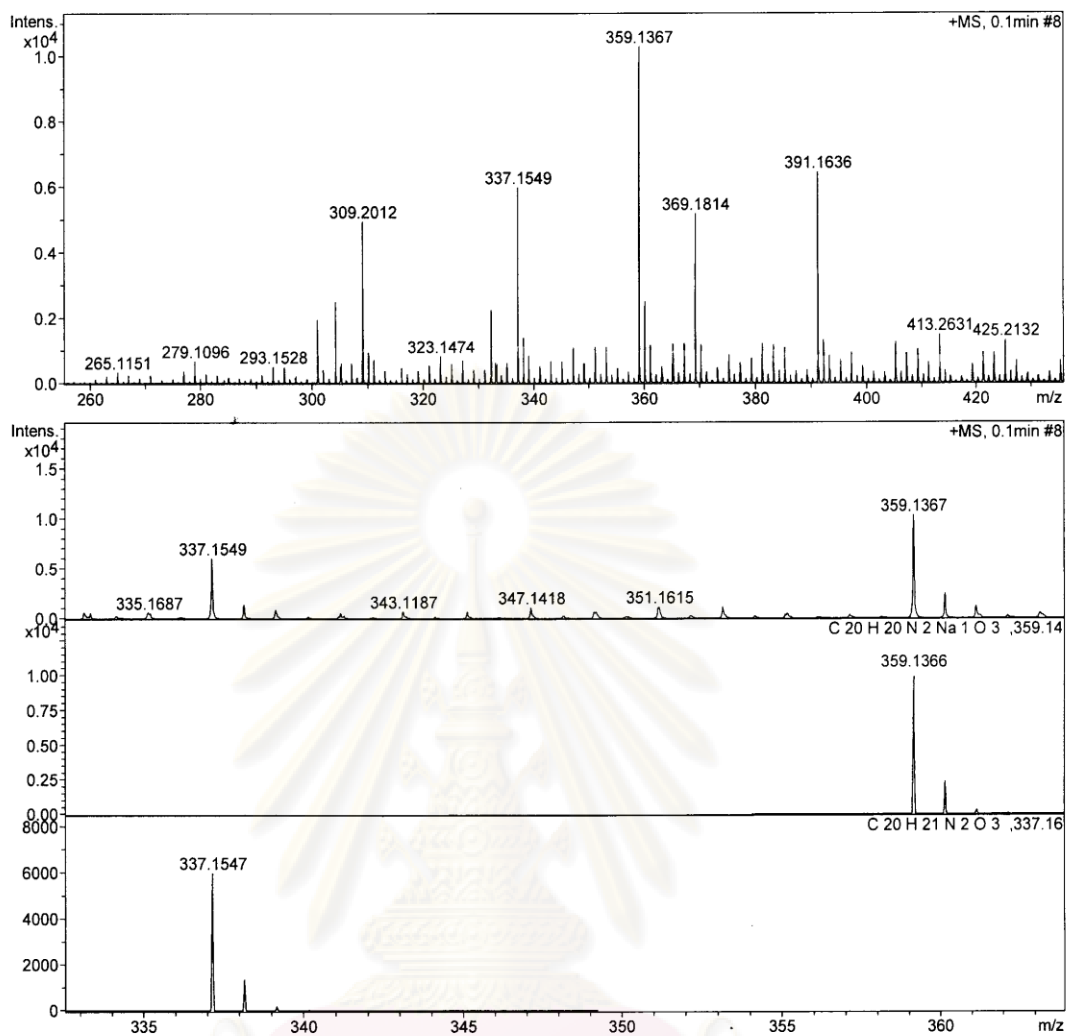

Figure B-1.7 High resolution mass spectrum of naucleaoral A (**5**).

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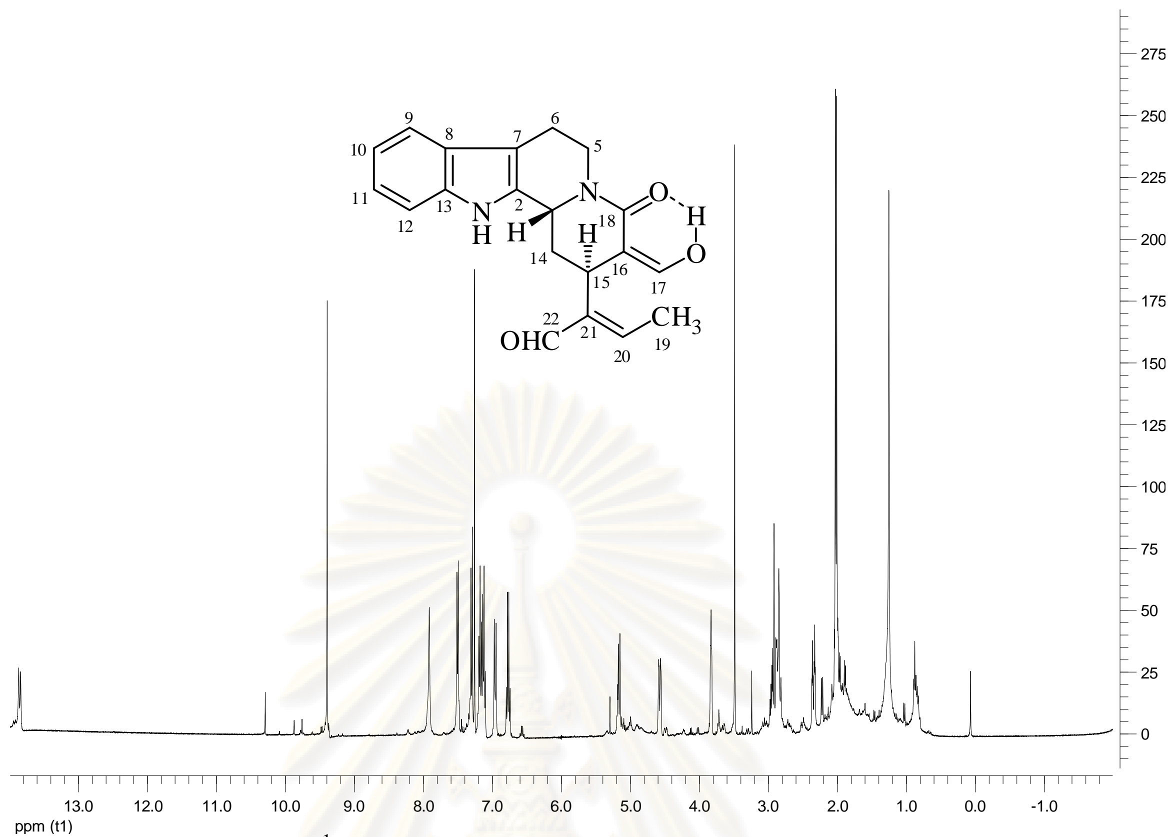

Figure C-1.1 ^{1}H NMR spectrum ($CDCl_3$-d_1) of naucleaoral B (**6**).

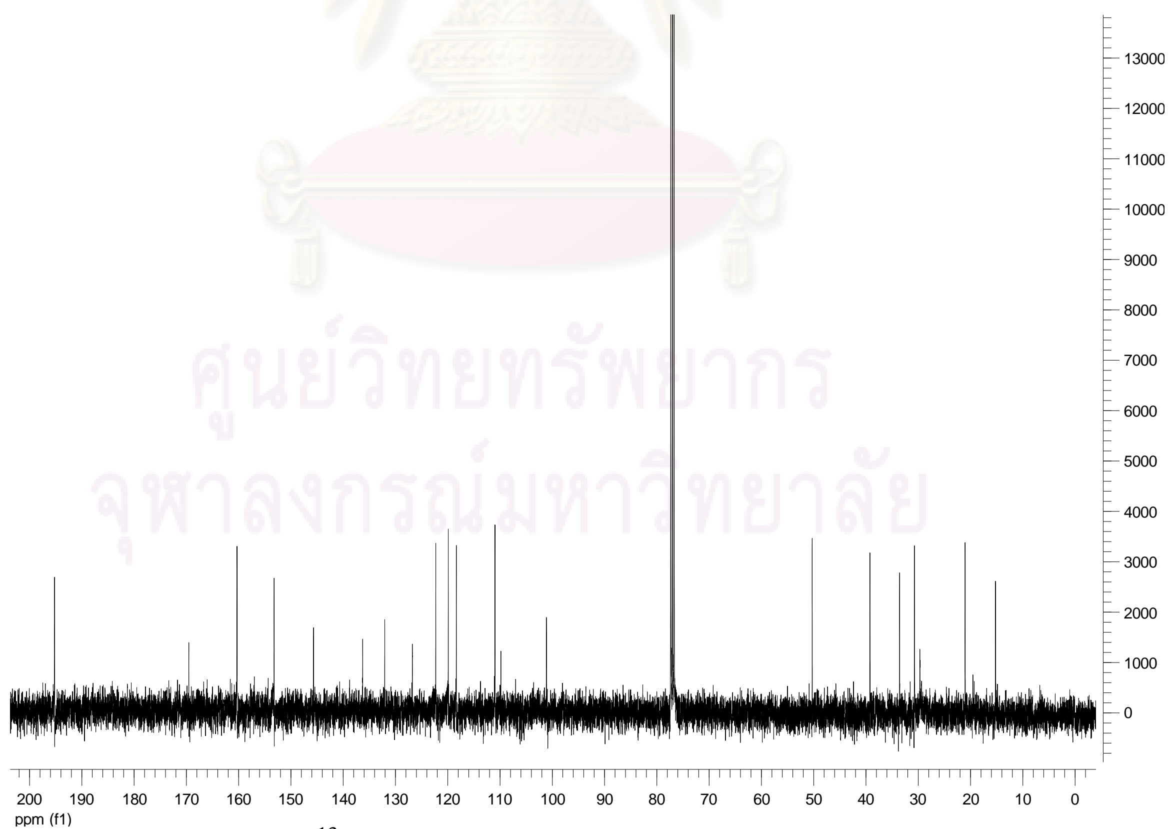

Figure C-1.2 ^{13}C NMR spectrum ($CDCl_3$-d_1) of naucleaoral B (**6**).

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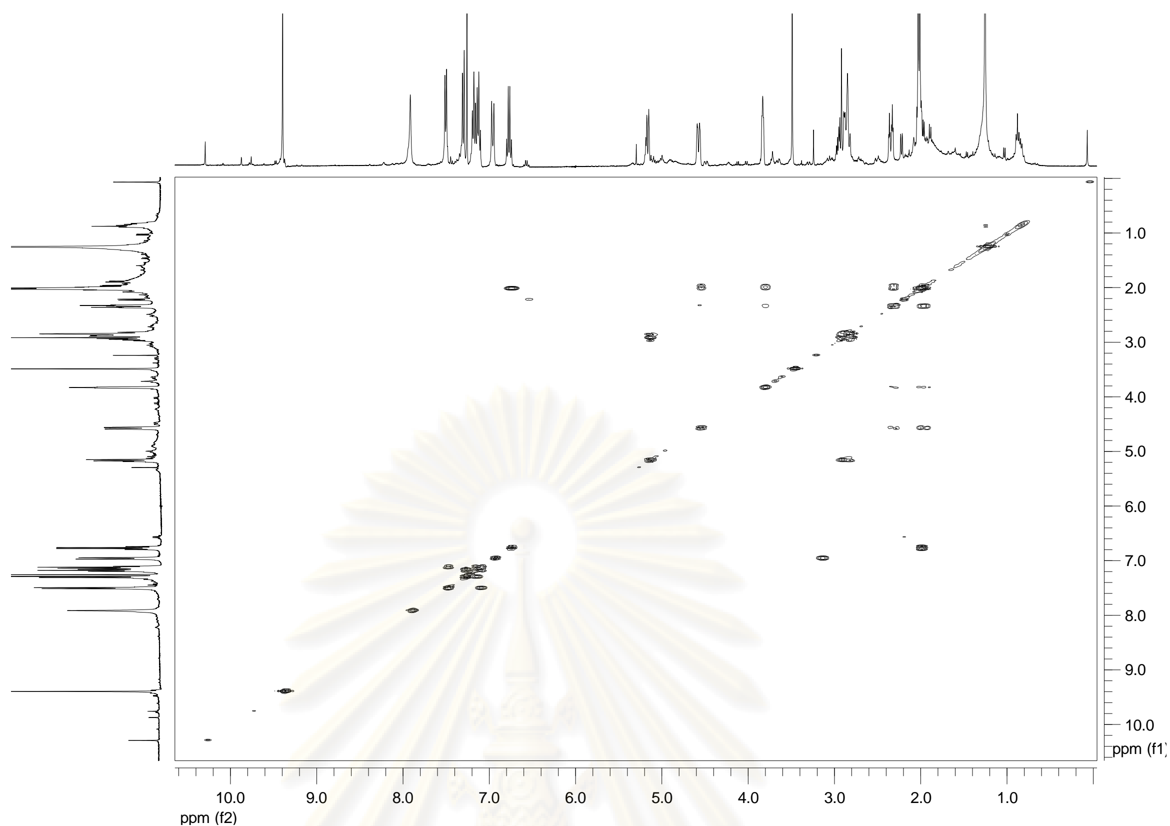

Figure C-1.3 COSY spectrum ($CDCl_3$-d_1) of naucleaoral B (**6**).

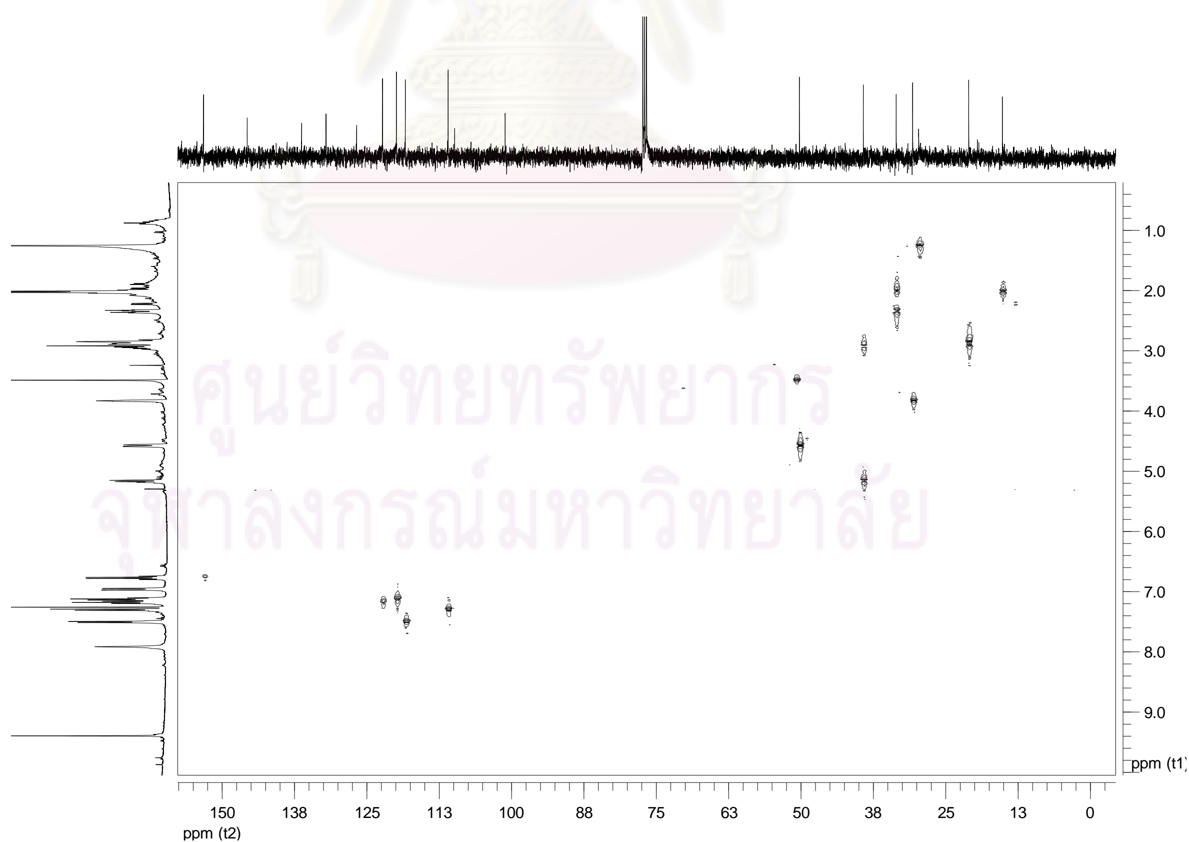

Figure C-1.4 HSQC spectrum ($CDCl_3$-d_1) of naucleaoral B (**6**).

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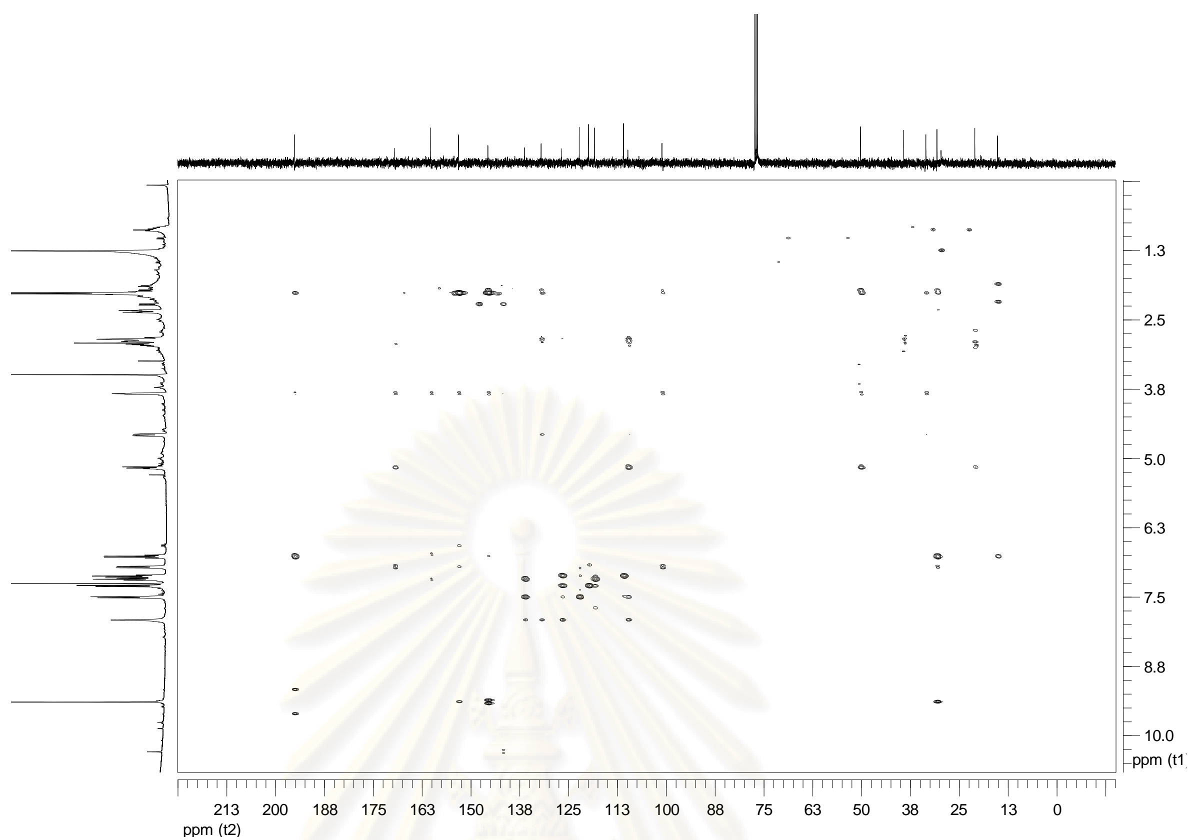


Figure C-1.5 HMBC spectrum ($CDCl_3$-d_1) of naucleaoral B (**6**).

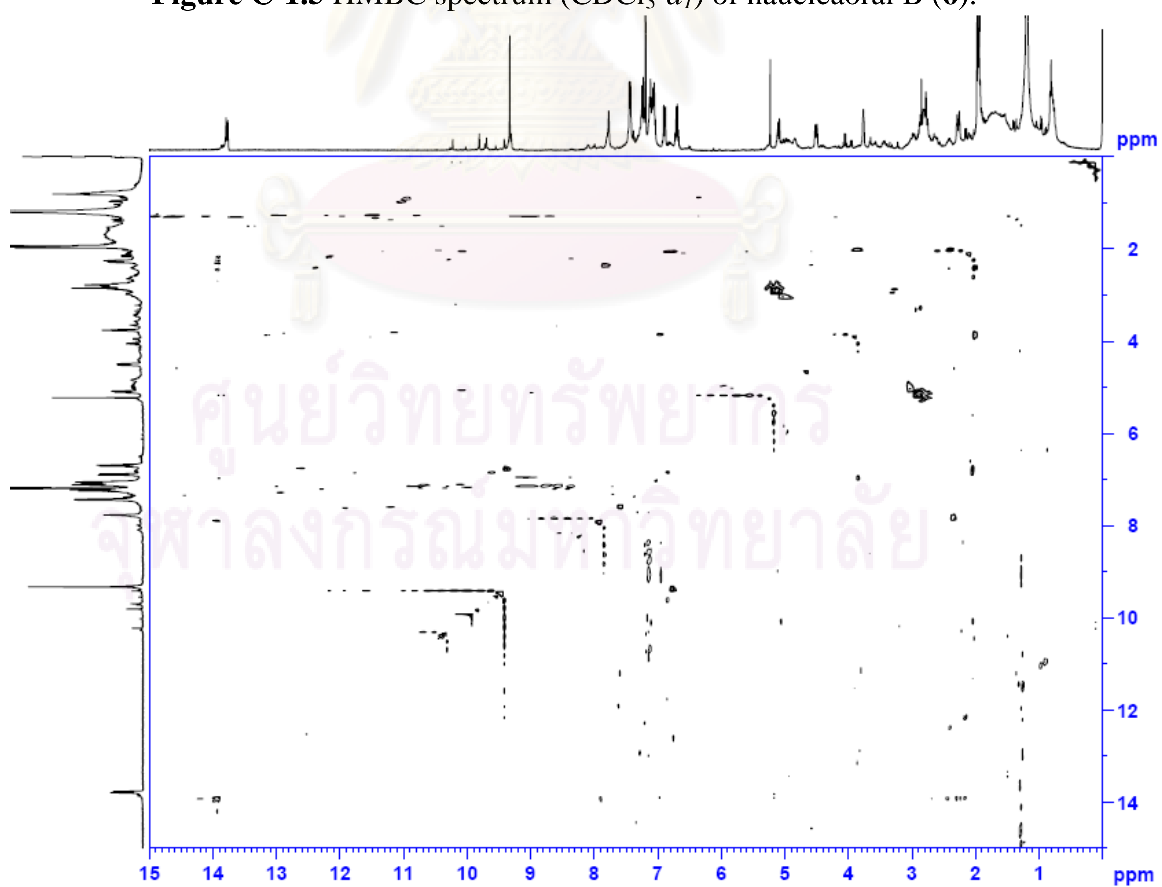
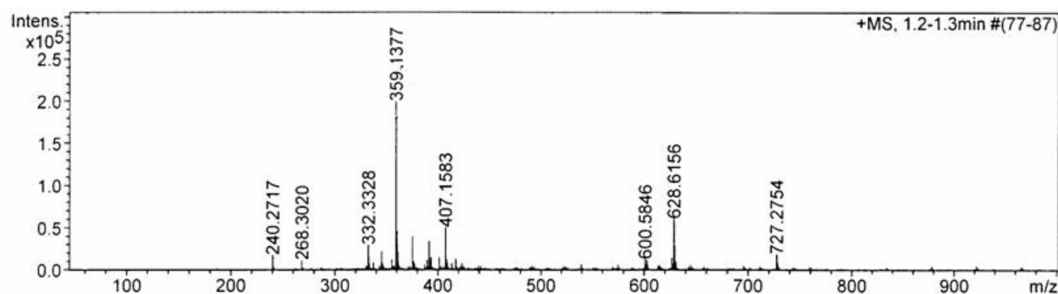


Figure C-1.6 NOESYspectrum ($CDCl_3$-d_1) of naucleaoral B (**6**).



#	m/z	Res.	S/N	I	FWHM
1	240.2717	7500	235.4	17493	0.0320
2	268.3020	7718	140.7	11513	0.0348
3	332.3328	7638	305.4	29528	0.0435
4	345.1225	7963	223.9	22122	0.0433
5	355.1070	7735	123.0	12190	0.0459
6	359.1377	7645	2050.9	199366	0.0470
7	360.1413	8055	468.5	45687	0.0447

Figure C-1.7 High resolution mass spectrum of naucleaoral B (**6**).

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VITA

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