Fungal Metabolites from the Endophytic Fungi *Aspergillus aculeatus* PSU-D2, *Pestalotiopsis microspora* PSU-A70, *Phomopsis* sp. PSU-D15 and Xylariaceae PSU-A80

Ubonta Sommart

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Organic Chemistry
Prince of Songkla University
2007

Thesis Title	Fungal Metabolites from the Endophytic Fungi *Aspergillus aculeatus* PSU-D2, *Pestalotiopsis microspora* PSU-A70, *Phomopsis* sp. PSU-D15 and Xylariaceae PSU-A80
Author	Miss Ubonta Sommart
Major Program	Organic Chemistry

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Krerkchai T. .....

(Assoc. Prof. Dr. Krerkchai Thongnoo)

Dean of Graduate School

ชื่อวิทยานิพนธ์	องค์ประกอบทางเคมีจากเชื้อราเอนโดไฟท์ *Aspergillus aculeatus* PSU-D2, *Pestalotiopsis microspora* PSU-A70, *Phomopsis* sp. PSU-D15 และ Xylariaceae PSU-A80
ผู้เขียน	นางสาวอุบลทา สมมารถ
สาขาวิชา	เคมีอินทรีย์
ปีการศึกษา	2549

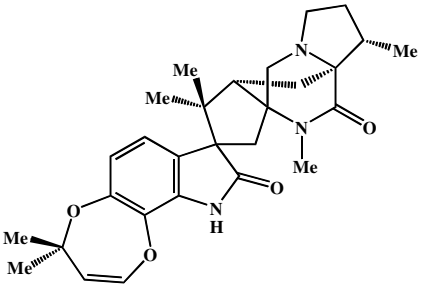
บทคัดย่อ

งานวิจัยนี้เป็นการศึกษาองค์ประกอบทางเคมีจากเชื้อราจำนวน 4 ชนิดได้แก่ *Aspergillus aculeatus* PSU-D2, *Pestalotiopsis microspora* PSU-A70, *Phomopsis* sp. PSU-D15 และ Xylariaceae PSU-A80 โดยนำส่วนสกัดหยาบเอทิลอะซิเตทจากส่วนน้ำเลี้ยงเชื้อและ/หรือเซลล์ของเชื้อราดังกล่าวมาทำให้บริสุทธิ์ด้วยวิธีทางโครมาโทกราฟี สามารถแยกสารบริสุทธิ์ประเภทต่างๆ ดังนี้

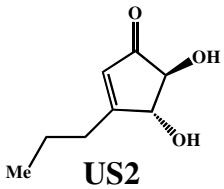
- สารที่มีการรายงานโครงสร้างแล้วคือ paraherquamide E (**US1**) จากส่วนสกัดหยาบจากเซลล์ของเชื้อรา *A. aculeatus* PSU-D2
- สารที่มีการรายงานโครงสร้างแล้วจำนวน 6 สารได้แก่ อนุพันธ์ของ terrein (**US2**) และ coumarin (**US3**) ประเภทละ 1 สาร และอนุพันธ์ของ phenol จำนวน 4 สาร (**US4-US7**) จากส่วนสกัดหยาบจากน้ำเลี้ยงเชื้อและเซลล์ของ เชื้อรา *P. microspora* PSU-A70
- สารใหม่จำนวน 4 สารได้แก่ อนุพันธ์ของ amide จำนวน 2 สาร (**US10** และ **US11**) อนุพันธ์ของ nitroester (**US13**) และ hydroxanthone dimer (**US15**) ประเภทละ 1 สาร และสารที่มีการรายงานโครงสร้างแล้วจำนวน 5 สารได้แก่ อนุพันธ์ของ tetralactone (**US12**) cyclohexane (**US8**) amide (**US9**) hydroxanthone dimer (**US14**) และ nucleoside (**US16**) ประเภทละ 1 สาร จากส่วนสกัดหยาบจากน้ำเลี้ยงเชื้อและเซลล์ของเชื้อรา *Phomopsis* sp. PSU-D15
- สารใหม่จำนวน 3 สารได้แก่ อนุพันธ์ของ pentacyclic จำนวน 1 สาร (**US20**) อนุพันธ์ของ dienoic acid (**US25**) และ tetralone (**US26**) ประเภทละ 1 สาร และสารที่มีการรายงานโครงสร้างแล้วจำนวน 10 สารได้แก่ อนุพันธ์ของ coumarin (**US3**) phenol

(**US4**) naphthol (**US17**) mellein (**US18**) tetralone (**US19**) pentacyclic (**US21**) octadien-2,3-diol (**US24**) และ lactone (**US27**) ประเภทละ 1 สาร และอนุพันธ์ของ benzylidene จำนวน 2 สาร (**US22** และ **US23**) จากส่วนสกัดหยาบจากน้ำเลี้ยงเชื้อและเซลล์ของเชื้อรา Xylariaceae PSU-A80

โครงสร้างของสารทั้งหมดวิเคราะห์โดยใช้ข้อมูลทางสเปกโทรสโกปี โดยเฉพาะ 1D และ 2D NMR สเปกโทรสโกปี และการเปรียบเทียบข้อมูลกับสารที่มีการรายงานโครงสร้างแล้ว



US1



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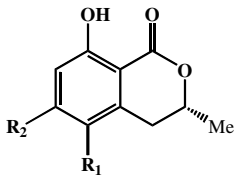
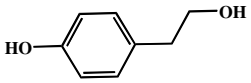
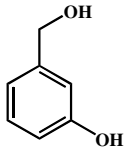


US3 : R_1 = H, R_2 = OH

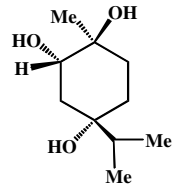
US18 : R_1 = Me, R_2 = H



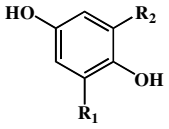
US4



US5

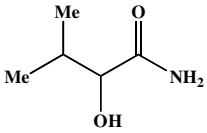


US8

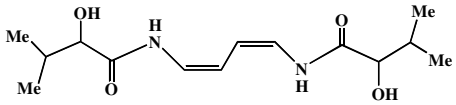


US6 : R_1 = CH_2OH, R_2 = Cl

US7 : R_1 = CO_2H, R_2 = H

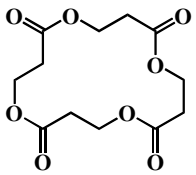


US9



US10

US11



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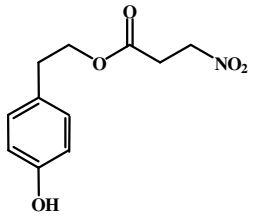

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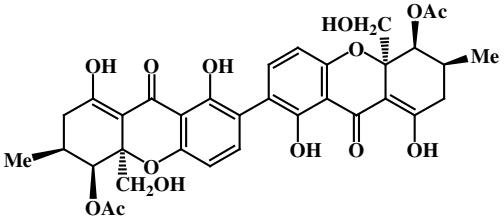
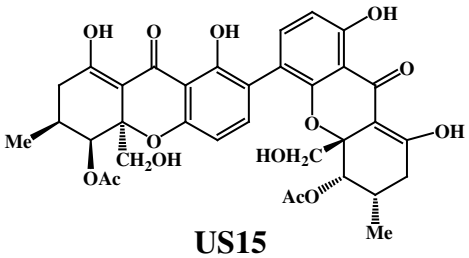
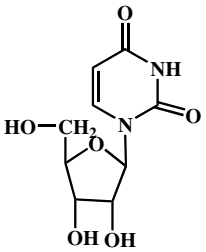


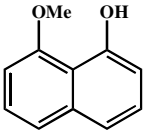
US14



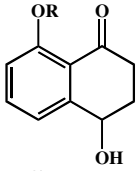
US15



US16

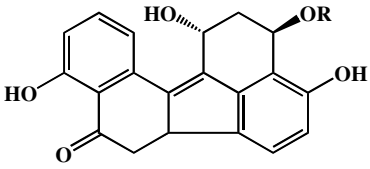


US17



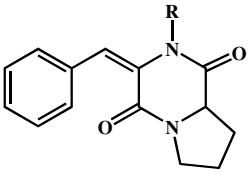
US19 : R = H

US26 : R = Me



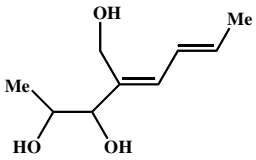
US20 : R = H

US21 : R = Me

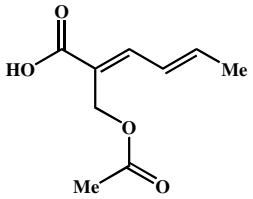


US22 : R = Me

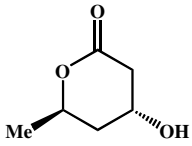
US23 : R = H



US24



US25



US27

Thesis Title	Fungal Metabolites from the Endophytic Fungi *Aspergillus aculeatus* PSU-D2, *Pestalotiopsis microspora* PSU-A70, *Phomopsis* sp. PSU-D15 and Xylariaceae PSU-A80
Author	Miss Ubonta Sommart
Major Program	Organic Chemistry
Academic Year	2006

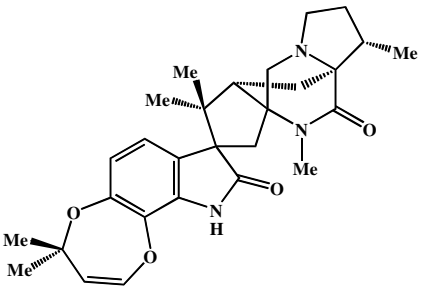
ABSTRACT

This work involved chemical investigation of the ethyl acetate extracts from culture broth and/or mycelia of four endophytic fungi: *Aspergillus aculeatus* PSU-D2, *Pestalotiopsis microspora* PSU-A70, *Phomopsis* sp. PSU-D15 and Xylariaceae PSU-A80. Each extract was subjected to various chromatographic techniques. Many types of compounds were isolated as follows.

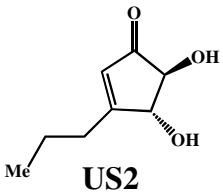
- The known paraherquamide E (**US1**) from the mycelial extract of *A. aculeatus* PSU-D2.
- Six known compounds: one terrein derivative (**US2**), one coumarin derivative (**US3**) and four phenol derivatives (**US4**-**US7**), from the broth and mycelial extracts of *P. microspora* PSU-A70.
- Four new compounds: two amide derivatives (**US10** and **US11**), one nitroester derivative (**US13**) and one hydroxanthone dimer (**US15**), and five known compounds: one tetralactone derivative (**US12**), one cyclohexane derivative (**US8**), one amide derivative (**US9**), one hydroxanthone dimer (**US14**) and one nucleoside derivative (**US16**), from the broth and mycelial extracts of *Phomopsis* sp. PSU-D15.
- Three new compounds: one pentacyclic derivative (**US20**), one dienoic acid derivative (**US25**) and one tetralone derivative (**US26**), and ten known compounds: one coumarin derivative (**US3**), one phenol derivative (**US4**), one naphthol derivative (**US17**), one mellein derivative (**US18**), one tetralone derivative (**US19**), one pentacyclic derivative (**US21**), one octadien-2,3-diol (**US24**), one lactone derivative

(**US27**) and two benzylidine derivatives (**US22** and **US23**), from the broth and mycelial extracts of Xylariaceae PSU-A80.

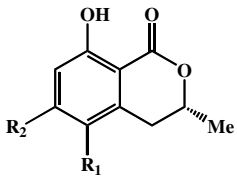
The structures were determined by analysis of 1D and 2D NMR spectroscopic data and comparison of the NMR data with those reported in the literatures.



US1



US2



US3 : R_1 = H, R_2 = OH

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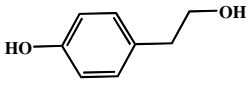
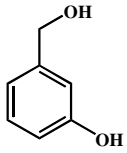
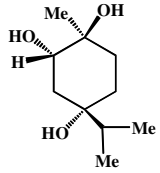


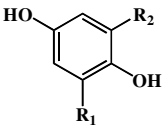
US4



US5



US8



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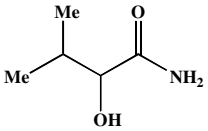
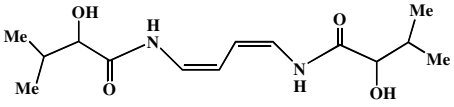
US7 : R_1 = CO_2H, R_2 = H

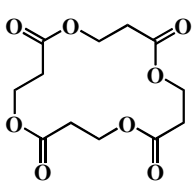


US9

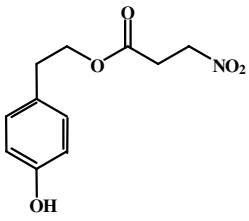


US10

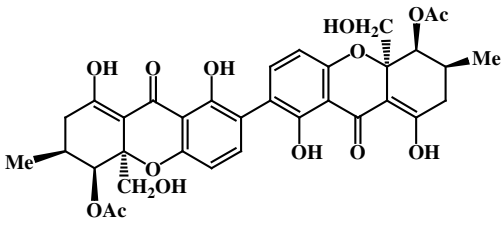
US11



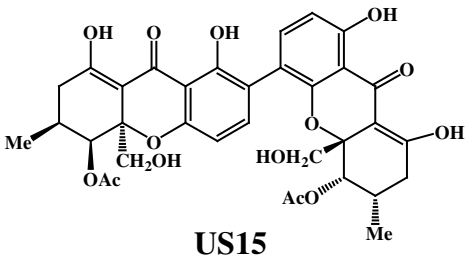
US12



US13



US14



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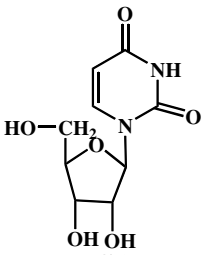
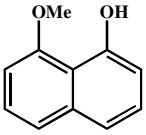
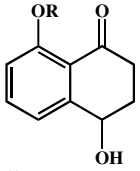


US16

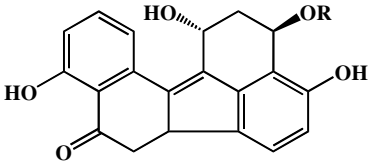


US17



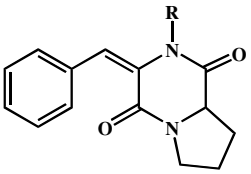
US19 : R = H

US26 : R = Me



US20 : R = H

US21 : R = Me



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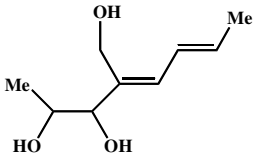
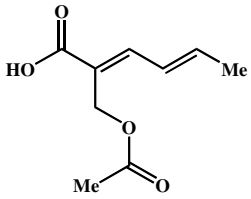
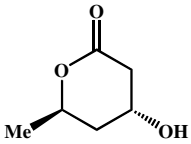
US23 : R = H



US24



US25



US27

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LIST OF ABBREVIATIONS AND SYMBOLS

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
q	=	*quartet*
m	=	*multiplet*
brs	=	*broad singlet*
dd	=	*doublet of doublet*
brd	=	*broad doublet*
brt	=	*broad triplet*
quint	=	*quintet*
sept	=	*septet*
ddd	=	*doublet of doublet of doublet*
tdd	=	*triplet of doublet of doublet*
dqd	=	*doublet of quartet of doublet*
dm	=	*doublet of multiplet*
δ	=	chemical shift relative to TMS
J	=	coupling constant
m/z	=	a value of mass divided by charge
°C	=	degree celcius
R_f	=	retention factor
g	=	gram
mL	=	milliliter
cm^{-1}	=	reciprocal centimeter (wavenumber)
nm	=	nanometer
λ_{max}	=	maximum wavelength
ν	=	absorption frequency
ε	=	Molar extinction coefficient
Hz	=	Hertz

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

MHz	=	megaHertz
ppm	=	part per million
$[\alpha]_D$	=	specific rotation
c	=	concentration
UV	=	Ultraviolet Visible
IR	=	Infrared
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
MS	=	Mass spectroscopy
HMQC	=	Heteronuclear Multiple Quantum Coherence
HMBC	=	Heteronuclear Multiple Bond Correlation
COSY	=	Correlation spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
NOE	=	Nuclear Overhauser Effect
TLC	=	Thin-Layer Chromatography
HCl	=	hydrochloric acid
NaOH	=	sodium hydroxide
$NaHCO_3$	=	sodium bicarbonate
MeOH	=	methanol
CD_3OD	=	deuteromethanol
$CDCl_3$	=	deuterochlorofrom
Acetone-d_6	=	hexadeuteroacetone
DMSO-d_6	=	hexadeuterodimethylsulphoxide
ASA	=	anisaldehyde-sulphuric acid in acetic acid solution

PART I

CHEMICAL CONSTITUENTS FROM THE ENDOPHYTIC FUNGUS *ASPERGILLUS ACULEATUS* PSU-D2

CHAPTER 1.1

INTRODUCTION

1.1.1 Introduction

Endophytic fungi grow in the intercellular spaces of higher plants. Many endophytic fungi have the ability to produce substances with antimicrobial (Phongpaichit, *et al*., 2006.), antioxidant (Strobel, *et al.*, 2002), antifungal (Li, *et al*., 2001), and herbicidal (Dai, *et al.*, 2005) activities. *Aspergillus aculeatus* produces various bioactive natural products as shown in **Table 1**. In an ongoing search for bioactive fungal metabolites, we discovered that the ethyl acetate extract from the culture broth of the endophytic fungus *Aspergillus aculeatus* PSU-D2 exhibited interesting antioxidation activity with the IC_{50} value of 0.21 mg/mL (DPPH assay) and antimycobacterial activity with a MIC value of 200 □g/mL. Moreover, the mycelial extract displayed antioxidation activity with the IC_{50} value of 0.37 mg/mL, antimycobacterial and antimalarial activities with the MIC values of 50 and 6.6 □g/mL, respectively. This fungus was isolated from the leaves of *Garcinia dulcis*.

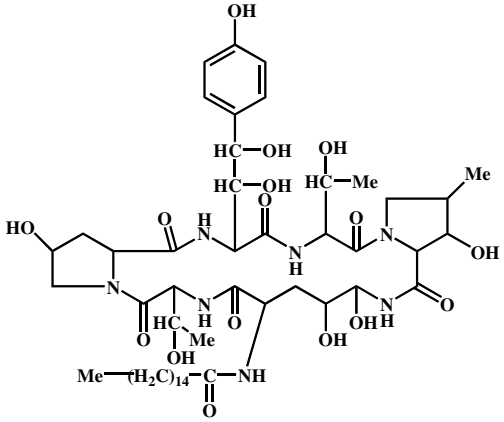
Table 1 Metabolites from the fungus *Aspergillus aculeatus* and the biological activity

Compound	Structure	Activity	Reference
aculeacin A	1	antibiotic	Mizuno, *et al*., 1977
aurasperone A	2	antitumor	Compos, *et al*., 2005, Akiyama and Sugita, 1991
fonsecinone A	3	inhibitory activity on Taq DNA polymerase	Compos, *et al*., 2005, Akiyama, *et al*., 2003

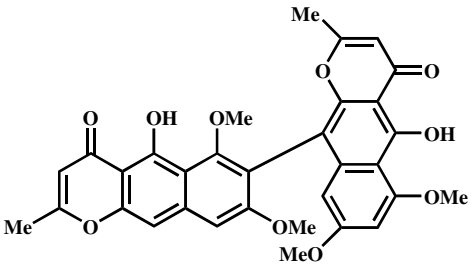
Table 1 (continued)

Compound	Structure	Activity	Reference
emodin	4	antitumour	Kurobane, *et al*., 1979, Lee, 2001
endocrocin	5	-	Kurobane, *et al*., 1979
secalonic acid B	6	cytostatic	Kurobane, *et al*., 1979, Kurobane, *et al*., 1987
secalonic acid D	7	antimicrobial, antitumor and immunostimulation	Anderson, *et al*., 1977, Ishida, 2000
secalonic acid F	8	inhibited the seedling growth of sorghum (*Sorghum vulgare* Pers.), hairy beggarticks (*Bidens pilosa* L.) and barnyard grass (*Echinochloa crus-galli* (L.) Beauv).	Anderson, *et al*., 1977, Zeng, *et al*., 2001
okaramine A	9	insecticidal	Hayashi *et al*., 1999
okaramine B	10	insecticidal	”
okaramine H	11	insecticidal	”
okaramine I	12	insecticidal	”

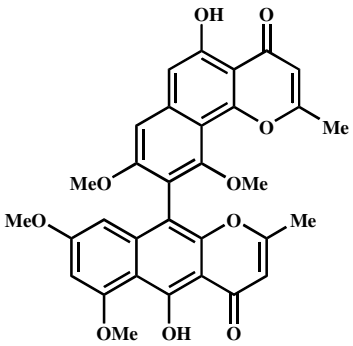
Structures of the metabolites from *A. aculeatus*



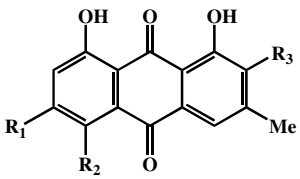
1 : aculeacin A



2 : aurasperone A

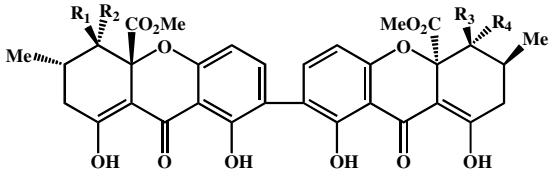


3 : fonsecinone A



4 : $R_1 = R_2 = H$, $R_3 = OH$: emodin

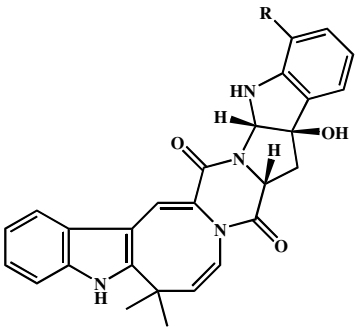
5 : $R_1 = OH$, $R_2 = H$, $R_3 = CO_2H$: endocrocin

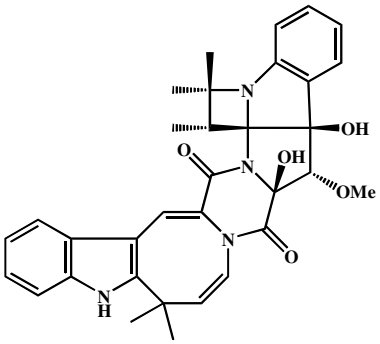


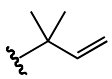
6 : $R_1 = R_4 = H$, $R_2 = R_3 = OH$: secalonic acid B

7 : $R_1 = R_4 = OH$, $R_2 = R_3 = H$: secalonic acid D

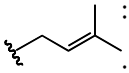
8 : $R_1 = R_3 = H$, $R_2 = R_4 = OH$: secalonic acid F





9 : R =  : okaramine A

10 : R = okaramine B

11 : R =  : okaramine H

12 : R = H : okaramine I

CHAPTER 1.2

EXPERIMENTAL

1.2.1 Instruments and chemicals

Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and reported without correction. Infrared spectra (IR) were obtained on a Perkin Elmer Spectrum GX FT-IR system and recorded on wavenumber (cm^{-1}). ^{1}H and ^{13}C Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on a FTNMR, Bruker Avance 300 MHz or 500 MHz spectrometers using tetramethylsilane (TMS) as an internal standard. Spectra were recorded as chemical shift parameter (□) value in ppm down field from TMS (□ 0.00). Ultraviolet spectra (UV) were measured with an UV-160A spectrophotometer (SHIMADSU). Principle bands ($□_{max}$) were recorded as wavelengths (nm) and log □ in MeOH solution. Specific rotations were measured in methanol solution or chloroform solution with sodium D line (590 nm) on a JASCO P-1020 automatic polarimeter. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for ethyl acetate which was an analytical grade reagent. Thin-layer chromatography (TLC) and precoated TLC plate were performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on

silica gel (Merck) type 100 (70-230 mesh ASTM), Sephadex LH-20 or reverse phase C_{18} silica gel.

1.2.2 Fermentation and extraction

The flask culture of the fungus *Aspergillus aculeatus* PSU-D2 (15 L) was filtered to separate into the filtrate and wet mycelia. The filtrate was divided into five portions (5 L each). Each portion was extracted three times with equal volumes of EtOAc (600 mL). The combined EtOAc extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to give a brown gum (260.0 mg). The wet mycelia were extracted twice with 500 mL of MeOH in 2 days. The aqueous MeOH layer was concentrated under reduced pressure. To the extract was added H_2O (50 mL), and the mixture washed with hexane (700 mL). The aqueous residue was extracted three times with equal volumes of EtOAc (300 mL). The combined EtOAc extracts were dried over anhydrous Na_2SO_4 and then evaporated to dryness under reduced pressure to give a brown gum (545.0 mg).

1.2.3 Purification of the broth extract

The broth extract of *A. aculeatus* PSU-D2 (260.0 mg) was chromatographed on Sephadex LH-20 column chromatography using 100% methanol as eluent to

afford thirty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford three fractions, as shown in **Table 2**.

Table 2 Fractions obtained from the broth extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	8.0	Dark-brown gum
B	167.6	Brown gum
C	5.9	Dark-brown gum

Fraction A Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated no definite spots under UV-S. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Fraction B was separated into two fractions by dissolving in methanol. The methanol soluble (31.1 mg) and insoluble (130.1 mg) fractions were obtained as a brown gum and a dark brown solid, respectively. Chromatogram characteristics of the soluble fraction on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.48 and 0.70 under UV-S and other spots with the R_f values of 0.75 and 0.78 as

violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 3**.

Table 3 Subfractions obtained from the methanol soluble fraction of fraction B by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B1	100% CH_2Cl_2-1% MeOH/CH_2Cl_2	1.7	Colorless gum
B2	1% MeOH/CH_2Cl_2	7.8	Pale brown gum
B3	2-3 % MeOH/CH_2Cl_2	12.9	Pale brown gum
B4	3% MeOH/CH_2Cl_2-100% MeOH	8.7	Brown gum

Subfraction B1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.50 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Subfraction B2 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated no definite spots. The ^{1}H NMR spectrum indicated that it contained many components. Therefore, it was not further investigated.

Subfraction B3 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction B4 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated no spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Fraction C Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated no definite spots under UV-S. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

1.2.4 Purification of the mycelial extract

The mycelial extract of *A. aculeatus* PSU-D2 (545.0 mg) was chromatographed on Sephadex LH-20 column chromatography using 100% methanol as eluent to afford twenty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram

characteristics and then evaporated to dryness under reduced pressure to afford five fractions, as shown in **Table 4**.

Table 4 Fractions obtained from the mycelial extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	41.5	Dark-brown gum
B	260.9	Dark-brown gum
C	36.4	Brown gum mixed with brown solid
D	2.8	Brown gum mixed with brown solid
E	2.0	Brown gum mixed with brown solid

Fraction A Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot near the baseline under UV-S and the other spot with the R_f value of 0.88 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Fraction B Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.44 and 0.65 under UV-S and other spots with the R_f values of 0.59 and 0.88 as violet spots after

dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane to yield seventy fractions. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give nine subfractions, as shown in **Table 5**.

Table 5 Subfractions obtained from fraction B by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B1	100% CH_2Cl_2- 0.5% MeOH/CH_2Cl_2	54.7	Yellow gum
B2	1% MeOH/CH_2Cl_2	10.7	Yellow gum mixed with white solid
B3	2-3% MeOH/CH_2Cl_2	43.4	Yellow gum
B4	3% MeOH/CH_2Cl_2	20.3	Colorless gum
B5	3% MeOH/CH_2Cl_2	6.6	Yellow gum
B6	3-4% MeOH/CH_2Cl_2	17.1	Brown gum
B7	5% MeOH/CH_2Cl_2	2.3	Yellow gum mixed with white solid
B8	6% MeOH/CH_2Cl_2	4.0	Pale brown gum
B9	7% MeOH/CH_2Cl_2- 100% MeOH	88.5	Brown gum

Subfraction B1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.53 and 0.71 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. The ^1H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Subfraction B2 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot under UV-S with the R_f value of 0.40 and other spots with the R_f values of 0.51 and 0.58 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 4% methanol-dichloromethane as a mobile phase (2 runs) afforded two bands.

Band 1 was obtained as a colorless gum (5.0 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated one spot under UV-S with the R_f value of 0.83 and the other spot with the R_f value of 0.70 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Band 2 was obtained as a colorless gum (2.0 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.56 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B3 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated two spots under UV-S with the R_f values of 0.23 and 0.36 and the other spot with the R_f value of 0.27 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the

major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Subfraction B4 (US1) Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.23 under UV-S.

$[\alpha]_D^{28}$	-18.16 (c = 0.43, MeOH)
UV (MeOH) λ_{max} nm (log ε)	224 (4.39), 260 (3.62), 296 (2.93)
FT-IR (neat) ν_{cm-1}	2929 (C-H stretching), 1696 (C=O stretching),
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	8.37 (s, 1H), 6.82 (*d*, *J* = 8.1 Hz, 1H), 6.68 (*d*, *J* = 8.1 Hz, 1H), 6.33 (*d*, *J* = 7.5 Hz, 1H), 4.90 (*d*, *J* = 7.5 Hz, 1H), 3.62 (*d*, *J* = 11.1 Hz, 1H), 3.20 (*td*, *J* = 8.7, 3.9 Hz, 1H), 3.08 (*m*, 1H), 3.04 (*s*, 3H), 2.70 (*d*, *J* = 15.6 Hz, 1H), 2.56 (*d*, *J* = 9.9 Hz, 1H), 2.32 (*dd*, *J* = 5.1, 9.3 Hz, 1H), 2.29 (m, 1H), 2.04 (*dd*, *J* = 10.2, 12.0 Hz, 1H), 1.95 (*m*, 1H), 1.88 (*d*, *J* = 15.6 Hz, 1H), 1.86 (*m*, 2H), 1.44 (*dd*, *J* = 9.0, 12.0 Hz, 1H), 1.43 (*s*, 3H), 1.42 (*d*, *J* = 7.5 Hz, 3H), 1.11 (*s*, 3H), 0.86 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm)	182.84, 177.00, 172.07, 146.04, 139.02, 135.28,

(75 MHz)		132.42, 125.35, 120.41, 117.27, 115.09, 79.75, 67.72, 65.15, 62.99, 59.87, 53.27, 52.09, 46.26, 40.17, 37.40, 30.16, 29.94, 29.77, 27.49, 25.54, 23.93, 20.70, 13.03
DEPT (135°) ($CDCl_3$)	CH :	139.02, 120.41, 117.27, 115.09, 52.09, 40.17, 34.50
	CH_2 :	59.87, 53.27, 37.40, 30.16, 27.49
	CH_3 :	29.94, 29.77, 25.54, 23.93, 20.70, 13.03

Subfraction B5 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.23 under UV-S. The ^{1}H NMR spectrum indicated that the major compounds was **US1**.

Subfraction B6 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated three spots with the R_f values of 0.11, 0.18 and 0.23 under UV-S. Further purification by precoated TLC with 4% methanol-dichloromethane as a mobile phase (5 runs) afforded three bands.

Band 1 was obtained as a yellow gum (2.9 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.43 under UV-S. The ^{1}H NMR spectrum indicated that the major compounds was **US1**.

Band 2 was obtained as a colorless gum (2.0 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile

phase demonstrated one spot with the R_f value of 0.29 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was obtained as a colorless gum (1.1 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.29 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B7 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.16 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B8 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated many spots near the baseline under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B9 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot near the baseline under UV-S and the other spot with the R_f value of 0.27 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Fraction C Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated three spots with the R_f values of 0.57, 0.71 and 0.77 under UV-S and the other spot with the R_f value of 0.88 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel using a gradient system of methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 6**.

Table 6 Subfractions obtained from fraction C by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
C1	100% CH_2Cl_2- 2% MeOH/CH_2Cl_2	2.5	Yellow gum
C2	2-3% MeOH/CH_2Cl_2	1.2	Yellow gum
C3	4-6% MeOH/CH_2Cl_2	6.0	Brown gum
C4	6-100% MeOH/CH_2Cl_2	11.1	Dark-brown gum

Subfraction C1 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated five spots with the R_f values of 0.19, 0.22, 0.29, 0.37 and 0.44 under UV-S. Because of the minute quantity, it was not further purified.

Subfraction C2 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.19 under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C3 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated three spots with the R_f values of 0.22, 0.24 and 0.46 and one spot near the baseline under UV-S. Because of the minute quantity, it was not further purified.

Subfraction C4 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Fraction D Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.62 and 0.64 under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Fraction E Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated no spots under UV-S. Because of the minute quantity, it was not further purified.

CHAPTER 1.3

RESULTS AND DISCUSSION

Attempted purification of the crude EtOAc extract from the broth of *A. aculeatus* PSU-D2 led to the isolation of a mixture of long-chain hydrocarbons. Compound **US1** was the only metabolite isolated from the mycelail extract. The structure was identified by spectroscopic methods.

1.3.1 Compound US1

Compound **US1** was obtained as a colorless gum. It exhibited UV absorption bands at 224, 260, and 296 nm (**Figure 1**) while a carbonyl absorption band was found at 1696 cm^{-1}, in the IR spectrum (**Figure 2**). The ^{1}H NMR spectrum (**Figure 3**) (**Table 7**) displayed characteristic signals for one amino proton (δ_H 8.37, *s*), two *ortho*-coupled aromatic protons [δ_H 6.82 (*d*, *J* = 8.1 Hz, 1H) and 6.68 (*d*, *J* = 8.1 Hz, 1H)], two *cis*-olefinic protons [δ_H 6.33 (*d*, *J* = 7.5 Hz, 1H) and 4.90 (*d*, *J* = 7.5 Hz, 1H)], two methine protons [δ_H 3.08 (*m*, 1H) and 1.95 (*m*, 1H)], four sets of nonequivalent methylene protons [δ_H 3.62 (*d, J* = 11.1 Hz, 1H) and 2.56 (*d*, *J* = 11.1 Hz, 1H),

3.20 (*td*, *J* = 8.7 and 3.9 Hz, 1H) and 2.29 (*m*, 1H), 2.70 (*d*, *J* = 15.6 Hz, 1H) and 1.88 (*d, J* = 15.6 Hz, 1H), and 2.04 (*dd*, *J* = 12.0 and 10.2 Hz, 1H) and 1.44 (*dd*, *J* = 12.0 and 9.0 Hz, 1H)], one set of equivalent methylene protons (δ_H 1.86, *m*, 2H) and six methyl groups [δ_H 3.04 (*s*, 3H) 1.45 (*s*, 3H), 1.43 (*s*, 3H), 1.42 (*s*, 3H), 1.11 (*s*, 3H) and 0.86 (*s*, 3H)]. The ^{13}C NMR spectrum (**Figure 6**) (**Table 7**) showed two carbonyl carbons (δ_C 182.84 and 172.07), six aromatic carbons (δ_C 146.04, 135.28, 132.42, 125.35, 120.41 and 117.27), two *cis*-olefinic carbons (δ_C 139.02 and 115.09), one oxyquaternary carbon (δ_C 79.75), four quaternary carbons (δ_C 67.72, 65.15, 62.99 and 46.26), two methine carbons (δ_C 52.09 and 40.17), five methylene carbons (δ_C 59.87, 53.27, 37.40, 30.16 and 27.49) and six methyl carbons (δ_C 29.94, 29.77, 25.54, 23.93, 20.70 and 13.03). Two *ortho*-coupled aromatic protons resonating at δ_H 6.82 and 6.68

were assigned as H-4 and H-5, respectively. ^3J HMBC correlations (**Figure 8**) (**Table 8**) of H-4/C-3 (δ_C 62.99), C-6 (δ_C 146.04) and C-8 (δ_C 132.42), H-5/ C-7 (δ_C 135.28) and those of the amino proton (δ_H 8.37) with C-3, C-8 and C-9 (δ_C 125.35) constructed an indoline-2-one skeleton with two oxysubstituents at C-6 and C-7. One of the *cis*-olefinic protons (H-24, δ_H 6.33) displayed HMBC cross peaks with C-7, C-26 (δ_C 79.75), C-27 (δ_C 29.94) and C-28 (δ_C 29.77). These data together with the chemical shift of C-26 resulted in the formation of ring A. HMBC cross peaks of H_{ab}-10 (δ_H 2.70 and 1.88), Me-22 (δ_H 1.11) and Me-23 (δ_H 0.86)/C-3, C-11 (δ_C 65.15), C-20 (δ_C 52.09) and C-21 (δ_C 46.26) linked a spirocyclopentane skeleton at C-3 of the indolinone unit having two methyl groups attached at C-21. In the ^{1}H-^{1}H COSY spectrum (**Figure 9**) (**Table 8**), H_2-15 (δ_H 1.86) were coupled with the H_{ab}-16 (δ_H 2.29 and 3.20) and H-14 (δ_H

1.95) which was further coupled with methyl protons (δ_H 1.42). These results revealed the presence of -$CH_2CH_2CH(CH_3)$- unit. HMBC cross peaks of H-14/C-13 (δ_C 62.72) and C-18 (δ_C 172.07), H_{ab}-16/C-13, H_{ab}-12 (δ_H 2.56 and 3.62)/C-13, H_{ab}-19 (δ_H 2.04 and 1.44)/C-14 (δ_C 40.17) and C-16 (δ_C 53.27) established a pyrrolidine skeleton having one methyl group at C-14, one amide carbonyl unit and one methylene group at C-13, and one methylene group attached at the nitrogen atom. HMBC cross peaks of H_{ab}-12/C-10 (δ_C 37.40), Me-29 (δ_H 3.04)/C-18 and H_{ab}-19/C-11 and C-20 of the cyclopentane unit constructed a spiro piperazinone at C-11 of the cyclopentane and fused 2-piperidinone with C-11 and C-20 of the cyclopentane. Therefore, the structure of **US1** was identical to that of paraherquamide E which was previously isolated from *Penicillium charlesii* (Liesch and Wichmann, 1990). Comparison of their specific rotation ($[\alpha]_D$ = -18.16, c = 0.43, MeOH for **US1** and $[\alpha]_D$ = -28.00, c = 0.43, MeOH for paraherquamide E) (Yamazaki and Okuyama, 1981) indicated that they possessed the same relative configuration.

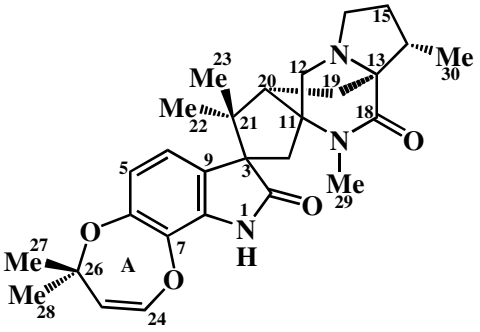


Table 7 The ^{1}H and ^{13}C NMR data of compound US1 in $CDCl_3$ and the ^{1}H NMR data of paraherquamide E in CD_2Cl_2

Position	US1		paraherquamide E
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)
1-NH	8.37 (*s*)		7.57 (*brs*)
2	-	182.84 (C=O)	-
3	-	62.99 (C)	-
4	6.82 (*d*, 8.1)	120.41 (CH)	6.84 (*d*, 8.5)
5	6.68 (*d*, 8.1)	117.27 (CH)	6.67 (*d*, 7.5)
6	-	146.04 (C)	-
7	-	135.28 (C)	-
8	-	132.42 (C)	-
9	-	125.35 (C)	-
10	a: 2.70 (*d*, 15.6)	37.40 (CH_2)	a: 2.66 (*d*, 15.9)
	b: 1.88 (*d,* 15.6)		b: 1.86 (*d,* 16.0)
11	-	65.15 (C)	-
12	a: 2.56 (*d*, 11.1)	59.87 (CH_2)	a: 2.51 (*brd*, 10.6)
	b: 3.62(*d,* 11.1)		b: 3.56 (*brd,* 10.7)
13	-	67.72 (C)	-
14	1.95 (*m*)	40.17 (CH)	1.89 (*m*)
15	1.86 (*m*)	30.16 (CH_2)	a: 1.75 (*dddd*, 4.5, 10.7, 10.7, 10.7)
			b: 1.97 (*m*)
16	a: 2.29 (*m*)	53.27 (CH_2)	a: 2.20 (*m*)
	b: 3.20 (*td*, 8.7, 3.9)		b: 3.13 (*m*)
18	-	172.07 (C=O)	-
19	a: 2.04 (*dd*, 12.0, 10.2)	27.49 (CH_2)	a: 2.00 (*dd*, 11.0, 11.2)
	b: 1.44 (*dd*, 12.0, 9.0)		b: 1.38 (*m*)

Table 7 (continued)

Position	US1		paraherquamide E
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)
21	-	46.26 (C)	-
22	1.11 (*s*)	20.70 (CH_3)	1.08 (*s*)
23	0.86 (*s*)	23.93 (CH_3)	0.84 (*s*)
24	6.33 (*d*, 7.5)	139.02 (CH)	6.33 (*d*, 8.2)
25	4.90 (*d*, 7.5)	115.09 (CH)	4.90 (*d*, 8.0)
26	-	79.75 (C)	-
27	1.45 (*s*)	29.94 (CH_3)	1.41 (*s*)
28	1.43 (*s*)	29.77 (CH_3)	1.43 (*s*)
29	3.04 (*s*)	25.54 (CH_3)	2.97 (*s*)
30	1.42 (*s*)	13.03 (CH_3)	1.36 (*d*, 6.8)

Proton	HMBC correlations	COSY	NOE
1-NH	C-3, C-8, C-9	-	-
H-4	C-3, C-6, C-8, C-9	H-5	-
H-5	C-3, C-6, C-7, C-9	H-4	-
H_a-10	C-2, C-3, C-9, C-11, C-12, C-20, C-21	H_b-10	H_b-10, H-14, H-29
H_b-10	C-2, C-3, C-9, C-11, C-12, C-20, C-21	H_a-10	H_a-10, H_a-12, H_a-16
H_a-12	C-10, C-11, C-13, C-20	H_b-12, H-20	H_b-10, H_b-12, H-22
H_b-12	C-10, C-11, C-13, C-20	H_a-12	H_a-12
H-14	C-13, C-14, C-18, C-30	H-15, H-30	H-30

Table 8 The HMBC, COSY and NOE data of compound US1 in $CDCl_3$

Table 8 (continued)

Proton	HMBC correlations	COSY	NOE
H-15	C-14, C-16, C-30	H-14, H_{ab}-16	-
H_a-16	C-13, C-14, C-15	H-15, H_b-16, H_a-19	H_a-12, H_b-16, H-23
H_b-16	C-13, C-14, C-15	H-15, H_a-16, H_a-19	H-15, H_a-16
H_a-19	C-11, C-13, C-14, C-16, C-20	H_{ab}-16, H-20	-
H_b-19	C-11, C-13, C-14, C-16, C-20	-	-
H-20	C-11, C-12, C-19, C-21, C-22, C-23	H_a-12, H_{ab}-19	H_a-10, H_a-12, H_{ab}-19, H-23
H-22	C-3, C-20, C-21, C-23	-	H-5, H_b-12, H-23
H-23	C-3, C-20, C-21, C-22	-	H_a-16, H-20
H-24	C-7, C-25, C-26, C-27, C-28	H-25	H-25
H-25	C-24, C-26, C-27, C-28	H-24	H-24, H-27, H-28
H-27	C-25, C-26, C-28	-	H-25, H-28
H-28	C-25, C-26, C-27	-	H-25, H-27
H-29	C-13, C-18	-	H-24
H-30	C-13, C-14, C-15	H-14	-

PART Ⅱ

CHEMICAL CONSTITUENTS FROM THE ENDOPHYTIC FUNGUS *PESTALOTIOPSIS MICROSPORA* PSU-A70

CHAPTER 2.1

INTRODUCTION

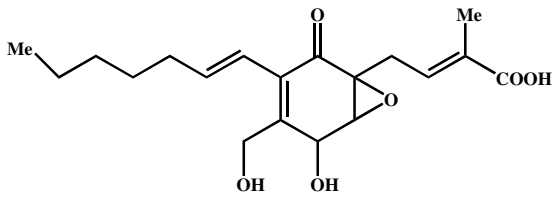
2.1.1 Introduction

Pestalotiopsis microspora is one of the most widely distributed endophytic fungi (Harper, *et al*., 2003). This microorganism produces bioactive natural products as summarized in **Table 9**. In an ongoing search for bioactive fungal metabolites, we discovered antioxidation activity with IC_{50} values of 0.17 and 0.22 mg/mL (DPPH assay) in the extracts of the broth and mycelia of *P. microspora* PSU-A70, respectively. *P. microspora* PSU-A70 was an endophytic fungus isolated from the leaves of *Garcinia atroviridis*.

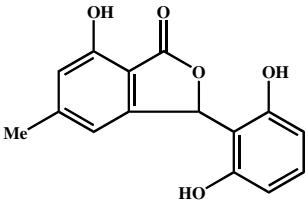
Table 9 Metabolites from the fungus *Pestalotiopsis microspora* and the biological activity

Compound	Structure	Activity	Reference
ambuic acid	13	antifungal	Li, *et al*., 2001
isopestacin	14	antifungal and antioxidant	Strobel, *et al*., 2002
pestacin	15	antioxidant and antimycotic	Harper, *et al*., 2003
taxol	16	anticancer	Strobel, *et al*., 1996, Metz, *et al*., 2000
torreyanic acid	17	cytotoxic	Lee, *et al*., 1996

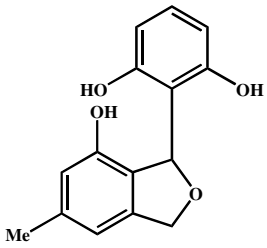
Structures of the metabolites from *P. microspora*



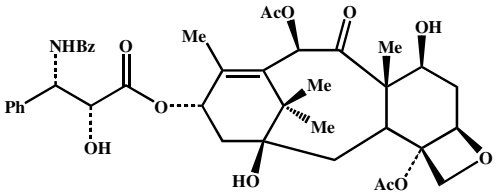
13 : ambuic acid



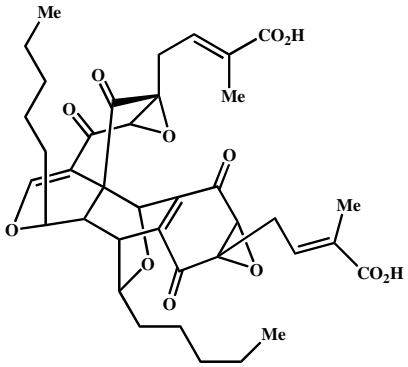
14 : isopestacin



15 : pestacin



16 : taxol



17 : torreyanic acid

CHAPTER 2.2

EXPERIMENTAL

2.2.1 Fermentation and extraction

The fermentation and extraction were performed using the same procedure as those of *A. aculeatus* PSU-D2. The crude EtOAc extracts from the culture broth and mycelia were obtained both as a brown gum in 1.20 g and 120.0 mg, respectively.

2.2.2 Purification of the broth extract

The broth extract of the fungus *P. microspora* PSU-A70 (1.20 g) was chromatographed on Sephadex LH-20 column chromatography using 100% methanol to afford seventy fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford six fractions, as shown in **Table 10**.

Table 10 Fractions obtained from the broth extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	250.3	Brown gum mixed with brown solid
B	407.6	Dark-brown gum
C	306.7	Dark-brown gum
D	100.8	Dark-brown gum
E	40.6	Brown gum mixed with brown solid
F	43.8	Brown gum mixed with brown solid

Fraction A Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Fraction B Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated a long tail and one major spot with the R_f value of 0.27 under UV-S. Further separation by column chromatography over silica gel using a gradient system of methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give five subfractions, as shown in **Table 11**.

Table 11 Subfractions obtained from Fraction B by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B1	4% MeOH/CH_2Cl_2	22.2	Brown gum
B2	4% MeOH/CH_2Cl_2	15.5	Brown gum
B3	4% MeOH/CH_2Cl_2	37.5	Colorless gum
B4	4-7% MeOH/CH_2Cl_2	32.5	Brown gum
B5	7% MeOH/CH_2Cl_2-100%MeOH	108.6	Dark-brown gum mixed with brown solid

Subfraction B1 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane as mobile phases demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B2 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane as mobile phases demonstrated one spot with the R_f value of 0.18 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US2.**

Subfraction B3 (US2) Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane as mobile phases demonstrated one spot with the R_f value of 0.18 under UV-S.

$[\alpha]_D^{28}$		+6.67 (c = 0.22, MeOH)
UV (MeOH) λ_{max} nm (log ε)		223 (4.16), 293 (3.42)
FT-IR (neat) ν_{cm-1}		3618 (OH stretching), 2928 (C-H stretching) 1697 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		5.86 (*s*, 1H), 5.23 (*brs,* 1H), 4.55 (*brs*, 1H), 4.21 (*d*, *J* = 2.7 Hz, 1H), 2.38 (*m*, 2H), 1.53 (*m*, 2H), 0.89 (*t*, *J* = 7.5 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		203.72, 179.66, 126.30, 81.38, 77.56, 31.75, 19.86, 13.84
DEPT (135°) ($CDCl_3$)	CH :	126.3, 81.38, 77.56
	CH_2 :	31.75, 19.86
	CH_3 :	13.84

Subfraction B4 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane as mobile phases demonstrated one spot with the R_f value of 0.18 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US2.**

Subfraction B5 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane as mobile phases demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction C Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated a long tail and two major spots with the R_f values of 0.23 and 0.32 under UV-S. Further separation by column chromatography over silica gel using a gradient system of methanol-dichloromethane was performed. Fractions, which

contained the similar components, were combined and evaporated to dryness under reduced pressure to give twelve subfractions, as shown in **Table 12**.

Table 12 Subfractions obtained from Fraction C by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
C1	4% MeOH/CH_2Cl_2	5.3	Brown gum
C2	4% MeOH/CH_2Cl_2	2.0	Brown gum
C3	4% MeOH/CH_2Cl_2	5.0	Brown gum
C4	4% MeOH/CH_2Cl_2	6.9	Brown gum
C5	4% MeOH/CH_2Cl_2	6.3	Brown gum
C6	6% MeOH/CH_2Cl_2	3.8	Brown gum
C7	6% MeOH/CH_2Cl_2	5.9	Brown gum
C8	6% MeOH/CH_2Cl_2	25.0	Dark-brown gum
C9	6% MeOH/CH_2Cl_2	8.3	Dark-brown gum
C10	6-10% MeOH/CH_2Cl_2	10.2	Dark-brown gum
C11	10-30% MeOH/CH_2Cl_2	69.8	Dark-brown gum mixed with brown solid
C12	30% MeOH/CH_2Cl_2-100% MeOH	16.7	Dark-brown gum mixed with brown solid

Subfraction C1 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction C2 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.54 under UV-S. Further purification by precoated TLC with 1% methanol-dichloromethane (7 runs) as a mobile phase afforded a colorless gum (0.7 mg) **(US3)**. Its chromatogram characteristics on normal phase TLC

with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.39 under UV-S.

$[\alpha]_D^{28}$		-40.66 (c = 0.07, MeOH)
UV (MeOH) λ_{max} nm (log ε)		215 (3.35), 268 (3.13)
FT-IR (neat) ν_{cm-1}		3741 (OH stretching), 2928 (C-H stretching) 1648 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		11.14 (*s*, 1H), 6.25 (*d*, *J* = 1.2 Hz, 1H), 6.14 (*brs*, 1H), 4.61 (*m*, 1H), 2.82 (*d*, *J* = 16.2 Hz, 1H), 2.78 (*d*, *J* = 16.2 Hz, 1H), 1.44 (*d*, *J* = 6.3 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		168.82, 163.50, 161.34, 140.71, 105.39, 100.99, 74.47, 33.80, 19.69
DEPT (135°) ($CDCl_3$)	CH :	105.39, 100.99, 74.47
	CH_2 :	33.80
	CH_3 :	19.69

Subfraction C3 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.54 under UV-S. Further purification by precoated TLC with 1% methanol-dichloromethane (7 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a colorless gum (1.2 mg). Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one UV-active spot with the same R_f value as **US3**. Thus, it was combined with **US3**.

Band 2 was obtained as a yellow gum (0.7 mg). Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction C4 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.54 under UV-S. Further purification by precoated TLC with 1% methanol-dichloromethane (7 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a yellow gum (1.0 mg). Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.39 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US3.**

Band 2 was obtained as a yellow gum (1.0 mg). Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction C5 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C6 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.25 under UV-S. Further purification by precoated TLC with 80% ethyl acetate-hexane (5 runs) as a mobile phase afforded a colorless gum (1.0 mg) **(US4)**. Its chromatogram characteristics on normal phase TLC with 70% ethyl acetate-hexane as a mobile phase demonstrated one spot with the R_f value of 0.20.

UV (MeOH) λ_{max} nm (log ε)	222 (2.94), 279 (2.36)
FT-IR (neat) ν_{cm-1}	3617 (OH stretching), 2925 (C-H stretching) 1699 (C=Cstretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	7.11 (*d*, *J* = 9.0 Hz, 2H), 6.79 (*d*, *J* = 9.0 Hz, 2H), 3.83 (*t*, *J* = 6.0 Hz, 2H), 2.81 (*t*, *J* = 6.0 Hz, 2H)

^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		154.22, 130.55, 130.15, 115.45, 63.80, 38.27
DEPT (135°) ($CDCl_3$)	CH :	130.15, 115.45
	CH_2 :	63.80, 38.27

Subfraction C7 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated a long tail and two major spots with the R_f values of 0.12 and 0.19 under UV-S. Further separation by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford three subfractions, as shown in **Table 13**.

Table 13 Subfractions obtained from Subfraction C7 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
C71	1.3	Brown gum
C72	1.7	Brown gum
C73	3.6	Brown gum

Subfraction C71 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane and 2% methanol-dichloromethane (3 runs) as mobile phases demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction C72 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane and 2% methanol-dichloromethane (3 runs) as mobile phases demonstrated one spot with the R_f value of 0.12 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US2.**

Subfraction C73 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane and 2% methanol-dichloromethane (3 runs)

as mobile phases demonstrated two spots with the R_f values of 0.14 and 0.23 under UV-S and the other spot near the baseline as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 2% methanol-dichloromethane (13 runs) as a mobile phase afforded four bands.

Band 1 was obtained as a yellow gum (1.3 mg). Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (3 runs) as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Band 2 (US5) was obtained as a colorless gum (0.9 mg). Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot with the R_f value of 0.26.

UV (MeOH) λ_{max} nm (log ε)		216 (3.80), 275 (3.29), 282 (3.21)
FT-IR (neat) ν_{cm-1}		3264 (OH stretching), 2925 (C-H stretching) 1593 (C=C stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		7.25 (*t*, *J* = 8.0 Hz, 1H), 6.92 (*brd*, *J* = 8.0 Hz, 1H), 6.87 (*brs*, 1H), 6.76 (*dd*, *J* = 8.0, 2.0 Hz, 1H), 4.67 (*s*, 2H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		155.85, 142.85, 129.85, 119.17, 114.56, 113.73, 65.06
DEPT (135°) ($CDCl_3$)	CH :	129.85, 119.17, 114.56, 113.73
	CH_2 :	65.06

Band 3 was obtained as a yellow gum (0.5 mg). Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot with the R_f value of 0.16. The ^{1}H NMR spectrum indicated that the major compound was **US2.**

Band 4 was obtained as a yellow gum (0.5 mg). Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one pale spot near the baseline as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction C8 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot with the R_f value of 0.16 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US2.**

Subfraction C9 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) and 4% methanol-dichloromethane (3 runs) as mobile phases demonstrated one major spot with the R_f value of 0.33 under UV-S. The ^{1}H NMR data indicated that it contained a mixture of **US2** and **US6**.

Subfraction C10 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) and 4% methanol-dichloromethane (3 runs) as mobile phases demonstrated one spot with the R_f value of 0.32 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US6.**

Subfraction C11 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) and 4% methanol-dichloromethane (3 runs) as mobile phases demonstrated a long tail and two major spots with the R_f values of 0.12 and 0.19 under UV-S. Further purification by column chromatography over reverse phase C_{18} silica gel was performed using 30% methanol-water and gradually enriched with methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give five subfractions, as shown in **Table 14**.

Table 14 Subfractions obtained from Subfraction C11 by column chromatography over reverse phase C_{18} silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
C11a	30% MeOH/H_2O	31.0	Brown gum
C11b	30% MeOH/H_2O	5.2	Brown gum
C11c	50-70% MeOH/H_2O	6.6	Brown gum
C11d	70% MeOH/H_2O	9.1	Brown gum
C11e	70% MeOH/H_2O-100% MeOH	13.9	Brown gum mixed with Brown solid

Subfraction C11a Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated three spots with the R_f values of 0.11, 0.23 and 0.32 under UV-S. Further separation on column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four subfractions, as shown in **Table 15**.

Table 15 Subfractions obtained from Subfraction C11a by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
C11a1	1.0	Brown gum
C11a2	15.9	Brown gum
C11a3	4.9	Brown gum
C11a4	6.0	Brown gum mixed with brown solid

Subfraction C11a1 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 5% methanol-dichloromethane as mobile phases demonstrated none of UV-active spots. The ^1H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction C11a2 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 5% methanol-dichloromethane as mobile phases demonstrated two spots with the R_f values of 0.14 and 0.23 and other spots with the R_f values of 0.18 and 0.43 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. This subfraction was dissolved in acetic anhydride (2 mL) in the presence of pyridine (0.5 mL). The reaction mixture was stirred at room temperature, overnight. The reaction mixture was poured into water and then extracted with ethyl acetate (3 x 15 mL). The combined ethyl acetate extracts were washed with 10% aqueous HCl (2 x 20 mL), 10% aqueous $NaHCO_3$ (3 x 20 mL) and water (3 x 20 mL), respectively, and then dried over anhydrous sodium sulphate. After removal of ethyl acetate, the acetate derivative (C11a21) was obtained as a pale brown gum (10.0 mg). Further purification by precoated TLC with 30% ethyl acetate-dichloromethane (3 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a pale brown gum (4.0 mg). Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.34 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a pale brown gum (3.7 mg). Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.18 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C11a3 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 5% methanol-dichloromethane as mobile phases demonstrated one spot with the R_f value of 0.20 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C11a4 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 5% methanol-dichloromethane as

mobile phases demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction C11b Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.32 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C11c Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.29 and 0.36 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. This subfraction was subjected to acetylation reaction. After work up, the acetate derivative (C11c1) was obtained as a pale brown gum (2.3 mg). Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C11d Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. This subfraction was subjected to acetylation reaction. After work up, the acetate derivative (C11d1) was obtained as a pale brown gum (3.6 mg). Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C11e Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction C12 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (3 runs) and 4% methanol-dichloromethane (3 runs) as mobile phases demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction D Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated a long tail and three major spots with the R_f values of 0.14, 0.23 and 0.32 under UV-S. Further separation by column chromatography over silica gel using a gradient system of methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give eight subfractions, as shown in **Table 16**.

Table 16 Subfractions obtained from Fraction D by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
D1	4% MeOH/CH_2Cl_2	4.8	Brown gum
D2	4% MeOH/CH_2Cl_2	6.8	Brown gum
D3	4% MeOH/CH_2Cl_2	1.1	Brown gum
D4	4% MeOH/CH_2Cl_2	1.6	Brown gum
D5	4% MeOH/CH_2Cl_2	9.7	Colorless gum
D6	6% MeOH/CH_2Cl_2	6.3	Brown gum
D7	6-10% MeOH/CH_2Cl_2	15.9	Brown gum
D8	20% MeOH/CH_2Cl_2 - 100% MeOH	10.2	Dark-brown gum

Subfraction D1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction D2 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated three spots with the R_f values of 0.32, 0.40 and 0.50 under UV-S. The ^{1}H NMR spectrum indicated that it was a mixture of **US3** and **US6**.

Subfraction D3 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.23 and 0.27 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US4**.

Subfraction D4 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction D5 (US6) Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S.

UV (MeOH) λ_{max} nm (log ε)		296 (3.18)
FT-IR (neat) ν_{cm-1}		3395 (OH stretching), 2931 (C-H stretching) 1651 (C=C stretching)
^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) (300 MHz)		6.69 (*d*, *J* = 2.7 Hz, 1H), 6.53 (*d*, *J* = 2.7 Hz,1H), 4.60 (*s*, 2H)
^{13}C NMR ($CDCl_3$ + CD_3OD) (δ ppm) (125 MHz)		148.94, 142.57, 127.33, 119.49, 114.22, 112.86, 61.09
DEPT (135°) ($CDCl_3$)	CH :	114.22, 112.86
	CH_2 :	61.09

Subfraction D6 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction D7 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Further separation by column chromatography over silica gel was performed using 70% ethyl acetate-dichloromethane and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions, which contained the

similar components, were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 17**.

Table 17 Subfractions obtained from Subfraction D7 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
D71	70-80% EtOAc/CH_2Cl_2	6.5	Dark-brown gum
D72	80% EtOAc/CH_2Cl_2-100% EtOAc	3.4	Dark-brown gum
D73	1% MeOH/EtOAc-100% MeOH	5.8	Dark-brown gum

Subfraction D71 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction D72 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated many spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction D73 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction D8 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction E Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated a long tail under UV-S. Further separation by column

chromatography over Sephadex LH-20 using 100% methanol was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four subfractions, as shown in **Table 18**.

Table 18 Subfractions obtained from Fraction E by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
E1	9.3	Dark-brown gum
E2	8.7	Dark-brown gum
E3	7.5	Colorless gum
E4	7.5	Dark-brown gum mixed with brown solid

Subfraction E1 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (4 runs) as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction E2 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction E3 (US7) Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.10 under UV-S.

UV (MeOH) λ_{max} nm (log ε)	233 (3.56), 323 (3.31)
FT-IR (neat) ν_{cm-1}	3400 (OH stretching), 2925 (C-H stretching) 1670 (C=O stretching)
^{1}H NMR ($CDCl_3$ + CD_3OD) (δ ppm) (300 MHz)	7.29 (*d*, *J* = 3.0 Hz, 1H), 6.96 (*dd*, *J* = 9.0, 3.0 Hz, 1H), 6.79 (*d*, *J* = 9.0 Hz, 1H)
^{13}C NMR ($CDCl_3$ + CD_3OD) (δ ppm)	73.10, 154.79, 148.71, 123.25, 117.52, 115.20,

(75 MHz)		114.28
DEPT (135°) ($CDCl_3$)	CH :	123.25, 117.52, 115.20

Subfraction E4 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction F Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

2.2.3 Purification of the mycelial extract

The mycelial extract of the fungus *P. microspora* PSU-A70 (120.0 mg) was chromatographed on Sephadex LH-20 column chromatography using 100% methanol to afford thirty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford three fractions, as shown in **Table 19**.

Table 19 Fractions obtained from the mycelial extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	79.9	Brown gum mixed with brown solid
B	10.1	Brown gum mixed with brown solid
C	8.6	Dark-brown gum

Fraction A Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 3% methanol-dichloromethane (3 runs) as mobile phases demonstrated one spot with the R_f value of 0.21 under UV-S and other violet spots with the R_f values of 0.28, 0.44 and 0.76 after dipping in ASA reagent and

subsequently heating the TLC plate. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give five subfractions, as shown in **Table 20**.

Table 20 Subfractions obtained from Fraction A by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
A1	3% MeOH/CH_2Cl_2	12.7	Brown gum
A2	3% MeOH/CH_2Cl_2	3.2	Brown gum
A3	3% MeOH/CH_2Cl_2	5.5	Brown gum
A4	7-30% MeOH/CH_2Cl_2	7.3	Brown gum
A5	30% MeOH/CH_2Cl_2-100%MeOH	9.6	Dark-brown gum

Subfraction A1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.78 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^1H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Subfraction A2 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated many spots under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction A3 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.16 under UV-S. The ^1H NMR spectrum indicated that the major compound was **US2**.

Subfraction A4 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated none of

definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction A5 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction B Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Fraction C Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated one major spot with the R_f value of 0.50 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US3**.

CHAPTER 2.3

RESULTS AND DISCUSSION

Six known compounds (**US2**-**US7**) were obtained from the broth extract of *P. microspora* PSU-A70. Only two compounds (**US2** and **US3**) were found in the mycelial extract. The structures were elucidated by spectroscopic methods.

2.3.1 Compound US2

Compound **US2** was obtained as a colorless gum. It exhibited UV absorption bands at 223 and 293 nm (**Figure 10**) while hydroxyl and carbonyl absorption bands were found at 3618 and 1697 cm^{-1}, respectively, in the IR spectrum (**Figure 11**). The ^{1}H NMR spectrum (**Figure 12**) (**Table 21**) contained signals of two hydroxy protons (δ_H 5.23, *brs*, 2H), one olefinic proton (δ_H 5.86, *s*, 1H), two oxymethine protons [δ_H 4.55 (*brs*, 1H) and 4.21 (*d*, *J* = 2.7 Hz, 1H)] and one propyl moiety [δ_H 2.38 (*m*, 2H), 1.53 (*m*, 2H) and 0.89 (*t*, *J* = 7.5 Hz, 3H)]. The ^{13}C NMR spectrum (**Figure 15**) (**Table 21**) showed one carbonyl carbon (δ_C 203.72), one quaternary carbon (δ_C 179.66), two oxymethine carbons (δ_C 81.38 and 77.56), two methylene carbons (δ_C 31.75 and 19.86) and one methyl carbon (δ_C 13.84). The signals at δ_C 31.75, 19.81 and 13.84 confirmed the presence of the propyl unit. In the COSY spectrum (**Figure 18**) (**Table 21**), ^{1}H-^{1}H couplings were observed between H-2 (δ_H 4.21) and H-3 (δ_H 4.55) and between H-3 and H-5 (δ_H 5.86). HMBC cross peaks of both H-2 and H-5 with C-1 (δ_C 203.72), C-3 (δ_C 77.56) and C-4 (δ_C 179.66) (**Figure 17**) (**Table 21**) constructed a cyclopentenone skeleton. Moreover, H_2-1′ (δ_H 2.38) of the propyl unit gave HMBC cross peaks with C-3, C-4 and C-5 (δ_C 126.30), indicating the attachment of the propyl moiety at C-4 of the cyclopentenone skeleton. Since H-2 was coupled with H-3 with a small coupling constant of 2.7 Hz, H-2 and H-3 had *trans* relationship. Therefore, **US2** was identified as dihydroterrein (Malmstrø m, *et al*., 2002).

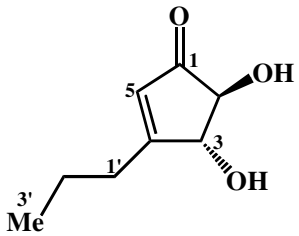


Table 21 The NMR data of compound US2 in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	COSY	NOE
1	-	203.72 (C=O)	-	-	-
2	4.21 (*d*, 2.7)	81.38 (CH)	C-1, C-3, C-4	H-3	H-3, H-5
2-OH	5.23 (*brs*)	-	-	-	-
3	4.55 (*brs*)	77.56 (CH)	C-2, C-4, C-5	H-2, H-5, H-1′	H-2, H-5, H-1′, H-2′
4	-	179.66 (C)	-	-	-
5	5.86 (*s*)	126.30 (CH)	C-1, C-2, C-3, C-4, C-1′	H-3, H-1′	H-2, H-3, H-1′, H-2′
1′	2.38 (*m*)	31.75 (CH_2)	C-3, C-4, C-5, C-2′, C-3′	H-3, H-5, H-2′	H-5
2′	1.53 (*m*)	19.86 (CH_2)	C-4, C-1′, C-3′	H-1′, H-3′	H-3, H-1′, Me-3′
3′	0.89 (*t*, 7.5)	13.84 (CH_3)	C-1′, C-2′	H-2′	H-3, H-5, H-1′, H-2′

2.3.2 Compound US3

Compound **US3** was obtained as a colorless gum. It exhibited an UV absorption band at 268 nm **(Figure 19)** while hydroxyl and carbonyl absorption bands were found at 3741 and 1648 cm^{-1}, respectively, in the IR spectrum **(Figure 20)**. The ^1H NMR spectrum (**Figure 21**) (**Table 22**) displayed characteristic signals for one

chelated hydroxyl proton at δ_H 11.14 (*s*) and a 1,2,3,5-tetrasubstituted benzene at δ_H 6.25 (*d*, *J* = 1.2 Hz, 1H) and 6.14 (*brs*, 1H). The chelated hydroxyl group was placed at C-8 (δ_C 163.50), *peri* position to a carbonyl group. This hydroxy proton gave a HMBC correlation with C-7 (δ_C 100.99) (**Figure 26**) (**Table 23**) which exhibited a HMQC cross peak with the aromatic proton at δ_H 6.25 (**Figure 25**) (**Table 22**). Thus, the remaining aromatic proton resonating at δ_H 6.14 was attributed to H-5. In addition, the ^{1}H NMR spectrum showed signals for one methyl group at δ_H 1.44 (*d*, *J* = 6.3 Hz, 3H), one methylene group at δ_H 2.82 (*d*, *J* = 16.2 Hz, 1H) and 2.78 (*d*, *J* = 16.2 Hz, 1H) and one methine group at δ_H 4.61 (*m*, 1H). In the ^{1}H-^{1}H COSY spectrum (**Figure 27**) (**Table 23**), H-3 (δ_H 4.61) was coupled with H_{ab}-4 (δ_H 2.78 and 2.82) and Me-9 (δ_H 1.44). These data together with the chemical shift of H-3 indicated the presence of –$CH_2CH(OR)CH_3$ unit. HMBC correlations of H_{ab}-4 with C-4a (δ_C 140.71), C-5 (δ_C 105.39) and C-8a (δ_C 100.99) linked this unit at C-4a of the aromatic ring. Comparison of the NMR data and specific rotation [**US3**: $[\alpha]_D$ -40.66 (c = 0.07, MeOH)] with 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin ($[\alpha]_D$ -33 (c = 0.07, MeOH) (Warashina, 2004) indicated that **US3** was (*R*)-(-)-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin, which was previously isolated from *Ceratocystis minor* (Ayer, *et al*., 1987).

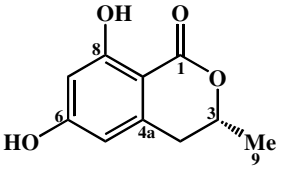


Table 22 The NMR data of compound US3 in $CDCl_3$

Position	US3		3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin	
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	-	168.82 (C=O)	-	170.69 (C=O)
3	4.61 (*m*)	74.47 (CH)	4.70 (*m*)	76.33 (CH)
4	a: 2.82 (*d*, 16.2)	33.80 (CH_2)	a: 2.85 (*d*, 16)	35.03 (CH_2)
	b: 2.78 (*d*, 16.2)		b: 2.96 (*d*, 16)	
4a	-	140.71 (C)	-	143.21 (C)

Table 22 (continued)

Position	US3		3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin	
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
5	6.14 (*brs*)	105.39 (CH)	6.27 (*s*)	107.41 (CH)
6	-	161.34 (C)	-	165.23 (C)
7	6.25 (*d*, 1.2)	100.99 (CH)	6.29 (*s*)	101.92 (CH)
8	-	163.50 (C)	-	165.09 (C)
8-OH	11.14 (*s*)	-	11.30 (*s*)	-
8a	-	100.99 (C)	-	101.71 (C)
9	1.44 (*d*, 6.3)	19.69 (CH_3)	1.45 (*d*, 6.3)	20.82 (CH_3)

Table 23 The HMBC, COSY and NOE data of compound US3 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-3	C-4a, C-9	H-4, H-9	H-4, H-9
H_a-4	C-3, C-4a, C-5, C-8a, C-9	H-3	H-3, H_b-4, H-5
H_b-4		H-3	H-3, H_a-4, H-5
H-5	C-4, C-6, C-7	H-7	H-4
H-7	C-5, C-6, C-8, C-8a	H-5	-
8-OH	C-7, C-8, C-8a	-	-
H-9	C-1, C-7, C-8	-	-

2.3.3 Compound US4

Compound **US4** was obtained as a colorless gum. It exhibited UV absorption bands at 222 and 279 nm (**Figure 28**) while a hydroxyl absorption band was found at 3617 cm^{-1} in the IR spectrum (**Figure 29**). The ^1H NMR spectrum (**Figure 30**) (**Table 24**) displayed characteristic signals for a 1,4-disubstituted benzene [δ_H 7.11 (*d*, *J* = 9.0 Hz, 1H) and 6.72 (*d*, *J* = 9.0 Hz, 1H)] and a hydroxyethyl group [δ_H 3.83 (*t*, *J* = 6.0 Hz, 2H) and 2.81 (*t*, *J* = 6.0 Hz, 2H)]. The ^{13}C NMR

spectrum (**Figure 32**) (**Table 24**) showed one oxyquaternary carbon (δ_C 154.22), one quaternary carbon (δ_C 130.55), two carbon resonances for four aromatic carbons (δ_C 130.15 and 115.45) and two methylene carbons (δ_C 63.80 and 38.27). These data revealed that one of the substitutents on the 1,4-disubstituted benzene was a hydroxyl group. The aromatic protons at δ_H 7.11 were attributed to H-3 and H-5, on the basis of their chemical shifts and HMBC correlations with C-1 (δ_C 154.22), C-2, C-6 (δ_C 115.45) and C-7 (δ_C 38.27) (**Figure 34**) (**Table 25**). Thus, the remaining aromatic protons resonating at δ_H 6.79 were attributed to H-2 and H-6 according to their multiplicity, coupling constant and HMBC correlations. Consequently, the hydroxyethyl group was attached at C-4 (δ_C 130.55). ^3J HMBC correlations of H_2-7 (δ_H 2.81) with C-3 and C-5 (δ_C 130.15) supported the assigned location. The chemical shifts of H_2-8 (δ_C 3.83) and C-8 (δ_C 63.80) confirmed the attachment of a hydroxyl group at C-8. Therefore, **US4** was identified as tyrosol (Capasso, *et al.*, 1992).

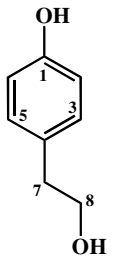


Table 24 The NMR data of compound US4 in $CDCl_3$

Position	US4		tyrosol
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_C (C-type)
1	-	154.22 (C)	156.70 (C)
2, 6	6.79 (*d*, 9.0)	115.45 (CH)	116.00 (CH)
3, 5	7.11 (*d*, 9.0)	130.15 (CH)	130.80 (CH)
4	-	130.55 (C)	130.90 (C)
7	2.81 (*t*, 6.0)	38.27 (CH_2)	39.30 (CH_2)
8	3.83 (*t*, 6.0)	63.80 (CH_2)	64.50 (CH_2)

Table 25 The HMBC and NOE data of compound US4 in $CDCl_3$

Proton	HMBC correlations	NOE
H-2, H-6	C-1, C-3, C-5	H-3, H-5
H-3, H-5	C-1, C-2, C-6, C-7	H-2, H-6, H-7
H-7	C-3, C-5, C-8	H-3, H-5, H-8
H-8	C-4, C-7	H-7

2.3.4 Compound US5

Compound **US5** was obtained as a colorless gum. It exhibited UV absorption bands at 275 and 282 nm (**Figure 35**) while a hydroxyl absorption band was found at 3264 cm^{-1} in the IR spectrum (**Figure 36**). The ^{1}H NMR spectrum (**Figure 37**) (**Table 26**) displayed typical signals for a 1,3-disubstituted benzene [δ_H 7.25 (*t*, *J* = 8.0 Hz, 1H), 6.92 (*brd*, *J* = 8.0 Hz, 1H), 6.87 (*brs*, 1H) and 6.76 (*dd*, *J* = 8.0 and 2.0 Hz, 1H)] and one hydroxymethyl group (δ_H 4.67, *s*, 2H). The ^{13}C NMR spectrum (**Figure 40**) (**Table 26**) showed one oxyquaternary carbon (δ_C 155.85), one quaternary carbon (δ_C 142.85), four methine carbons (δ_C 129.85, 119.17, 114.56 and 113.73) and one hydroxymethylene carbon (δ_C 65.06). The aromatic proton at δ_H 6.76 was attributed to H-4 on the basis of HMBC correlations with C-2 (δ_C 113.73), C-3 (δ_C 155.85) and C-6 (δ_C 119.17) (**Figure 42**) (**Table 26**). Thus, the remaining aromatic protons resonating at δ_H 7.25, 6.92 and 6.87 were assigned as H-5, H-6 and H-2, respectively, according to their multiplicity, coupling constants and HMBC correlations. From these results, the hydroxymethyl group became a C-1 substituents. This was confirmed by HMBC correlations of H_2-7(δ_H 4.67)/ C-1 (δ_C 142.85), C-2 and C-6. Therefore, **US5** was identified as 3-hydroxybenzyl alcohol (Alfaro, *et al.*, 2003).

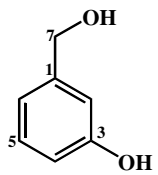


Table 26 The NMR data of compound US5 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	HMBC correlations	COSY
1	-	142.85(C)	-	-
2	6.87 (*brs*)	113.73 (CH)	C-3, C-4, C-6, C-7	H-4, H-6, H-7
3	-	155.85 (C)	-	-
4	6.76 (*dd*, 8.0, 2.0)	114.56 (CH)	C-2, C-3, C-6	H-2, H-5
5	7.25 (*t*, 8.0)	129.85 (CH)	C-1, C-3, C-4, C-6	H-4, H-6
6	6.92 (*brd*, 8.0)	119.17 (CH)	C-2, C-4, C-5, C-7	H-5, H-7
7	4.67 (*s*)	65.06 (CH_2)	C-1, C-2, C-6	H-2, H-6

2.3.5 Compound US6

Compound **US6** was obtained as a colorless gum. It exhibited UV **(Figure 44)** and IR **(Figure 45)** absorption bands similar to those of **US5**. The ^{1}H NMR spectrum (**Figure 46**) (**Table 27**) indicated that **US6** was a derivative of **US5**. In addition, the ^{1}H NMR spectrum displayed the replacement of characteristic signals of the 1,3-disubstituted benzene in **US5** with typical signals for a 1,2,3,5-tetrasubstituted benzene [δ_H 6.69 (*d*, *J* = 2.7 Hz, 1H), and 6.53 (*d*, *J* = 2.7 Hz, 1H)] in **US6**. The ^{13}C NMR spectrum (**Figure 47**) (**Table 27**) showed two oxyquaternary carbons (δ_C 148.94 and 142.57), two quaternary carbons (δ_C 127.33 and 119.49), two methine carbons (δ_C 114.22 and 112.86) and one hydroxymethylene carbon (δ_C 61.09). The aromatic proton at δ_H 6.53 was attributed to H-6 on the basis of its HMBC correlations with C-2 (δ_C 142.57), C-4 (δ_C 114.22) and C-5 (δ_C 148.94) (**Figure 49**) (**Table 27**). Thus, the other aromatic proton resonating at δ_H 6.69 was attributed to H-4 according to its multiplicity, coupling constant and HMBC correlations. HMBC correlations of H_2-7 (δ_H 4.60) with C-1 (δ_C 127.33), C-2 and C-6 (δ_C 112.86) linked the hydroxymethyl group at C-1. According to the carbon chemical shifts, the substituents at C-2 and C-5 were hydroxyl groups. The low resolution mass spectrum (**Figure 50**) showed the molecular ion at *m/z* 174/176 in a ratio of 3:1, thus indicating that a chlorine atom was attached at C-3. Therefore, **US6** was identified as 3-chlorogentisyl alcohol (McCorkindale, *et al.*, 1972).

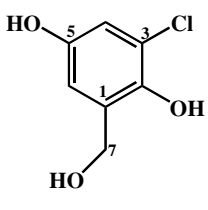


Table 27 The NMR data of compound US6 in $CDCl_3$+CD_3OD

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations
1	-	127.33 (C)	-
2	-	142.57 (C)	-
3	-	119.49 (C)	-
4	6.69 (*d*, 2.7)	114.22 (CH)	C-2, C-3, C-5, C-6
5	-	148.94 (C)	-
6	6.53 (*d*, 2.7)	112.86 (CH)	C-2, C-4, C-5
7	4.60 (*s*)	61.09 (CH_2)	C-1, C-2, C-6

2.3.6 Compound US7

Compound **US7** was obtained as a colorless gum. The IR spectrum (**Figure 52**) showed typical absorption bands of a benzoic acid derivative at 3400 and 1670 cm^{-1}. The ^{13}C resonance at δ_C 172.00 (**Figure 56**) (**Table 28**) supported the IR data. The ^1H NMR spectrum (**Figure 53**) (**Table 28**) displayed typical signals for a 1,2,5-trisubstituted benzene [δ_H 7.29 (*d*, *J* = 3.0 Hz, 1H), 6.96 (*dd*, *J* = 9.0 and 3.0 Hz, 1H) and 6.79 (*d*, *J* = 9.0 Hz, 1H)]. The aromatic proton at δ_H 7.29 was attributed to H-6 on the basis of HMBC correlations with C-1 (δ_C 114.28), C-2 (δ_C 154.79), C-5 (δ_C 148.71) and C-7 (δ_C 172.00) (**Figure 58**) (**Table 27**). Thus, the remaining aromatic protons resonating at δ_H 6.96 and 6.79 were attributed to H-3 and H-4, respectively, according to their multiplicity, coupling constants and HMBC correlations. The substituents at C-2 and C-5 were the hydroxyl groups on the basis of their carbon chemical shifts. Therefore, **US7** was identified as gentisic acid (Marzouk, *et al.*, 1999).

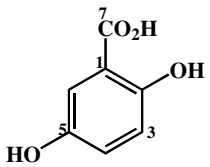


Table 28 The NMR data of compound US7 in $CDCl_3$+CD_3OD

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	COSY
1	-	114.28 (C)	-	-
2	-	154.79 (C)	-	-
3	6.79 (*d*, 9.0)	117.52 (CH)	C-1, C-2, C-5, C-7	H-4
4	6.96 (*dd*, 3.0, 9.0)	123.25 (CH)	C-2, C-5, C-6	H-3, H-6
5	-	148.71 (C)	-	-
6	7.29 (*d*, 3.0)	115.20 (CH)	C-1, C-2, C-5, C-7	H-4
7	-	172.00 (C=O)	-	-

PART Ⅲ

CHEMICAL CONSTITUENTS FROM THE ENDOPHYTIC FUNGUS *PHOMOPSIS* SP. PSU-D15

CHAPTER 3.1

INTRODUCTION

3.1.1 Introduction

The genus *Phomopsis* has been known to be a rich source of bioactive secondary metabolites of diverse structures (Isaka, *et al.*, 2001). This microorganism produces bioactive natural products as summarized in **Table 29**. In connection with our ongoing research for bioactive fungal metabolites, the ethyl acetate extracts from the culture broth and mycelia of the endophytic fungus *Phomopsis* sp. PSU-D15 was found to exhibit interesting antimycobacterial activity with MIC values of 12.5 and 100 μg/mL, respectively. This fungus was isolated from the leaves of *Garcinia dulcis*.

Table 29 Metabolites from the fungus *Phomopsis* sp. and the biological activity

Compound	Structure	Activity	Reference
chaetoglobosin M	18	-	Burlot, *et al*, 2003
chaetoglobosin N	19	-	,,
chaetoglobosin O	20	growth-inhibition	Burlot, *et al*, 2003, Ichihar, *et al*, 1996
chaetoglobosin 510	21	antimicrofilament and cytotoxic	Christain, *et al*, 2005
chaetoglobosin 540	22	,,	,,
chaetoglobosin 542	23	,,	,,
convolvulanic acid A	24	phytotoxic	Tsantrizos, *et al*, 1992

Table 29 (continued)

Compound	Structure	Activity	Reference
convolvulanic acid B	25	phytotoxic	Tsantrizos, *et al*, 1992
onvolvulol	26	,,	,,
cytochalasin H	27	inhibited the growth of wheat coleoptiles	Izawa, *et al.*, 1989, Cole, *et al.*, 1981
cytochalasin J	28	-	Izawa, *et al.*, 1989
epoxcytochalasin H	29	-	,,
epoxcytochalasin J	30	-	,,
cytochalasin N	31	-	,,
cytochalasin O	32	-	,,
cytochalasin P	33	-	,,
cytochalasin Q	34	-	,,
cytochalasin R	35	-	,,
cytochalasin S	36	-	,,
dicerandrol A	37	antibiotic and cytotoxic	Wagenaar and Clardy, 2001
dicerandrol B	38	,,	,,
dicerandrol C	39	,,	,,
phomodiol	40	antifungal	Horn, *et al.*, 1994
phomol	41	antiinflammatory	Weber, *et al*, 2004
phomopsichalasin	42	antimicrobial	Horn, *et al.*, 1995
phomopsidin	43	anti-microtubule	Kobayashi , *et al.*, 2003

Table 29 (continued)

Compound	Structure	Activity	Reference
phomopsin A	44	anti-microtubule	Lacey, *et al*, 1987, luduena, 1989
phomopsin B	45	anti-microtubule	Lacey, *et al*, 1987
phomosine A	46	antibacterial, fungicidal and algicidal activity	Krohn, *et al.*, 1995
phomosine B	47	,,	,,
phomosine C	48	,,	,,
phomosine D	49	antibacterial, fungicidal and algicidal activity	Dai, *et al*, 2005
phomosine E	50	,,	,,
phomosine F	51	,,	,,
phomosine G	52	,,	,,
Phomoxanthone A	53	antimalarial, antitubercular and cytotoxicity	Isaka, *et al.*, 2001
Phomoxanthone B	54	,,	,,
phenochalasin A	55	Inhibitor of lipid droplet formation	Tomoda, *et al*, 1999
phenochalasin B	56	,,	,,
(+)-fusicocca-3(16),10(14)-diene	57	-	Sassa, *et al*, 2004
(+)-β-araneosene	58	-	,,
(+)-δ-araneosene	59	-	,,

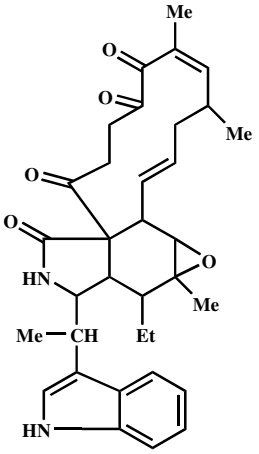
Table 29 (continued)

Compound	Structure	Activity	Reference
1-Methyl-3-(4'-hydroxy-phenyl)propyl caffeate	60	antifungal	Svetaz, *et al*, 2004
α-pyrone convolvulopyrone	61	phytotoxic	Tsantrizos, *et al*, 1992
(1a*S*,3*R*,4*R*,4a*R*,6*S*,7*R*,8a*S*)-7-chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octa-hydro-1a*H*-naphtho[1-b]-oxirene-4-carboxylic acid	62	antibacterial, fungicidal and algicidal activity	Dai, *et al.*, 2005
(2*S*,3*S*)-3,6-dihydro-6-oxo-2-(1*E*)-1-propenyl-2*H*-pyran-3-yl 4,6-dimethyl-2,4-dodecadienoate	63	cytokine production inhibitory	Wrigley, *et al.*, 1999
(2*S*,3*S*)-2-[(1*E*)-2-carboxy-ethenyl]-3,6-dihydro-6-oxo-2*H*-pyran-3-yl 4,6-dimethyl-2,4-dodecadienoate	64	,,	,,
(2*S*,3*S*)-2-[(1*E*)-2-carboxy-ethenyl]-3,6-dihydro-6-oxo-2*H*-pyran-3-yl 11-hydroxy-4,6-dimethyl-2,4-dodeca-dienoate	65	,,	,,
3a,5a,6,7,8,9,9a,9b-octa-hydro-7,9b-dimethyl-naphtho[1,2-c]furan-1(3*H*)-one	66	-	Begley and Grove 1985

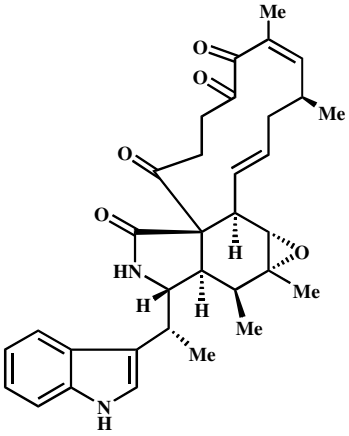
Table 29 (continued)

Compound	Structure	Activity	Reference
6α,7β,9α-trihydroxy-8 (14),15-isopimaradiene-20,6γ-lactone	67	cytotoxic	Morooka, *et al.*, 1986
6-hydroxy-6-isopropylcyclo-hex-1-enecarboxylic acid	68	antibacterial, fungicidal and algicidal activity	Dai, *et al*, 2005
Phomozin	69	phytotoxin	Mazars, *et al*, 1990

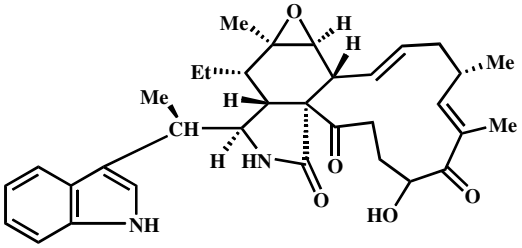
Structures of the metabolites from *Phomopsis* sp.



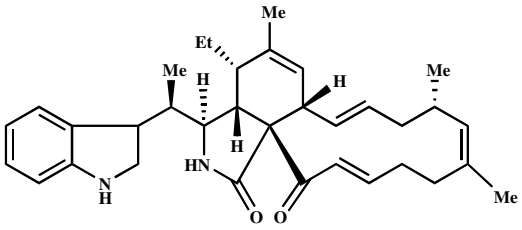
18 : chaetoglobosin M



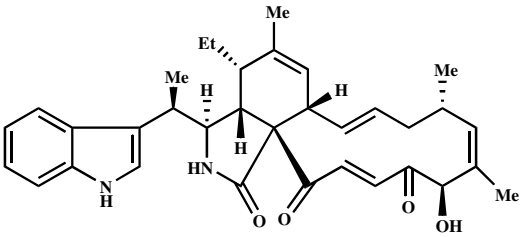
19 : chaetoglobosin N



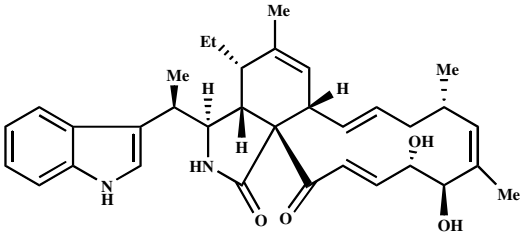
20 : chaetoglobosin O



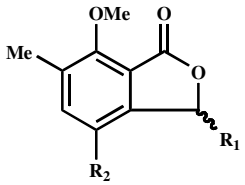
21 : chaetoglobosin 510



22 : chaetoglobosin 540



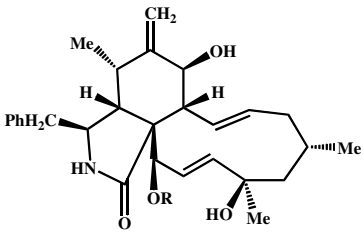
23 : chaetoglobosin 542



24 : R_1 = OH, R_2 = CO_2H : convolvulanic acid A

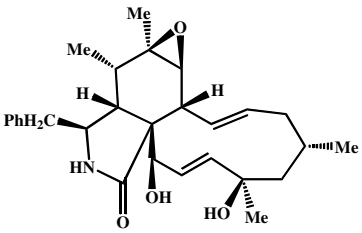
25 : R_1 = H, R_2 = CO_2H : convolvulanic acid B

26 : R_1 = OH, R_2 = CH_2OH : convolvulol



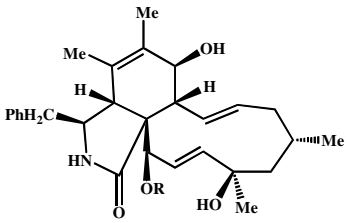
27 : R = Ac : cytochalasin H

28 : R = H : cytochalasin J



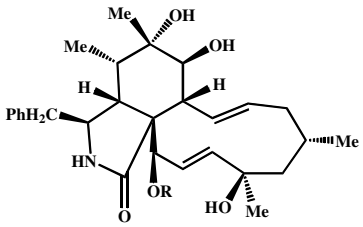
29 : R = Ac : epoxcytochalasin H

30 : R = H : epoxcytochalasin J



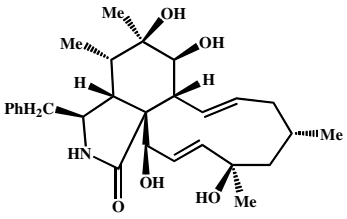
31 : R = Ac : cytochalasin N

32 : R = H : cytochalasin O

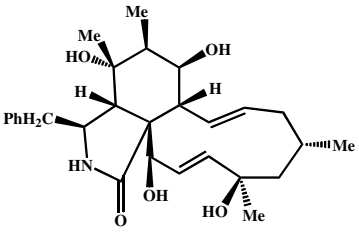


33 : R = Ac : cytochalasin P

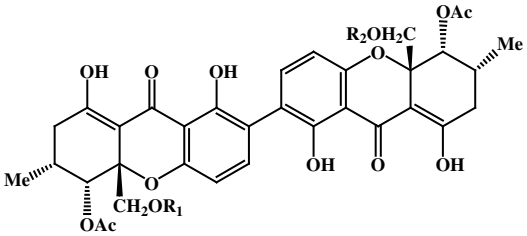
34 : R = H : cytochalasin Q



35 : cytochalasin R



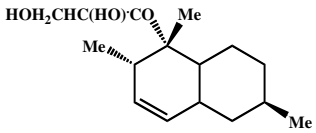
36 : cytochalasin S



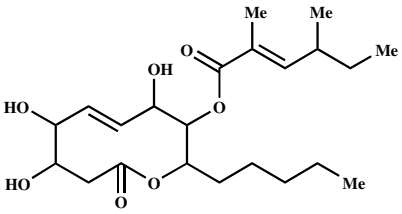
37 : dicerandrol A : $R_1 = R_2 = H$

38 : dicerandrol B : R_1 = Ac, R_2 = H

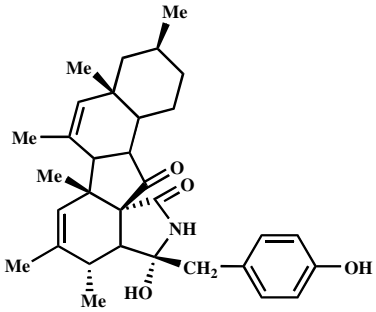
39 : dicerandrol C : $R_1 = R_2$ = Ac



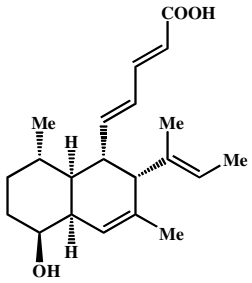
40 : phomodiol



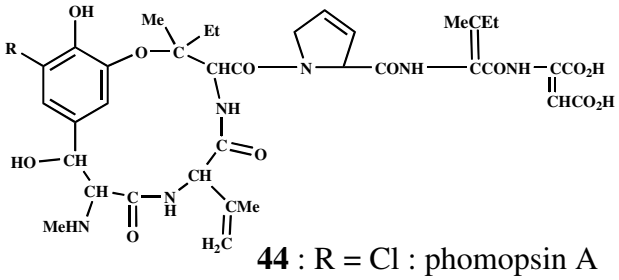
41 : phomol



42 : phomopsichalasin

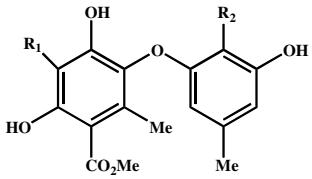


43 : phomopsidine



44 : R = Cl : phomopsin A

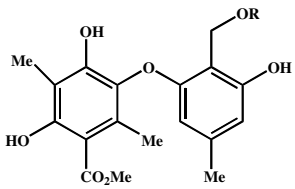
45 : R = H : phomopsin B



46 : R_1 = Me, R_2 = CHO : phomosine A

47 : R_1 = Me, R_2 = CH_2OMe : phomosine B

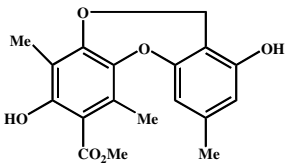
48 : R_1 = H, R_2 = CHO : phomosine C



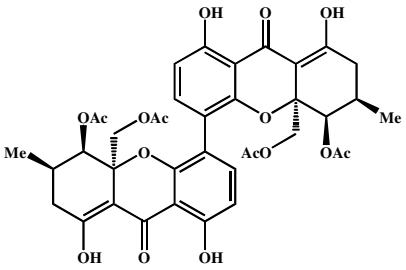
49 : R = H : phomosine D

50 : R = CH_2OMe : phomosine E

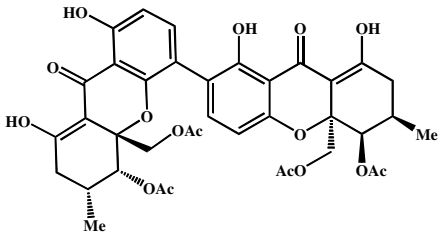
51 : R = Ac : phomosine F



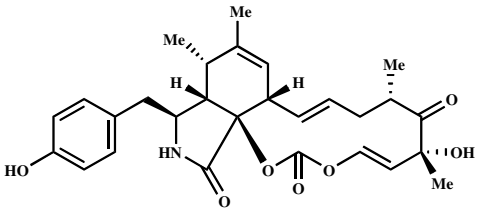
52 : phomosine G



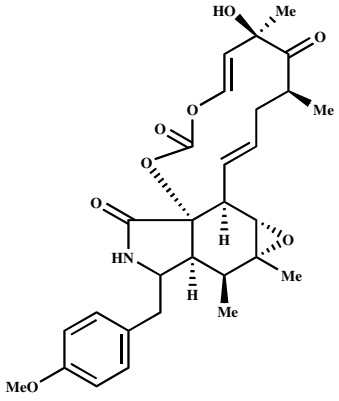
53 : phomoxanthones A



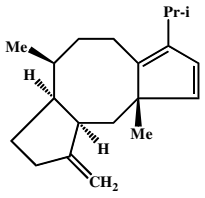
54 : phomoxanthones B



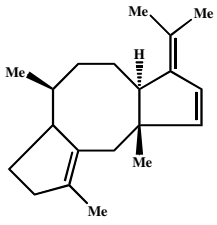
55 : phenochalasin A



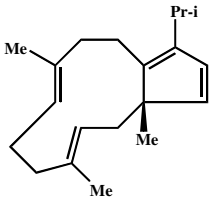
56 : phenochalasin B



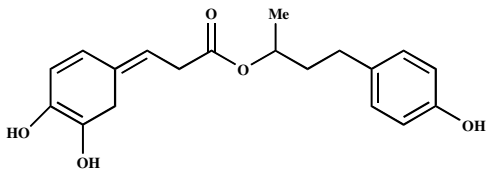
57 : (+)-fusicocca-3(16),10(14)-diene



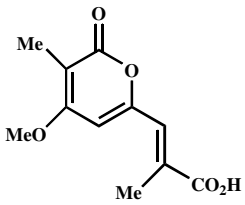
58 : (+)-β-araneosene



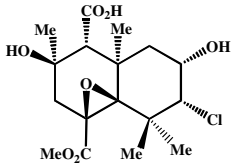
59 : (+)-δ-araneosene



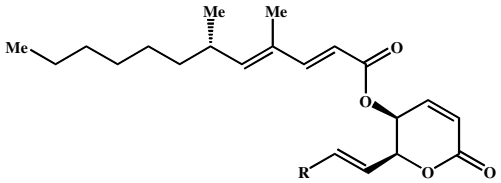
60 : 1-Methyl-3-(4'-hydroxyphenyl)propyl caffeate



61 : α-pyrone convolvulopyrone

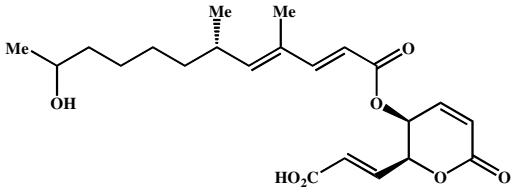


62 : (1a*S*,3*R*,4*R*,4a*R*,6*S*,7*R*,8a*S*)-7-chloro-3,6-dihydroxy-3,4a,8,8-tetramethyloctahydro-1a*H*-naphtho[1-b]oxirene-4-carboxylic acid

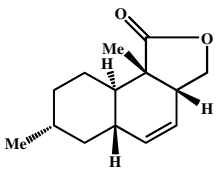


63 : R = Me : (2*S*,3*S*)-3,6-dihydro-6-oxo-2-(1*E*)-1-propenyl-2*H*-pyran-3-yl 4,6-dimethyl-2,4-dodecadienoate

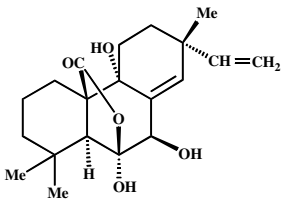
64 : R = CO_2H : (2*S*,3*S*)-2-[(1*E*)-2-carboxyethenyl]-3,6-dihydro-6-oxo-2*H*-pyran-3-yl 4,6-dimethyl-2,4-dodecadienoate



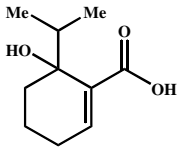
65 : (2*S*,3*S*)-2-[(1*E*)-2-carboxy-ethenyl]-3,6-dihydro-6-oxo-2*H*-pyran-3-yl 11-hydroxy-4,6-dimethyl-2,4-dodecadienoate



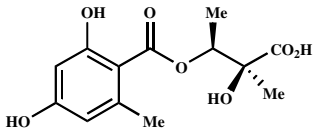
66 : 3a,5a,6,7,8,9,9a,9b-octahydro-7,9b-dimethylnaphtho[1,2-c]furan-1(3*H*)-one



67 : 6α,7β,9α-trihydroxy-8(14),15-isopimaradiene-20,6γ-lactone



68 : 6-hydroxy-6-isopropylcyclohex-1-enecarboxylic acid



69 : phomozin

CHAPTER 3.2

EXPERIMENTAL

3.2.1 Fermentation and extraction

The fermentation and extraction were performed using the same procedure as those of *A. aculeatus* PSU-D2. The crude EtOAc extracts from the culture broth and mycelia were obtained in 1.00 g and 340.0 mg, respectively, both as a brown gum.

3.2.2 Purification of the broth extract

The broth extract of *Phomopsis* sp. PSU-D15 (1.00 g) was chromatographed on Sephadex LH-20 using 100% methanol to afford forty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four fractions, as shown in **Table 30**.

Table 30 Fractions obtained from the broth extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	32.6	Dark-brown gum
B	785.9	Dark-brown gum
C	46.2	Dark-brown gum
D	7.2	Dark-brown gum

Fraction A Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Further separation on column chromatography over Sephadex LH 20 using 50%

methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 31**.

Table 31 Subfractions obtained from fraction A by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
A1	0.8	Pale brown gum
A2	5.3	Brown gum mixed with brown solid
A3	9.9	Brown gum mixed with brown solid
A4	1.6	Brown gum mixed with brown solid
A5	4.7	Brown gum
A6	0.7	Brown gum
A7	1.9	Brown gum mixed with brown solid

Subfraction A1 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (2 runs) as a mobile phase showed one pale purple spot with the R_f value of 0.61 after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction A2 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (2 runs) as a mobile phase showed one pale purple spot with the R_f value of 0.57 after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction A3 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (2 runs) as a mobile phase showed one spot with the R_f value of 0.57 after dipping in ASA reagent and subsequently heating the TLC plate. Further separation with precoated TLC on silica gel plates with 6% methanol-dichloromethane (2 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a colorless gum (1.0 mg). Its chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one pale spot under UV-S with the R_f value of 0.84. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a colorless gum (0.7 mg). Its chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed one pale spot under UV-S with the R_f value of 0.81. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction A4 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (2 runs) as a mobile phase showed one pale spot under UV-S with the R_f value of 0.24 and the other spot with the R_f value of 0.53 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction A5 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (2 runs) as a mobile phase showed one pale spot with the R_f value of 0.53 after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^1H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction A6 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (2 runs) as a mobile phase showed one pale spot under UV-S with the R_f value of 0.10 and the other spot with the R_f value of 0.53 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction A7 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (2 runs) as a mobile phase showed one pale spot with the R_f value of 0.55 after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^1H NMR spectrum showed signals at the high field, it was not further investigated.

Fraction B Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase showed a long tail and a major spot

under UV-S with the R_f value of 0.10. Further separation on column chromatography over Sephadex LH-20 using 100% methanol was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 32**.

Table 32 Subfractions obtained from the fraction B by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B1	132.1	Dark-brown gum
B2	59.3	Brown gum
B3	157.4	Brown gum
B4	135.6	Brown gum
B5	136.3	Brown gum
B6	208.4	Brown gum
B7	15.7	Dark-brown gum

Subfraction B1 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S and showed two spots with the R_f values of 0.27 and 0.51 and three spots near baseline after dipping in ASA reagent and subsequently heating the TLC plate. Further separation on column chromatography over reverse-phase C_{18} silica gel using a gradient system of methanol-water was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give eleven subfractions, as shown in **Table 33**.

Table 33 Subfractions obtained from subfraction B1 by column chromatography over reverse phase C_{18} silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
BA	20% MeOH/H_2O	3.0	Brown gum
BB	20% MeOH/H_2O	6.4	Pale brown gum
BC	20% MeOH/H_2O	4.4	Pale brown gum
BD	20-40% MeOH/H_2O	6.1	Pale brown gum
BE	20-40% MeOH/H_2O	3.4	Pale brown gum
BF	20-40% MeOH/H_2O	4.8	Pale brown gum
BG	20-40% MeOH/H_2O	5.0	Pale brown gum
BH	50% MeOH/H_2O	4.3	Pale brown gum
BI	50% MeOH/H_2O	9.2	Pale brown gum
BJ	50-70% MeOH/H_2O	7.5	Brown gum
BK	90% MeOH/H_2O - 100%MeOH	45.0	Brown gum

Subfraction BA Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated a long tail under UV-S. Because of the minute quantity, it was not further purified.

Subfraction BB Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase showed one spot near the baseline under UV-S and other spots with the R_f values of 0.16, 0.25 and 0.43 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. The ^1H NMR spectral data indicated that the major compounds were **US9** and **US12.** Because of the minute quantity, it was not further purified.

Subfraction BC Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase showed one spot under UV-S with the same R_f value as **US9**. Thus, it was combined with **US9**.

Subfraction BD Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot near the baseline under UV-S and other spots with the R_f values of 0.14 and 0.27

as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane as a mobile phase (7 runs) afforded three bands.

Band 1 was obtained as a colorless gum (1.3 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated one pale spot with the R_f value of 0.4 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a brown gum (1.0 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated one major spot with the R_f value of 0.40 and two pale spots with the R_f values of 0.11 and 0.22 under UV-S. Because of the minute quantity, it was not further purified.

Band 3 was obtained as a brown gum (0.8 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated one pale spot with the R_f value of 0.11 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction BE Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one violet spot near the baseline under UV-S and the other spot with the R_f value of 0.27 after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane as a mobile phase (7 runs) afforded three bands.

Band 1 was obtained as a yellow viscous liquid mixed with a white solid (1.5 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase showed one violet spot with the R_f value of 0.53 after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a brown gum (0.5 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile

phase demonstrated two spots near the baseline under UV-S and the other spot with the R_f value of 0.22 after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Band 3 was obtained as a brown gum (0.2 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated one pale spot near the baseline under UV-S. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction BF Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot near the baseline under UV-S and the other violet spot with the R_f value of 0.27 after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane as a mobile phase (7 runs) afforded two bands.

Band 1 was obtained as a yellow gum mixed with a colorless solid (1.9 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase showed one pale violet spot with the R_f value of 0.53 after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that it was **US8**.

Band 2 was obtained as a pale brown gum (0.9 mg). Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase showed one spot with the R_f value of 0.24 and two spots near the baseline under UV-S. Because of the minute quantity, it was not further purified.

Subfraction BG Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot near the baseline under UV-S and the other violet spot with the R_f value of 0.27 after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane as a mobile phase (7 runs) afforded two bands.

Band 1 was obtained as a brown gum mixed with a brown solid (1.6 mg). Chromatogram characteristics on normal phase TLC with 6%

methanol-dichloromethane as a mobile phase showed three spots with the R_f values of 0.24, 0.56 and 0.62. Because of the minute quantity, it was not further purified.

Band 2 was obtained as a pale yellow gum (0.6 mg). Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase showed two spots near the baseline. Because of the minute quantity, it was not further purified.

Subfraction BH Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f values of 0.71 and two spots near the baseline under UV-S and other spots with R_f values of 0.31 and 0.36 after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction BI Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated two spots with the R_f values of 0.14 and 0.32 under UV-S and other spots with the R_f values of 0.54 and 0.61 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane as a mobile phase (10 runs) afforded three bands.

Band 1 was obtained as a colorless gum (1.0 mg). Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane (2 runs) as mobile phases showed no definite spots under UV-S. Because of the minute quantity, it was not further purified.

Band 2 was obtained as a colorless gum mixed with a white solid (0.6 mg). Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as (2 runs) showed one spot with the R_f value of 0.19 and one spot near the baseline under UV-S. Because of the minute quantity, it was not further purified.

Band 3 was obtained as a pale yellow gum (0.8 mg). Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane (2 runs) showed one spot near the baseline under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction BJ Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated two spots near the baseline under UV-S and the other spot with the R_f value of 0.36 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane as a mobile phase (10 runs) afforded two bands.

Band 1 was obtained as a white gum mixed with a white solid (1.3 mg). Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane (2 runs) showed no definite spots under UV-S. Because of the minute quantity, it was not further purified.

Band 2 was obtained as a brown gum mixed with a brown solid (3.0 mg). Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane (2 runs) showed one major spot near the baseline under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction BK Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated a long tail and one spot near the baseline under UV-S and the other spot near the baseline as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give seven subfractions, as shown in **Table 34**.

Table 34 Subfractions obtained from subfraction BK by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
BK1	4.8	Brown gum mixed with brown solid
BK2	16.7	Brown gum mixed with brown solid
BK3	11.9	Brown gum
BK4	1.9	Brown gum

Table 34 (continued)

Subfraction	Weight (mg)	Physical appearance
BK5	2.6	Brown gum
BK6	1.7	Brown gum
BK7	2.7	Brown gum

Subfraction BK1 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail under UV-S. The ^{1}H NMR spectrum indicated that the major component was **US8**.

Subfraction BK2 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction BK3 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail and one spot near the baseline under UV-S and four spots with the R_f values of 0.38, 0.71, 0.83 and 0.95 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction BK4 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail and one spot near the baseline under UV-S and three spots with the R_f values of 0.38, 0.71 and 0.95 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction BK5 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail and one spot near the baseline under UV-S and many violet spots after dipping in ASA reagent subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction BK6 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot near the baseline under UV-S and the other spot near the baseline as a violet spot after dipping in ASA reagent subsequently heating the TLC plate. The ^{1}H NMR spectral data indicated that it contained a mixture of **US9** and **US11**. Because of the minute quantity, it was not further purified.

Subfraction BK7 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated no UV-active spots. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B2 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Further purification by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 35**.

Table 35 Subfractions obtained from subfraction B2 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B21	21.4	Dark-brown gum
B22	5.0	Dark-brown gum
B23	9.5	Dark-brown gum
B24	2.9	Dark-brown gum

Subfraction B21 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.20 and 0.50 under UV-S. The ^{1}H NMR spectrum indicated that it was a mixture. It was further combined with **B31** and then purified by column chromatography over silica gel using a gradient system of methanol-dichloromethane.

Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give ten subfractions, as shown in **Table 36**.

Table 36 Subfractions obtained from subfraction B21 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B211	100% CH_2Cl_2-2% MeOH/CH_2Cl_2	1.1	Colorless gum mixed with white solid
B212	2% MeOH/CH_2Cl_2	3.1	Pale brown gum
B213	3% MeOH/CH_2Cl_2	3.0	Pale brown gum
B214	3% MeOH/CH_2Cl_2	1.5	Pale brown gum
B215	3-5% MeOH/CH_2Cl_2	1.5	Colorless gum
B216	3-5% MeOH/CH_2Cl_2	3.7	Pale brown gum mixed with white solid
B217	5-7% MeOH/CH_2Cl_2	1.1	Colorless gum
B218	7-10% MeOH/CH_2Cl_2	1.2	Pale brown gum mixed with pale brown solid
B219	10% MeOH/CH_2Cl_2	2.1	Pale brown gum mixed with pale brown solid
B2110	10% MeOH/CH_2Cl_2 - 100% MeOH	11.8	Dark-brown gum

Subfraction B211 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) as a mobile phase demonstrated five spots with the R_f values of 0.24, 0.31, 0.40, 0.47 and 0.64 under UV-S and two other spots with the R_f values of 0.17 and 0.52 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction B212 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane as mobile phases demonstrated one spot with the R_f value of 0.21 under UV-S and the

other violet spot with the R_f value of 0.17 after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further purified.

Subfraction B213 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.19 under UV-S. Further purification by precoated TLC with 4% methanol-dichloromethane (12 runs) as a mobile phases afforded a colorless gum (1.0 mg). Its chromatogram characteristic on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated two pale spots with the R_f values of 0.57 and 0.67 under UV-S. Because of the minute quantity, it was not further investigated.

Subfraction B214 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane demonstrated one spot with the R_f value of 0.21 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B215 (US8) Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane demonstrated one spot with the R_f value of 0.19 after dipping in ASA reagent and subsequently heating the TLC plate.

$[\alpha]_D^{28}$	+5.11 (c = 0.15, EtOH)
FT-IR (neat) ν_{cm-1}	3391 (OH stretching), 2925 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	4.02 (*brd*, 1H), 3.66 (*s*, 1H), 3.54 (*brs*, 1H), 3.52 (*s*, 1H), 2.02 (*dt*, *J* = 13.5, 3.9 Hz; 1H), 1.95 (*dd*, *J* = 13.5, 3.9 Hz, 1H), 1.82 (*dt*, *J* = 13.5, 3.9 Hz, 1H), 1.75 (*dd*, 13.5, 3.9 Hz, 1H), 1.60 (*septet*, *J* = 6.9 Hz, 1H), 1.45 (*m*, 1H), 1.33 (*s*, 3H), 0.93 (*d*, *J* = 6.9 Hz, 6H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)	75.31, 74.85, 71.62, 38.44, 33.78, 29.55, 29.17, 27.68, 16.66, 16.63

DEPT (90°) ($CDCl_3$)	CH :	74.84, 38.44
DEPT (135°) ($CDCl_3$)	CH_2 :	33.78, 29.55, 29.17
	CH_3 :	27.68, 16.66, 16.63

Subfraction B216 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.19 under UV-S. Further purification by precoated TLC with 4% methanol-dichloromethane (8 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a pale yellow gum (1.1 mg). It chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.51 after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained a brown gum (1.9 mg). It chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.42 and 0.51 after dipping in ASA reagent and subsequently heating the TLC plate. Because of the minute quantity, it was not further investigated.

Subfraction B217 (US9) Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane (2 runs) demonstrated one spot with the R_f value of 0.12 under UV-S.

$[\alpha]_D^{28}$	-8.33 (c = 0.10, MeOH)
UV (MeOH) λ_{max} nm (log ε)	233 (3.66)
FT-IR (neat) ν_{cm-1}	3354 (OH stretching), 1667 (C=O stretching), 1278 (C-N stretching)
^1H NMR ($CDCl_3$) (δ ppm) (300 MHz)	6.25 (*brs*, 1H), 5.65 (*brs*, 1H), 3.95 (*brs*, 1H), 2.10 (*m*, 1H), 0.98 (*d*, *J* = 9.0 Hz, 3H), 0.84 (*d*, *J* = 6.0 Hz, 3H)

^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		175.89, 76.20, 31.91, 19.06, 15.45
DEPT (135°) ($CDCl_3$)	CH :	76.20, 31.91
	CH_3 :	19.06, 15.45

Subfraction B218 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane (2 runs) demonstrated one spot with the R_f value of 0.12 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B219 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane (2 runs) demonstrated no UV-active spots. Because of the minute quantity, it was not further investigated.

Subfraction B2110 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane (2 runs) demonstrated two spots near the baseline under UV-S, and other two spots with the R_f values of 0.36 and 0.45 as violet spots after dipping in ASA reagent and subsequently heating. Further purification by precoated TLC with 4% methanol-dichloromethane (10 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a pale yellow gum (1.0 mg). Its chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because of the minute quantity, it was not further investigated.

Band 2 was obtained as a pale yellow gum (1.2 mg). Its chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because of the minute quantity, it was not further investigated.

Subfraction B22 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane demonstrated one spot with the R_f value of 0.50 under UV-S. The ^{1}H NMR spectrum indicated that the major component was **US3**.

Subfraction B23 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane (2 runs) demonstrated two spots with the R_f values of 0.20 and 0.43 under UV-S. Further purification by precoated TLC with 5% methanol-dichloromethane (8 runs) as a mobile phase affords three bands.

Band 1 was obtained as a brown gum (4.6 mg). Its chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) and 6% methanol-dichloromethane (2 runs) demonstrated one spot with the R_f value of 0.39 under UV-S. The ^{1}H NMR spectrum indicated that the major component was **US3**.

Band 2 was obtained as a colorless gum (0.3 mg). Its chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) and 6% methanol-dichloromethane (2 runs) demonstrated one spot near the baseline under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was obtained as a colorless gum (0.3 mg). Its chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (3 runs) and 6% methanol-dichloromethane (2 runs) demonstrated one spot near the baseline under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B24 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane (2 runs) as a mobile phase demonstrated two spots with the R_f values of 0.20 and 0.43 under UV-S. Because of the minute quantity, it was not further investigated.

Subfraction B3 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Further purification by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give five subfractions, as shown in **Table 37**.

Table 37 Subfractions obtained from subfraction B3 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B31	13.4	Brown gum
B32	24.7	Brown gum
B33	37.9	Brown gum
B34	17.6	Brown gum
B35	3.0	Brown gum

Subfraction B31 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated two spots with the same R_f values as **B21**. Thus, it was combined with **B21**.

Subfraction B32 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 1:2.

Subfraction B33 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S. The ^{1}H NMR spectrum indicated that it consisted of a mixture of **US10** and **US11** in the ratio of 4:3.

Subfraction B34 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S. It was separated by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give five subfractions, as shown in **Table 38**.

Table 38 Subfractions obtained from subfraction B34 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B341	3.1	Yellow gum
B342	2.8	Colorless gum
B343	5.0	Colorless gum
B344	9.9	Colorless gum
B345	3.0	Colorless gum

Subfraction B341 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated no UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Therefore, it was not further studied.

Subfraction B342 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.27 under UV-S. The ^{1}H NMR spectrum indicated that the major component was **US10**.

Subfraction B343 (US10) Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.24.

$[\alpha]_D^{26}$	+32.64 (c = 0.13, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)	260 (3.97)
FT-IR (neat) ν_{cm-1}	3374 (OH, NH stretching), 1665 (C=O stretching), 1622 (N-H bending), 1023 (C-N stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	10.97 (*d*, *J* = 10.8 Hz, 1H), 7.50 (*dd*, *J* = 10.8, 8.7 Hz, 1H), 6.49 (*brs*, 1H), 5.15 (*d*, *J* = 8.7 Hz, 1H), 4.07 (*d*, *J* = 3.3 Hz, 1H), 2.13 (*m*, 1H), 0.99 (*d*, *J* = 6.9 Hz, 3H), 0.82 (*d*, *J* = 6.9 Hz, 3H)

^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	172.41, 138.63, 96.80, 76.16, 32.17, 18.90, 15.69
EIMS *m/z* (% relative intensity):	284 (0.4), 146 (5.5), 98 (8), 96 (49), 87 (100), 73 (74)

Subfraction B344 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.27 under UV-S. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 2:1.

Subfraction B345 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.27 under UV-S. The ^{1}H NMR spectrum indicated that it consisted of a mixture of **US10** and **US11** in the ratio of 2:3.

Subfraction B35 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction B4 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated a long tail. Further purification by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give six subfractions, as shown in **Table 39**.

Table 39 Subfractions obtained from subfraction B4 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B41	3.4	Brown gum
B42	22.7	Brown gum
B43	50.1	Brown gum
B44	27.8	Brown gum

Table 39 (continued)

Subfraction	Weight (mg)	Physical appearance
B45	9.9	Brown gum
B46	2.8	Brown gum

Subfraction B41 Chromatogram characteristics on normal phase TLC with 7% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot near the baseline under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B42 Chromatogram characteristics on normal phase TLC with 7% methanol-dichloromethane (3 runs) as a mobile phase demonstrated a long tail and one major spot with the R_f value of 0.15 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US10**.

Subfraction B43 Chromatogram characteristics on normal phase TLC with 7% methanol-dichloromethane (3 runs) as a mobile phase demonstrated a long tail and two major spots with the R_f values of 0.15 and 0.29 under UV-S. Further purification by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give six subfractions, as shown in **Table 40**.

Table 40 Subfractions obtained from subfraction B43 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B431	3.2	Brown gum
B432	3.9	Pale brown gum
B433	17.4	Pale brown gum
B434	14.7	Pale brown gum
B435	3.1	Colorless gum
B436	1.8	Brown gum

Subfraction B431 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction B432 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.24 under UV-S. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 2:1.

Subfraction B433 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.24 under UV-S. The ^{1}H NMR spectrum indicated that it consisted of a mixture of **US10** and **US11** in the ratio of 1:1.

Subfraction B434 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.24 under UV-S. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 2:3.

Subfraction B435 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.24 under UV-S. The ^{1}H NMR spectrum indicated that it consisted of a mixture of **US10** and **US11** in the ratio of 1:2.

Subfraction B436 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S. The ^{1}H NMR spectrum indicated the presence of many compounds. Thus, it was not further investigated.

Subfraction B44 Chromatogram characteristics on normal phase TLC with 7% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot with the R_f value of 0.15 under UV-S. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10**, **US11** and **US12** in the ratio of 3:1:2.

Subfraction B45 Chromatogram characteristics on normal phase TLC with 7% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot with the R_f value of 0.15 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US12**.

Subfraction B46 Chromatogram characteristics on normal phase TLC with 7% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot near the baseline under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US12**.

Subfraction B5 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Further purification by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give seven subfractions, as shown in **Table 41**.

Table 41 Subfractions obtained from subfraction B5 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B51	0.8	Pale brown gum
B52	2.6	Dark-brown gum
B53	17.3	Pale brown gum
B54	13.6	Yellow gum
B55	14.9	Brown gum
B56	32.4	Dark-brown gum
B57	7.4	Colorless gum

Subfraction B51 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one pale spot with the R_f value of 0.17 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B52 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one pale spot with the R_f value of 0.17 under UV-S. The ^{1}H NMR spectral data indicated that it contained a mixture of **US10** and **US11** in the ratio of 2:1**.**

Subfraction B53 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and two major spots under UV-S with the R_f values of 0.32 and 0.41. Further purification by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give ten subfractions, as shown in **Table 42**.

Table 42 Subfractions obtained from subfraction B53 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B531	4.5	Brown gum
B532	0.2	Brown gum
B533	0.9	Pale Brown gum
B534	0.9	Pale Brown gum
B535	4.4	Pale Brown gum
B536	2.6	Pale Brown gum
B537	2.5	Colorless gum
B538	0.3	Pale Brown gum
B539	0.5	Pale Brown gum
B5310	1.1	Brown gum

Subfraction B531 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed no UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction B532 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one pale spot under UV-S with the R_f value of 0.12. According to its ^{1}H NMR spectrum, it was not further isolated.

Subfraction B533 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one pale spot under UV-S with the R_f value of 0.21. The ^{1}H NMR spectrum indicated that the major compound was **US10**.

Subfraction B534 Chromatogram characteristics on normal phase TLC with Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and one major spot under UV-S with the R_f value of 0.21. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 2:1.

Subfraction B535 Chromatogram characteristics on normal phase TLC with Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one spot under UV-S with the R_f value of 0.21. The ^{1}H NMR spectrum indicated that it consisted of a mixture of **US10** and **US11** in the ratio of 5:4.

Subfraction B536 Chromatogram characteristics on normal phase TLC with Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one spot under UV-S with the R_f value of 0.21. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 4:5.

Subfraction B537 (US11) Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase showed one spot under UV-S with the R_f value of 0.18.

$[\alpha]_D^{27}$	+137.84 (c = 0.13, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)	260 (3.87)
FT-IR (neat) ν_{cm-1}	3362 (OH, NH stretching), 1665 (C=O stretching), 1625 (N-H bending), 1024 (C-N stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	11.05 (*d*, *J* = 10.8 Hz, 1H), 7.55 (*dd*, *J* = 10.8, 9.0 Hz, 1H), 6.43 (*brs*, 1H), 6.10 (*brs*, 1H), 5.23

	(*d*, *J* = 9.0 Hz, 1H), 4.03 (*d*, *J* = 3.3 Hz, 1H), 2.15 (*m*, 1H), 1.06 (*d*, *J* = 6.9 Hz, 3H), 0.91 (*d*, *J* = 6.9 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)	176.43, 138.16, 97.11, 76.43, 31.86, 19.05, 15.44
EIMS *m/z* (% relative intensity):	284 (0.1), 146 (1), 98 (4), 87 (84), 73 (100)

Subfraction B538 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one spot under UV-S with the R_f value of 0.23. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B539 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one pale spot under UV-S with the R_f value of 0.14. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B5310 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one pale spot under UV-S with the R_f value of 0.16. The ^{1}H NMR spectrum indicated that the major compound was **US11**.

Subfraction B54 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and three major spots under UV-S with the R_f values of 0.19, 0.32 and 0.44. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 3:2.

Subfraction B55 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and two major spots under UV-S with the R_f values of 0.32 and 0.44. The ^{1}H NMR spectrum indicated that the major compounds were a mixture of **US10**, **US11** and **US12** in the ratio of 1:2:4.

Subfraction B56 Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed a long tail and the major spot under UV-S with the R_f value of 0.44. The ^{1}H NMR spectrum indicated that the major compound was **US12**.

Subfraction B57 (US12) Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane as a mobile phase showed one spot under UV-S with the R_f value of 0.36.

FT-IR (neat) ν_{cm-1}	2927 (C-H stretching), 1717 (C=O stretching), 1402 (C-O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	4.65 (*t*, *J* = 6.0 Hz, 2H), 3.05 (*t*, *J* = 6.0 Hz, 2H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)	174.49, 69.30, 30.73
EIMS *m/z* (% relative intensity):	287 (10), 120 (100), 107 (48)

Subfraction B6 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase showed a long tail after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR data indicated that it consisted of a mixture of **US10** and **US12.**

Subfraction B7 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane showed two spots near the baseline under UV-S. The ^{1}H NMR data indicated that the major component was **US12**.

Fraction C Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase showed two major spots with the R_f values of 0.17 and 0.29 after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane (7 runs) as mobile phase afforded five bands.

Band 1 was obtained as a brown gum (1.4 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 2% methanol-dichloromethane showed no spots after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Band 2 was obtained as a brown gum (1.3 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 2% methanol-dichloromethane as mobile phases showed one spot with the R_f value of 0.33 after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated the presence of many components. Therefore, it was not further studied.

Band 3 was obtained as a pale brown gum (1.3 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 2% methanol-dichloromethane showed no spots after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Therefore, it was not further studied.

Band 4 (US13) was obtained as a colorless gum (1.3 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 2% methanol-dichloromethane showed one spot with the R_f value of 0.38.

UV (MeOH) λ_{max} nm (log ε)		221 (3.83), 277 (2.96)
FT-IR (neat) ν_{cm-1}		3409 (OH stretching), 1730 (C=O stretching), 1613 (C=C stretching), 1556 (N=O stretching), 1377 (C-N stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		7.00 (*d*, *J* = 8.4 Hz, 1H), 6.70 (*d*, *J* = 8.4 Hz, 1H), 4.55 (*t*, *J* = 6.3 Hz, 2H), 4.25 (*t*, *J* = 7.2 Hz, 2H), 2.89 (*t*, *J* = 6.3 Hz, 2H), 2.80 (*t*, *J* = 7.2 Hz, 2H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		169.00, 153.90, 130.00, 129.99, 115.45, 69.66, 66.11, 34.06, 31.09
DEPT (135°) ($CDCl_3$)	CH :	129.99, 115.45
	CH_2 :	69.66, 66.11, 34.06, 31.09
EIMS *m/z* (% relative intensity):		239 (5), 193 (1), 121 (97), 107 (100), 77 (40)

Band 5 was obtained as a dark-brown gum (10.1 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and

2% methanol-dichloromethane showed two spots near the baseline after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 5% methanol-dichloromethane (17 runs) as mobile phase afforded five bands.

Band 51 was obtained as a brown gum mixed with a brown solid (1.0 mg). Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase showed one spot with the R_f value of 0.38. The ^{1}H NMR spectrum suggested that it was a mixture. Because of the minute quantity, it was not further separated.

Band 52 was obtained as a brown gum mixed with a brown solid (1.0 mg). Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase showed one spot with the R_f value of 0.16. The ^{1}H NMR spectrum suggested that the major component was **US12**. Because of the minute quantity, it was not further separated.

Band 53 was obtained as a brown gum mixed with a brown solid (2.0 mg). Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) as a mobile phase showed one spot with the R_f value of 0.13. The ^{1}H NMR spectrum suggested that it was **US12**.

Band 54 was obtained as a brown gum mixed with a brown solid (1.5 mg). Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane showed one spot with the R_f value of 0.18. The ^{1}H NMR spectrum suggested that it was a mixture. Because of the minute quantity, it was not further separated.

Band 55 was obtained as a brown gum mixed with a brown solid (0.7 mg). Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane showed no spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Therefore, it was not further studied.

Fraction D Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase showed no spots after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum

indicated the absence of olefinic and aromatic protons. Thus it was not further studied.

3.2.3 Purification of the mycelial extract

The mycelial extract of *Phomopsis* sp. PSU-D15 (335.0 mg) was chromatographed on Sephadex LH-20 using 100% methanol to afford fifty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford ten fractions, as shown in **Table 43**.

Table 43 Fractions obtained from the mycelial extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	99.9	Brown gum mixed with brown solid
B	41.3	Brown gum mixed with brown solid
C	23.1	Brown gum mixed with brown solid
D	2.6	Brown gum mixed with pale brown solid
E	4.9	Brown gum mixed with brown solid
F	4.1	Brown gum mixed with brown solid
G	4.6	Brown gum mixed with brown solid

Fraction A Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase showed no UV-active spots. The ^{1}H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Fraction B Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated four spots with the R_f values of 0.45, 0.52, 0.60, 0.75 and one spots near the baseline under UV-S. Further separation on by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined

on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 44**.

Table 44 Subfractions obtained from Fraction B by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B1	1.9	Brown gum
B2	8.7	Yellow gum
B3	2.4	Pale brown gum
B4	4.5	Colorless gum
B5	22.2	Colorless gum

Subfraction B1 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated no UV-active spots. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction B2 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane as a mobile phase demonstrated three spots with the R_f values of 0.41, 0.43 and 0.90 under UV-S. The ^{1}H NMR spectrum indicated that it was a mixture. It was further combined with **fraction C**.

Subfraction B3 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot near the baseline under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B4 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one spot with the R_f value of 0.28 under UV-S. The ^{1}H NMR spectrum indicated that it contained a mixture of **US10** and **US11** in the ratio of 2:1.

Subfraction B5 Chromatogram characteristics on normal phase TLC with 6% methanol-dichloromethane (3 runs) as a mobile phase demonstrated one

spot with the R_f value of 0.28 under UV-S. The ^{1}H NMR spectrum indicated that it consisted of a mixture of **US10** and **US11** in the ratio of 1:2.

Fraction C Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated three spots with the same R_f values as **B2**. Thus, it was combined with **B2** and separated by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford six subfractions, as shown in **Table 45**.

Table 45 Subfractions obtained from Fraction C by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
C1	1.2	Brown gum
C2	9.0	Yellow gum
C3	6.0	Yellow gum
C4	5.7	Yellow gum
C5	3.1	Colorless gum
C6	4.8	Pale brown gum

Subfraction C1 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated no spots under UV-S. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction C2 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated three spots with the R_f values of 0.48, 0.68 and 0.88 under UV-S. Further separation on column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford two subfractions, as shown in **Table 46**

Table 46 Subfractions obtained from Subfraction C2 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
C21	1.4	Yellow gum
C22	7.4	Yellow gum

Subfraction C21 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated no UV-active spots. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C22 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated three spots with the R_f values of 0.24, 0.36 and 0.50 under UV-S. Further purification by precoated TLC with 2% methanol-dichloromethane (7 runs) as a mobile phase afforded three bands.

Band 1 was obtained as a yellow gum (1.1 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated one spot with the R_f value of 0.50. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a yellow gum (1.2 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated one spot with the R_f value of 0.36. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 (US14) was obtained as a yellow gum (1.2 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated one spot with the R_f value of 0.30.

$[\alpha]_D^{26}$		-61.67 (c = 0.01, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)		218 (4.69), 237 (4.61), 346 (4.63)
FT-IR (neat) ν_{cm-1}		3501 (OH stretching), 2925 (C-H stretching), 1740 and 1606 (C=O stretching), 1402 (C-O stretching), 1024 (C-N stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		13.92 (*s*, 1H), 11.86 (*s*, 1H), 7.33 (*d*, *J* = 8.4 Hz, 1H), 6.44 (*d*, *J* = 8.4 Hz, 1H), 5.69 (*s*, 1H), 4.03 (*d*, *J* = 13.2 Hz, 1H), 3.47 (*brd*, *J* = 13.2 Hz, 1H), 2.41 (*m*, 1H), 2.36 (*m*, 1H), 2.32 (*m*, 1H), 2.03 (*s*, 3H), 1.01 (*d*, *J* = 6.0 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		187.73, 178.02, 170.51, 159.50, 157.12, 140.07, 117.92, 107.83, 106.42, 100.83, 82.49, 70.24, 65.56, 33.40, 27.67, 20.83, 17.53
DEPT (135°) ($CDCl_3$)	CH :	140.07, 107.83, 70.24, 27.67
	CH_2 :	65.56, 33.40
	CH_3 :	20.83, 17.53
EIMS *m/z* (% relative intensity):		664 (1), 648 (2), 647 (7), 430 (6), 396 (5), 368 (7), 290 (8), 284 (11), 279 (15), 256 (18), 149 (100)

Subfraction C3 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated two spots with the R_f values of 0.42 and 0.48 under UV-S. Further purification by precoated TLC with 2% methanol-dichloromethane (13 runs) as a mobile phase afforded four bands.

Band 1 (US15) was obtained as a yellow gum (1.5 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated one spot with the R_f value of 0.36.

$[\alpha]_D^{26}$		+106.8 (c = 0.10, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)		212 (4.45), 243 (4.35), 345 (4.30)
FT-IR (neat) ν_{cm-1}		3345 (OH stretching), 2926 (C-H stretching), 1740 and 1606 (C=O stretching), 1402 (C-O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		13.85 (*s*, 1H), 13.82 (*s*, 1H), 11.95 (*s*, 1H), 11.36 (*s*, 1H), 7.21 (*d*, *J* = 8.5 Hz, 1H), 7.06 (*d*, *J* = 8.5 Hz, 1H), 6.52 (*d*, *J* = 8.5 Hz, 1H), 6.41 (*d*, *J* = 8.5 Hz, 1H), 5.59 (*s*, 1H), 5.46 (*d*, *J* = 1.5 Hz, 1H), 3.95 (*d*, *J* = 10.0 Hz, 1H), 3.92 (*d*, *J* = 12.0 Hz, 1H), 3.58 (*d*, *J* = 10.0 Hz, 1H), 3.33 (*d*, *J* = 12.0 Hz, 1H), 2.39 (*m*, 1H), 2.38 (*m*, 1H), 2.37 (*m*, 1H), 2.31 (*m*, 2H), 2.20 (*m*, 1H), 2.07 (*s*, 3H), 1.90 (*s*, 3H), 1.01(*d*, *J* = 6.0 Hz, 3H), 0.92 (*d*, *J* = 6.5 Hz, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		186.98, 177.33, 176.64, 169.70, 169.40, 160.93, 158.44, 156.63, 153.30, 138.12, 137.44, 117.29, 115.86, 109.15, 107.21, 105.59, 100.11, 99.99, 81.69, 81.22, 70.13, 68.31, 64.67, 64.02, 32.49, 32.12, 26.71, 26.64, 19.89, 19.73, 16.52, 16.29
DEPT (135°) ($CDCl_3$)	CH :	138.12, 137.44, 109.15, 107.21, 70.13, 68.31, 26.71, 26.64,16.52
	CH_2 :	64.67, 64.02, 32.49, 32.12
	CH_3 :	19.89, 19.73, 16.52, 16.29
EIMS *m/z* (% relative intensity):		666 (7), 665 (26), 635 (31), 634 (100)

Band 2 was obtained as a yellow gum (0.4 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated two spots with the R_f values of 0.43 and 0.32. The ^{1}H NMR spectrum indicated that major component was **US14**. Because of the minute quantity, it was not further investigated.

Band 3 was obtained as a yellow gum (1.0 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated one spot with the R_f value of 0.23. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 4 was obtained as a yellow gum (1.2 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated a long tail from the baseline. Because the ^1H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction C4 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail from the baseline under UV-S. Further separation on column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford three subfractions, as shown in **Table 47**.

Table 47 Subfractions obtained from Subfraction C4 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
C41	0.7	Dark-brown gum
C42	1.6	Brown gum
C43	3.7	Brown gum

Subfraction C41 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane (2 runs) demonstrated no UV-active spots. Because the ^1H NMR spectrum showed signals at the high field, it was not further investigated.

Subfraction C42 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane

(2 runs) demonstrated one spot with the R_f value of 0.12. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C43 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 4% methanol-dichloromethane (2 runs) demonstrated one spot with the R_f value of 0.14. Further purification by precoated TLC with 4% methanol-dichloromethane (10 runs) and 6% methanol-dichloromethane (3 runs) afforded two bands.

Band 1 was obtained as a brown gum (1.6 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane, 4% methanol-dichloromethane and 6% methanol-dichloromethane (3 runs) demonstrated one spot with the R_f value of 0.45. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

Band 2 was obtained as a brown gum (1.5 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane and 4% methanol-dichloromethane demonstrated no UV-active spots. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C5 (US16) Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S.

$[\alpha]_D^{29}$	+20.50 (c = 0.11, MeOH)
UV (MeOH) λ_{max} nm (log ε)	215 (3.35), 260 (3.33)
FT-IR (neat) ν_{cm-1}	3399 (OH stretching), 2926 (C-H stretching), 1606 (C=O stretching), 1402 (C-O stretching)
^{1}H NMR (Acetone-d_6) (δ ppm) (500 MHz)	0.09 (*brs*), 7.99 (*d*, *J* = 8.5 Hz, 1H), 5.92 (*d*, *J* = 5.0 Hz, 1H), 5.60 (*d*, *J* = 8.5 Hz, 1H), 4.29 (*t*, *J* = 5.0 Hz, 1H), 4.26 (*t*, *J* = 5.0 Hz, 1H), 4.02 (*td*, *J* = 2.5, 5.0 Hz, 1H), 3.84 (*dd*, *J* = 12.0, 2.5 Hz, 1H), 3.78 (*dd*, *J* = 12.0, 2.5 Hz, 1H)
^{13}C NMR (Acetone-d_6) (δ ppm) (125 MHz)	163.63, 151.69, 140.79, 101.61, 89.25, 85.17, 74.43, 70.27, 61.25

DEPT (135°) (Acetone-d_6) CH : 140.79, 101.61, 89.25, 85.17, 74.43, 70.27

CH_2 : 61.25

EIMS *m/z* (% relative intensity): 244 (2), 226 (19), 141 (22), 133 (69), 73 (70), 113 (100)

Subfraction C6 Chromatogram characteristics on normal phase TLC with 3% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.20 under UV-S. The ^{1}H NMR spectrum indicated that the major component was **US9.**

Fraction D Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated three spots with the R_f values of 0.20, 0.29 and 0.34 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Fraction E Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.20 and 0.36 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Fraction F Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.27 under UV-S. Because the ^{1}H NMR spectrum showed signals at the high field, it was not further investigated.

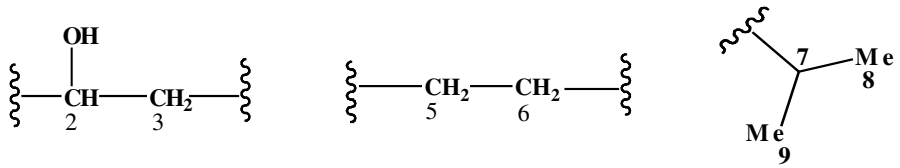
CHAPTER 3.3

RESULTS AND DISCUSSION

Three new compounds (**US10**, **US11** and **US13**) were isolated from the broth extract together with four known compounds (**US8**, **US9**, **US12** and **US16**) while one new unsymmetrical dihydroxanthone dimer (**US15**) and one known symmetrical dimer (**US14**) were obtained from the mycelial extract. The structures were identified by spectroscopic methods.

3.3.1 Compound US8

Compound **US8** was obtained as a colorless gum. It exhibited a hydroxyl absorption band at 3391 cm^{-1} in the IR spectrum (**Figure 61**). The ^{1}H NMR spectrum (**Figure 62**) (**Table 48**) contained signals for one isopropyl group [δ_H 1.60 (*septet*, J = 6.9 Hz, 1H) and δ_H 0.93 (*d*, J = 6.9 Hz, 6H)], one oxymethine proton [δ_H 3.54 (*brs*, 1H)], two sets of nonequivalent methylene protons [δ_H 2.02 (*dt*, J = 3.9 and 13.5 Hz, 1H), 1.82 (*td*, J = 3.9 and 13.5 Hz, 1H) and δ_H 1.95 (*dd*, J = 3.9 and 13.5 Hz, 1H), 1.75 (*brd*, J = 13.5 Hz, 1H)], one set of equivalent mehtylene protons [δ_H 1.45 (*m*, 2H)] and one methyl group [δ_H 1.33 (*s*, 3H)]. The ^{13}C NMR spectrum (**Figure 65**) (**Table 48**) showed two quaternary carbons (δ_C 75.31 and 71.62), one oxymethine carbon (δ_C 74.85), one methine carbon (δ_C 38.44), three methylene carbons (δ_C 33.78, 29.55 and 29.17) and three methyl carbons (δ_C 27.68, 16.66 and 16.63). In the ^{1}H-^{1}H COSY spectrum (**Figure 68**) (**Table 48**), the oxymethine proton, H-2 (δ_H 3.54), showed cross peaks with H_{ab}-3 (δ_H 1.95 and 1.75) while the methylene protons, H_2-5 (δ_H 1.45), caused cross peaks with the methylene protons, H_{ab}-6 (δ_H 2.02 and 1.82). These indicated the presence of three subunits in the structure of **US8**.



unit 1 unit 2 unit 3

The units 1 and 2 were jointed together with two oxyquaternary carbons, C-1 (δ_C 71.62) and C-4 (δ_C 75.31), according to the HMBC cross peaks of H_{ab}-3 and H_{ab}-6 with C-1 and C-4 (**Figure 67**) (**Table 49**) to form a 1,2,4-trihydroxycyclohexane skeleton. In addition, both Me-8 (δ_H 0.93) and Me-9 (δ_H 0.93) of unit 3 showed correlations with C-4, in the HMBC spectrum, indicating that the isopropyl group was located at C-4. HMBC correlations of Me-10 (δ_H 1.33) with C-1, C-2 (δ_C 74.85) and C-6 (δ_C 29.17) connected the methyl group at C-1 of the cyclohexane skeleton. The relative stereochemistry of **US8** was established by the NOEDIFF results and coupling constant. Since H-2 appeared as a broad singlet, it was assigned at equatorial position. Irradiation of H-5 did not enhance the signal intensity of Me-10 (**Figure 69**) (**Table 49**), indicating that Me-10 was at equatorial position. Therefore, the hydroxyl groups at C-1 and C-2 had *trans* diaxial relationship. The isopropyl group at C-4 was also at equatorial position according to the enhancement of Me-8 and Me-9 signals upon irradiation of H_b-3 (**Figure 69**) (**Table 49**). Therefore, **US8** had the same structure as (1*S*,2*S*,4*S*)-*p*-mentane-1,2,4-triol which was previously reported (Thappa, *et al*., 1976). Since **US8** displayed the same sign of the specific rotation, $[\alpha]_D$ +5.11 (c = 0.15, EtOH), as that of (1*S*,2*S*,4*S*)-*p*-mentane-1,2,4-triol, $[\alpha]_D$ +24.00 (c = 0.15, EtOH), all chiral carbons in **US8** might have *S* configuration.

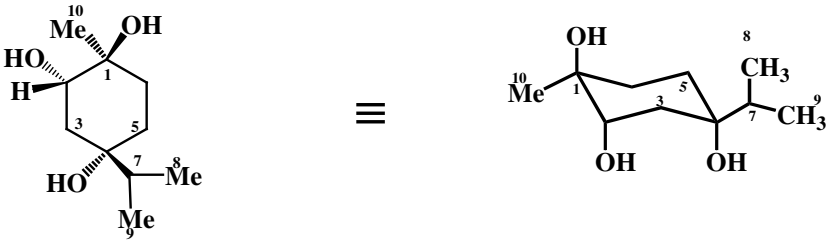


Table 48 The NMR data of compound US8 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	-	71.62 (C)
2	3.54 (*brs*)	74.85 (CH)
3	a: 1.95 (*dd*, 13.5, 3.9)	33.78 (CH_2)
	b: 1.75 (*brd*, 13.5)	-
4	-	75.31 (C)
5	1.45 (*m*)	29.55 (CH_2)
6	a: 2.02 (*dt*, 13.5, 3.9)	29.17 (CH_2)
	b: 1.82 (*dt,* 13.5, 3.9)	-
7	1.60 (*septet*, 6.9)	38.44 (CH)
8	0.93 (*d*, 6.9)	16.63 (CH_3)
9	0.93 (*d*, 6.9)	16.66 (CH_3)
10	1.33 (*s*)	27.68 (CH_3)
1,2,4-OH	3.52 (*s*), 3.66 (*s*), 4.02(*s*)	-

Table 49 The HMBC, COSY and NOE data of compound US8 in $CDCl_3$

Position	HMBC correlations	COSY	NOE
H-2	-	H_{ab}-3	H_{ab}-3
H_a-3	C-1, C-2, C-4	H-2, H_b-3	-
H_b-3	C-1, C-2, C-4	H-2, H_a-3	Me-8, Me-9, H-5
H-5	C-3, C-4, C-6	H_{ab}-6	H_{ab}-6, Me-8, Me-9
H_a-6	C-1, C-4, C-5	H-5, H_b-6	H-5, H_b-6, Me-8, Me-9
H_b-6	C-1, C-4, C-5	H-5, H_a-6	H-5, H_a-6, Me-8, Me-9
H-7	C-3, C-4, C-5, C-8, C-9	H-8, H-9	-
H-8	C-4, C-7, C-9	H-7	H_{ab}-3, H-5, H-7
H-9	C-4, C-7, C-8	H-7	H_{ab}-3, H-5, H-7
H-10	C-1, C-2, C-6	-	-

3.3.2 Compound US9

Compound **US9** was obtained as a colorless gum. It exhibited UV absorption band at 233 nm (**Figure 70**) while hydroxyl and carbonyl absorption bands were found at 3354 and 1667 cm^{-1}, respectively, in the IR spectrum (**Figure 71**). The ^1H NMR spectrum (**Figure 72**) (**Table 50**) contained signals of amino protons [δ_H 6.25 (*brs*) and 5.65 (*brs*)], one oxymethine proton (δ_H 3.95, *brs*, 1H), one methine proton (δ_H 2.10, *m*), and two methyl groups [δ_H 0.98 (*d*, *J* = 9.0 Hz, 3H) and 0.84 (*d*, *J* = 6.0 Hz, 3H)]. The ^{13}C NMR spectrum (**Figure 74**) (**Table 50**) showed one carbonyl carbon (δ_C 175.89), one oxymethine carbon (δ_C 76.20), one methine carbon (δ_C 31.91) and two methyl carbons (δ_C 19.06 and 15.45). The correlations of H-3 (δ_H 2.10) with H-2 (δ_H 3.95), Me-4 (δ_H 0.98) and Me-5 (δ_H 0.84) in the ^1H-^1H COSY spectrum (**Figure 77**) (**Table 50**) indicated the presence of a 1-hydroxy-2-methylpropyl unit. The appearance of H-2 and C-2 at low field connected this unit with the amide carbonyl C-1 (δ_C 175.89). Therefore, **US9** was identified as 2-hydroxy-3-methylbutanamide (Nakajima and Ubukata, 1998).

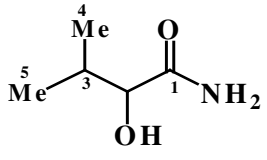


Table 50 The NMR data of compound US9 in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	COSY
1	-	175.89 (C=O)	-	-
2	3.95 (*brs*)	76.20 (CH)	-	H-3
3	2.10 (*m*)	31.91 (CH)	-	H-2, H-4, H-5
4	0.98 (*d*, 9.0)	19.06 (CH_3)	C-2, C-3, C-5	H-3
5	0.84 (*d*, 6.0)	15.45 (CH_3)	C-2, C-3, C-4	H-3
NH_2	6.25 (*brs*)	-	-	-
	5.65 (*brs*)	-	-	-

3.3.3 Compound US10

Compound **US10** with the molecular formula $C_{14}H_{24}N_2O_4$ from EIMS (*m/z* 284) (**Figure 85**) was obtained as a colorless gum with $[\alpha]_D$ +32.64 (c = 0.13, $CHCl_3$). It exhibited an UV absorption band at 260 nm (**Figure 78**). Hydroxyl and amino absorption bands were found at 3374 cm^{-1} while carbonyl absorption band was observed at 1665 cm^{-1}, in the IR spectrum (**Figure 79**). The ^1H NMR spectrum (**Figure 80**) (**Table 51**) contained signals of the 1-hydroxy-2-methylpropyl group [δ_H 4.07 (*d*, *J* = 3.3 Hz, 1H), 2.13 (*m*, 1H), 0.99 (*d*, *J* = 6.9 Hz, 3H) and 0.82 (*d*, *J* = 6.9 Hz, 3H)], one amino proton (δ_H 10.97, *d*, *J* = 10.8 Hz), two *cis*-olefinic protons [δ_H 7.50 (*dd*, *J* = 10.8 and 8.7 Hz, 1H) and 5.15 (*d*, *J* = 8.7 Hz, 1H)] and one hydroxy proton (δ_H 6.49, *brs*). Apart from four carbon signals of the 1-hydroxy-2-methylpropyl group at δ_C 76.16, 32.17, 18.90 and 15.69, the ^{13}C NMR spectrum (**Figure 81**) (**Table 51**) showed one carbonyl carbon (δ_C 172.41) and two olefinic carbons (δ_C 138.63 and 96.80). The lower-field olefinic proton, H-6 (δ_H 7.50), gave a ^1H-^1H COSY cross peak with the amino proton, H-5 (δ_H 10.97) (**Figure 84**) (**Table 51**), suggesting the presence of *cis*-aminovinyl moiety. HMBC correlations of both H-6 of the aminovinyl

group and H-3 (δ_H 4.07) of the 1-hydroxy-2-methylpropyl group with the carbonyl carbon (δ_C 172.41) (**Figure 83**) (**Table 51**) established the attachment of both units with the same carbonyl carbon. These data together with the molecular formula $C_{14}H_{24}N_2O_4$ suggested that compound **US10** was a symmetrical molecule. Therefore, compound **US10** was identified as a new enamide dimer.

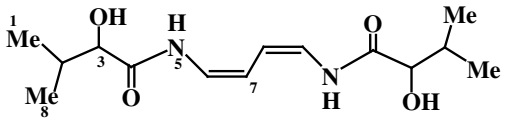


Table 51 The NMR data of compound US10 in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	COSY	NOE
1	0.82 (*d*, 6.9)	15.69 (CH_3)	C-2, C-3, C-8	H-2	H-2, H-3, H-8
2	2.13 (*m*)	32.17 (CH)	C-1, C-3, C-8	H-1, H-3, H-8	H-1, H-3, H-8
3	4.07 (*d*, 3.3)	76.16 (CH)	C-1, C-2, C-4 C-8	H-2	H-1, H-2, H-8
3-OH	6.49 (*brs*)	-	-	-	-
4	-	172.41 (C=O)	-	-	-
5-NH	10.97 (*d*, 10.8)	-	-	H-6	-
6	7.50 (*dd*, 10.8, 8.7)	138.63 (CH)	C-4, C-7	5-NH, H-7	H-5, H-7
7	5.15 (*d*, 8.7)	96.80 (CH)	C-4, C-6	H-6	H-6
8	0.99 (*d*, 6.9)	18.90 (CH_3)	C-1, C-2, C-3	H-2	H-1, H-2, H-3

3.3.4 Compound US11

Compound **US11** with the molecular formula $C_{14}H_{24}N_2O_4$ from EIMS (*m/z* 284) (**Figure 93**) was obtained as a colourless gum $[\alpha]_D$ +137.84 (c = 0.13, $CHCl_3$). It exhibited similar UV (**Figure 86**) and IR (**Figure 87**) absorption bands to those of **US10**. The ^1H (**Figure 88**) (**Table 52**) and ^{13}C (**Figure 89**) (**Table 52**) NMR data were almost identical to those of compound **US10** except for the appearance of 3-OH as two broad singlets. In addition, compound **US11** demonstrated HMBC correlations (**Figure 91**) (**Table 52**) similar to those of compound **US10**. However, they gave different specific rotation. Therefore, compound **US11** had the same structure as compound **US10** with the difference in the configuration of one of the hydroxycarbons.

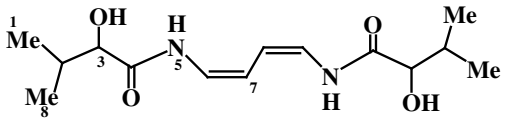
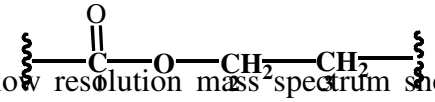


Table 52 The NMR data of compound US11 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	HMBC correlations	COSY
1	0.91 (*d*, 6.9)	15.44 (CH_3)	C-2, C-3, C-8	H-2
2	2.15 (*m*)	31.86 (CH)	C-1, C-3, C-4, C-8	H-1, H-3, H-8
3	4.03 (*d*, 3.3)	76.43 (CH)	C-1, C-2, C-4, C-8	H-2
3-OH	6.43 (*brs*), 6.10 (*brs*)	-	-	-
4	-	176.43 (C=O)	-	-
5	11.05 (*d*, 10.8)	-	-	-
6	7.55 (*dd*, 10.8, 9.0)	138.16 (CH)	C-7	H-5, H-7
7	5.23 (*d*, 9.0)	97.11 (CH)	C-6	H-6
8	1.06 (*d*, 6.9)	19.05 (CH_3)	C-1, C-2, C-3	H-2

3.3.5 Compound US12

Compound **US12** with the molecular formula $C_{12}H_{16}O_8$ from EIMS (*m/z* 288) (**Figure 100**) was obtained as a colorless gum. It exhibited a carbonyl absorption band at 1717 cm^{-1} in the IR spectrum (**Figure 95**). The ^1H NMR spectrum (**Figure 96**) (**Table 53**) contained signals for two sets of coupled methylene protons [δ_H 4.65 (*t*, *J* = 6.0 Hz, 2H) and δ_H 3.05 (*t*, *J* = 6.0 Hz, 2H)]. The ^{13}C NMR spectrum (**Figure 98**) (**Table 53**) showed one ester-carbonyl carbon (C-1, δ_C 174.49), one oxymethylene carbon (C-2, δ_C 69.30) and one methylene carbon (C-3, δ_C 30.73). HMBC cross peaks (**Figure 99**) (**Table 53**) of H-2 with C-1 and C-3 indicated the presence of the following structural unit with the molecular weight of 72 mass unit.



The low resolution mass spectrum showed the molecular ion at *m/z* 288 established the structure of compound **US12** to be a tetralactone (Zhang, *et al*., 2004).

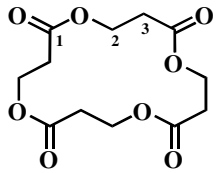


Table 53 The NMR data of compound US12 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δc (C-type)	HMBC correlations
1	-	174.49 (C=O)	-
2	4.65 (*t*, 6.0)	69.30 (CH_2)	C-1, C-3
3	3.05 (*t*, 6.0)	30.73 (CH_2)	C-1, C-2

3.3.6 Compound US13

Compound **US13** with the molecular formula $C_{11}H_{13}NO_5$ from EIMS (*m/z* 239) (**Figure 109**) was isolated as a colorless gum. It exhibited UV (**Figure 101**) absorption bands at 221 and 277 nm while hydroxyl and carbonyl absorption bands

were found at 3409 and 1730 cm^{-1}, respectively, in the IR spectrum (**Figure 102**). The ^{1}H NMR spectrum (**Figure 103**) (**Table 54**) revealed the presence of a 1,4-disubstituted benzene [δ_H 7.00 (*d*, *J* = 8.4 Hz, 2H) and 6.70 (*d*, *J* = 8.4 Hz, 2H)] and four sets of methylene protons [δ_H 4.55 (*t*, *J* = 6.3 Hz, 2H), 4.25 (*t*, *J* = 7.2 Hz, 2H), 2.89 (*t*, *J* = 6.3 Hz, 2H) and 2.80 (*t*, *J* = 7.2 Hz, 2H)]. The ^{13}C NMR spectrum (**Figure 105**) (**Table 54**) showed one carbonyl carbon (δ_C 169.00), one oxyquaternary carbon (δ_C 153.90), one quaternary carbon (δ_C 130.00), two carbon resonances for four aromatic carbons (δ_C 115.45 and 129.99) and four methylene carbons (δ_C 31.09, 34.06, 66.11 and 69.66). These data revealed that one of the substituents on the 1,4-disubstituted benzene was a hydroxyl group. In the ^{1}H-^{1}H COSY spectrum (**Figure 108**) (**Table 54**), two sets of coupled methylene protons were observed: H-1 (δ_H 4.55) with H-2 (δ_H 2.89) and H-5 (δ_H 4.25) with H-6 (δ_H 2.80). HMBC correlations (**Figure 107**) (**Table 54**) of H-5 with C-3 (δ_C 169.00) and C-7 (δ_C 130.00) of the 4-hydroxyphenyl unit and that of H-2 with C-3 established the ester linkage between C-2 and C-5 with an oxygen atom attached to C-5 and further linked C-6 with C-7 of the 4-hydroxyphenyl unit. The chemical shifts of C-2 and C-5 supported the assigned linkage. These results together with the molecular formula $C_{11}H_{13}NO_5$ suggested that the substituent at C-1 was a nitro group. Therefore, compound **US13** was identified as a new nitroester compound.

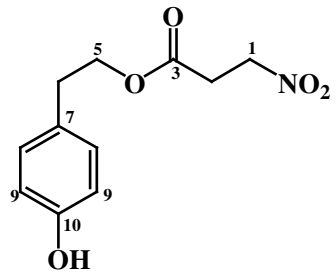


Table 54 The NMR data of compound US13 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	HMBC correlations	COSY	NOE
1	4.55 (*t*, 6.3)	69.66 (CH_2)	C-2, C-3	H-2	H-2
2	2.89 (*t*, 6.3)	31.09 (CH_2)	C-1, C-3	H-1	H-1
3	-	169.00 (C=O)	-	-	-
5	4.25 (*t*, 7.2)	66.11 (CH_2)	C-3, C-6, C-7	H-6	H-6
6	2.80 (*t*, 7.2)	34.06 (CH_2)	C-5, C-8	H-5	H-5, H-8
7	-	130.00 (C)	-	-	-
8	7.00 (*d*, 8.4)	129.99 (CH)	C-6, C-9, C-10	H-9	H-6, H-9
9	6.70 (*d*, 8.4)	115.45 (CH)	-	H-8	H-8
10	-	153.90 (C)	C-7, C-10	-	-

3.3.7 Compound US14

Compound **US14** with the molecular formula $C_{34}H_{34}O_{14}$ from EIMS (**Figure 120**) was isolated as a yellow gum. The ^{13}C NMR spectrum (**Figure 115**) (**Table 55**) consisted of 17 carbon resonances, indicating that compound **US14** must be symmetrical. It exhibited UV (**Figure 110**) absorption bands at 218, 237 and 346 nm while hydroxyl and carbonyl absorption bands were found at 3501 and 1606 cm^{-1}, respectively, in the IR spectrum (**Figure 111**). The ^1H NMR spectrum (**Figure 112**) (**Table 55**) contained signals of two H-bonded hydroxyl groups [δ_H 13.92 (*s*, 1H) and 11.86 (*s*, 1H)], two methyl protons [δ_H 2.03 (*s*, 3H) and 1.01 (*d*, *J* = 6.0 Hz, 3H)], two sets of nonequivalent methylene protons [(δ_H 4.03, *d*, *J* = 13.2 Hz, 1H and 3.47, *brd*, *J* = 13.2 Hz, 1H) and (δ_H 2.41, *m*, 1H and 2.32, *m*, 1H)], one oxymethine proton [δ_H 5.69 (*s*, 1H)], two *ortho*-coupled aromatic protons [δ_H 7.33 (*d*, *J* = 8.4 Hz, 1H) and 6.44 (*d*, *J* = 8.4 Hz, 1H)] and one methine proton (δ_H 2.36, *s*) in each monomer. The connectivity of the spin systems into the carbon skeleton of compound **US14** was established using HMBC correlations (**Figure 117**) (**Table 56**). One of the chelated hydroxy protons at δ_H 11.86 gave ^3J correlation with C-2 (δ_C 117.92) and C-9a (δ_C 106.42) while one of the *ortho*-coupled aromatic protons resonating at δ_H 6.44

showed these correlations with C-2 and C-9a. These established the location of the hydroxyl group and the aromatic proton at C-1 (δ_C 159.50) and C-4 (δ_C 107.83), respectively. The remaining *ortho*-coupled proton (δ_H 7.33) was then placed at C-3. In addition, HMBC correlations of H-4 to a carbonyl carbon (δ_C 187.73) and an oxygenated carbon (C-4a, δ_C 157.12) confirmed the presence of the carbonyl group at C-9a and established an oxysubstituent at C-4a. The substituent at C-2 remained unidentified at this stage. The other chelated hydroxyl group, resonating at δ_H 13.92, was assigned to be located at C-8 (δ_C 178.02) as the ^{13}C NMR spectrum consisted of only one carbonyl carbon of an unsaturated ketone. This hydroxy proton gave ^3J HMBC correlations with C-7 (δ_C 33.40) and C-8a (δ_C 100.83). The nonequivalent methylene protons (δ_H 2.32 and 2.41), which showed a HMQC cross peak with C-7, exhibited HMBC cross peaks with C-5 (δ_C 70.24) and C-6 (δ_C 27.67). These data together with HMBC correlations of the doublet methyl protons (δ_H 1.01) with C-5, C-6 and C-7 indicated the presence of -C=C(OH)CH_2CH(Me)CH- unit. The chemical shifts of H-5 (δ_H 5.69) and H_{ab}-12 (δ_H 4.03 and 3.47), the ^3J HMBC correlations of H-5/C-8a, C-11 (δ_C 17.53), C-12 (δ_C 65.56), and C-13 (δ_C 170.51) and that of Me-14/C-13 established the cyclohexene ring having an acetoxyl group at C-5 and a hydroxymethyl group at C-10a. The other substituent at C-10a was an oxysubstituent according to its chemical shift. On the basis of the molecular formula, each monomer contained 9 degrees of unsaturation, thus indicating an ether linkage between C-4a and C-10a to form a hydroxanthone unit. Therefore, the remaining substituent at C-2 must be the other identical hydroxyxanthone unit. The relative stereochemistry of **US14** was established by the NOEDIFF results. The signal intensity of H-6 was enhanced when either H-5 or H-12_b was irradiated (**Figure 119**) (**Table 56**), indicating their *cis*-relationship. The appearance of H-5 as a singlet established the location of H-5 at equatorial position. Therefore, the structure of **US14** was identical to that of dicerandrol A which was previously isolated from *Phomopsis longicalla* (Wagenaar and Clardy, 2001). They also possessed similar specific rotation (**US14**: $[\alpha]_D$ -61.67, c = 0.01, $CHCl_3$ and dicerandrol A: $[\alpha]_D$ -50.9, c = 0.01, $CHCl_3$).

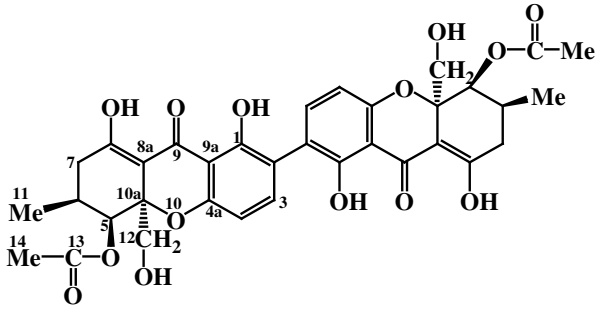


Table 55 The ^{1}H and ^{13}C NMR data of compound US14 in $CDCl_3$ and the ^{1}H NMR data of dicerandrol A in CD_3CN

Position	US14		dicerandrol A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1, 1′-OH	11.86 (*s*)	159.50 (C)	11.83 (*s*)	160.3 (C)
2, 2′	-	117.92 (C)	-	118.5 (C)
3, 3′	7.33 (*d*, 8.4)	140.07 (CH)	7.39 (*d*, 8.5)	141.1 (CH)
4, 4′	6.44 (*d*, 8.4)	107.83(CH)	6.43 (*d*, 8.5)	109.0 (CH)
4a, 4a′	-	157.12 (C)	-	158.9 (C)
5, 5′	5.69 (*s*)	70.24 (CH)	5.61 (*d,* 1.5)	71.8 (CH)

Table 55 (continued)

Position	US14		dicerandrol A	
	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
6, 6′	2.36 (*m*)	27.67 (CH)	2.46 (*m*)	28.5 (CH)
7, 7′	a: 2.32 (*m*)	33.40 (CH_2)	a: 2.33 (*m*)	34.1 (CH_2)
	b: 2.41 (*m*)		b: 2.50 (*m*)	-
8, 8′-OH	13.92 (*s*)	178.02 (C)	13.94 (*bs*)	179.9 (C)
8a, 8a′	-	100.83 (C)	-	102.3 (C)
9, 9′	-	187.73 (C=O)	-	189.1 (C=O)
9a, 9a′	-	106.42 (C)	-	107.3 (C)
10a, 10a′	-	82.49 (C)	-	83.8 (C)

11, 11′	1.01 (*d*, 6.0)	17.53 (CH_3)	1.00 (*d*, 6.5)	17.8 (CH_3)
12, 12′	a: 4.03 (*d*, 13.2)	65.56 (CH_2)	a: 3.90 (*dd*, 4.5, 13.0)	65.8 (CH_2)
	b: 3.47 (*brd*, 13.2)		b: 3.55 (*dd*, 7.0, 13.0)	-
13, 13′	-	170.51 (C=O)	-	171.4 (C=O)
14, 14′	2.03 (*s*)	20.83 (CH_3)	2.03 (*s*)	21.0 (CH_3)

Table 56 The HMBC, COSY and NOE data of compound US14 in $CDCl_3$

Position	HMBC correlations	COSY	NOE
1-OH	C-1, C-2, C-9a	-	-
H-3	C-1, C-2, C-4a	H-4	H-4
H-4	C-2, C-4a, C-9, C-9a	H-3	H-3
H-5	C-6, C-7, C-8a, C-9, C-10a, C-11, C-12, C-13	-	H-6, H-11
H-6	C-5, C-11	H-11	H-5, H-11
H_a-7	C-5, C-6	H_b-7	H_b-7
H_b-7	C-5, C-6	H_a-7	H_a-7, H-11
8-OH	C-7, C-8, C-8a	-	-
H-11	C-5, C-6, C-7	H-6	H-5, H-6, H_a-7

Table 56 (continued)

Position	HMBC correlations	COSY	NOE
H_a-12	C-5, C-10a	H_b-12	H_b-12
H_b-12	C-5, C-10a	H_a-12	H-6, H_a-12
H-14	C-13		

3.3.8 Compound US15

Compound **US15** with the molecular formula $C_{34}H_{34}O_{14}$ from EIMS (*m/z* 666) (**Figure 130**) was isolated as a yellow gum. Its UV (**Figure 121**) and IR (**Figure 122**) absorption bands were similar to those of **US14**. These results suggested the presence of a dimer-tetrahydroxanthone as found in **US14**. The ^{1}H NMR spectrum

(**Figure 123**) (**Table 57**) revealed two sets of signals as follows: four H-bonded hydroxy protons [δ_H 13.85 (*s*, 1H), 13.82 (*s*, 1H), 11.95 (*s*, 1H) and 11.36 (*s*, 1H)], four methyl protons [δ_H 2.07 (*s*, 3H), 1.90 (*s*, 3H), 1.01 (*d*, *J* = 6.0 Hz, 3H) and 0.92 (*d*, *J* = 6.5 Hz, 3H)], four sets of nonequivalent methylene protons [δ_H 3.95 (*d*, *J* = 10.0 Hz, 1H) and 3.58 (*d*, *J* = 10.0 Hz, 1H)], [δ_H 3.92 (*d*, *J* = 12.0 Hz, 1H) and 3.33 (*d*, *J* = 12.0 Hz, 1H)], [δ_H 2.39 (*m*, 1H) and 2.31 (*m*, 1H)], [δ_H 2.37 (*m*, 1H) and 2.20 (*m*, 1H)], two oxymethine protons [δ_H 5.59 (*s*, 1H) and 5.46 (*d*, *J* = 1.5 Hz, 1H)], four *ortho*-coupled aromatic protons [δ_H 7.21 (*d*, *J* = 8.5 Hz, 1H) and 6.52 (*d*, *J* = 8.5 Hz, 1H), δ_H 7.06 (*d*, *J* = 8.5 Hz, 1H) and 6.41 (*d*, *J* = 8.5 Hz, 1H)] and two methine protons [δ_H 2.38 (*m*, 1H) and 2.31 (*m*, 1H)]. The ^{13}C NMR spectrum (**Figure 126**) (**Table 57**) confirmed the above conclusion by the presence of 32 carbon resonances for 34 carbon atoms. These results indicated that **US15** was unsymmetrical. The HMBC correlations of 1-OH with C-1 (δ_C 158.44), C-2 (δ_C 117.29) and C-9a (δ_C 105.59) (**Figure 128**) (**Table 57**) indicated that two *ortho*-coupled aromatic protons were attributed to H-3 (δ_H 7.06, δ_C 137.44) and H-4 (δ_H 6.41, δ_C 107.21). The position of the attachment of the left-handed tetrahydroxanthone monomer must be C-2, the same position as found in **US14**. In addition, the HMBC cross peaks of 15-OH with C-15 (δ_C 160.93), C-16 (δ_C 109.15) and C-23a (δ_C 105.59) revealed that two *ortho*-coupled aromatic protons were H-16 (δ_H 6.52, δ_C 109.15) and H-17 (δ_H 7.21, δ_C 138.12). Consequently, the position of the attachment of the other tetrahydroxanthone monomer must be C-18. Bond formation between C-2 of the left-handed tetrahydroxanthone monomer and C-18 of the other tetrahydroxanthone monomer was confirmed by ^3J HMBC correlation of H-3/C-18 and H-17/C-2. Therefore, **US15** was assigned as the new tetrahydroxanthone dimer. Because of the overlap of proton signals in **US15**, its relative stereochemistry was determined by comparison of proton chemical shifts and coupling constants with those of compound **US14**. Compounds **US14** and **US15** were assumed to have the same relative stereochemistry on the basis of these similar data.

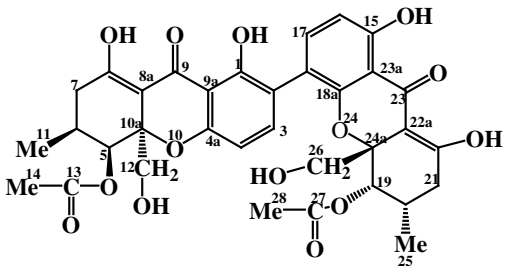


Table 57 The NMR data of compound US15 in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	COSY	NOE
1-OH	11.95 (*s*)	158.44 (C)	C-1, C-2, C-9a	-	-
2	-	117.29 (C)	-	-	-
3	7.06 (*d,* 8.5)	137.44 (CH)	C-2, C-4a, C-18	H-4	H-4
4	6.41 (*d*, 8.5)	107.21 (CH)	C-2, C-4a, C-9a	H-3	H-3
4a	-	156.63 (C)	-	-	-

Table 57 (continue)

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	COSY	NOE
5	5.46 (*d*, 1.5)	68.31 (CH)	C-7, C-8a, C-10a, C-11, C-13	-	-
6	2.31 (*m*)	26.71 (CH)	C-5, C-11	H-11	-
7	a : 2.31 (*m*)	32.12 (CH_2)	C-5, C-6, C-8a	-	-
	b : 2.39 (*m*)			-	-
8-OH	13.85 (*s*)	177.33 (C)	C-7, C-8, C-8a	-	-
8a	-	99.99 (C)	-	-	-
9	-	186.98 (C=O)	-	-	-
9a	-	105.59 (C)	-	-	-
10a	-	81.22 (C)	-	-	-
11	0.92 (*d*, 6.5)	16.29 (CH_3)	C-5, C-6, C-7	H-6	H-6, H_a-7
12	a : 3.33 (*d*, 12.0)	64.02 (CH_2)	C-5, C-10a	-	-
	b : 3.92 (*d*, 12.0)			-	-
13	-	169.40 (C=O)	-	-	-
14	1.90 (*s*)	19.73 (CH_3)	C-13	-	-
15-OH	11.36 (*s*)	160.93 (C)	C-15, C-16, C-23a	-	-
16	6.52 (*d*, 8.5)	109.15 (CH)	C-18, C-23a	H-17	H-17
17	7.21 (*d*, 8.5)	138.12 (CH)	C-2, C-15, C-18a	H-16	H-16
18	-	115.86 (C)	-	-	-
18a	-	153.30 (C)	-	-	-
19	5.59 (*s*)	70.13 (CH)	C-6, C-7, C-22a, C-24a, C-25, C-27	-	-
20	2.38 (*m*)	26.64 (CH)	C-19, C-25	H-25	
21	a : 2.20 (*m*)	32.49 (CH_2)	C-20	-	-
	b : 2.37 (*m*)			-	-
22-OH	13.82 (*s*)	176.64 (C)	C-21, C-22, C-22a	-	-

Table 57 (Continued)

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	COSY	NOE
22a	-	100.11 (C)	-	-	-
23	-	186.98 (C=O)	-	-	-
23a	-	105.59 (C)	-	-	-
24a	-	81.69 (C)	-	-	-
25	1.01 (*d*, 6.0)	16.52 (CH_3)	C-19, C-20, C-21	H-20	H-19
26	a: 3.58 (*d*, 10.0)	64.67 (CH_2)	C-19, C-24a	-	-
	b: 3.95 (*d*, 10.0)			-	-
27	-	169.70 (C=O)	-	-	-
28	2.07 (*s*)	19.89 (CH_3)	C-27	-	-

3.3.9 Compound US16

Compound **US16** with the molecular formula $C_9H_{12}N_2O_6$ from EIMS (*m/z* 244) (**Figure 140**) was obtained as a colorless gum with $[\alpha]_D$ +20.50 (c = 0.11, MeOH). It exhibited UV (**Figure 131**) absorption bands at 215 and 260 nm while hydroxyl and carbonyl absorption bands were found at 3399 and 1606 cm^{-1}, in the IR spectrum (**Figure 132**). The ^1H NMR spectrum (**Figure 133**) (**Table 58**) contained signals of a ribose moiety [four methine protons at δ_H 5.92 (*d*, *J* = 5.0 Hz, 1H), 4.29 (*t*, *J* = 5.0 Hz, 1H), 4.26 (*t*, *J* = 5.0 Hz, 1H), 4.02 (*td*, *J* = 2.5 and 5.0 Hz, 1H) and one set of methylene protons at δ_H 3.84 (*dd*, *J* = 2.5 and 12.0 Hz, 1H), 3.78 (*dd*, *J* = 2.5 and 12.0 Hz, 1H) and a cytosine moiety [δ_H 10.09 (*brs*, 1H), 7.99 (*d*, *J* = 8.5 Hz, 1H) and 5.60 (*d*, *J* = 8.5 Hz, 1H)]. The ^{13}C NMR spectrum (**Figure 136**) (**Table 58**) showed two carbonyl carbons (δ_C 163.63 and 151.69), six methine carbons (δ_C 140.79, 101.61, 89.25, 85.17, 74.43, 70.27) and one methylene carbon (δ_C 61.25). HMBC cross peaks (**Figure 138**) (**Table 58**) of H-7 of the ribose moiety with the carbonyl carbon, C-6 (δ_C 151.69), and the methine carbon, C-4 (δ_C 140.79), linked C-7 of the ribose unit with N-5 of the cytosine group. Therefore, compound **US16** was identified as uridine (Rosemeyer, *et al*., 1990).

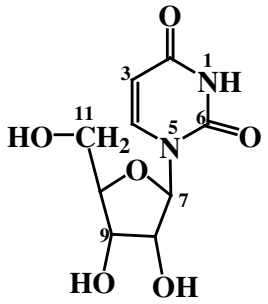


Table 58 The NMR data of compound US16 in Acetone-d_6

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	HMBC correlations	NOE
1	10.09 (*brs*)	-	-	-
2	-	163.63 (C=O)	-	-
3	5.60 (*d*, 8.5)	-	C-2, C-4	H-4
4	7.99 (*d*, 8.5)	101.61 (CH)	C-2, C-3, C-6, C-7	H-3, H-7, H-8
6	-	140.79 (CH)	-	-
7	5.92 (*d*, 5.0)	151.69 (C=O)	C-4, C-6, C-8, C-9	H-4, H-8
8	4.29 (*t*, 5.0)	89.25 (CH)	C-10	H-4
9	4.26 (*t*, 5.0)	74.43 (CH)	C-7, C-11	H_b-11
10	4.02 (*dt*, 5.0, 2.5)	70.27 (CH)	C-9	H_{ab}-11
11	a: 3.84 (*dd*, 12.0, 2.5)	85.17 (CH)	C-9, C-13	H-10, H_b-11
	b: 3.78 (*dd*, 12.0, 2.5)	61.25 (CH_2)	-	H-10, H_a-11

PART Ⅳ

CHEMICAL CONSTITUENTS FROM THE ENDOPHYTIC FUNGUS XYLARIACEAE PSU-A80

CHAPTER 4.1

INTRODUCTION

4.1.1 Introduction

The Xylariaceae is a large family (Xylariales, Ascomycotina) of 36 or more genera (Whalley and Edwards, 1995). The perithecia and stromata are typically dark with black spores (http://www. hiddenforest.co.nz/fungi/family/xylariaceae/-xylariaceae.htm). Secondary metabolites produced by this family are summarized in **Table 59**. In an ongoing search for bioactive fungal metabolites, we discovered antioxidation activity with IC_{50} values of 0.20 and 1.37 mg/mL (DPPH assay) in the extracts of the broth and mycelia of the Xylariaceae fungus PSU-A80, respectively. The Xylariaceae fungus PSU-A80 was an endophytic fungus isolated from the leaves of *Garcinia atroviridis*.

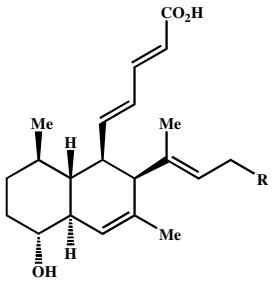
Table 59 Metabolites from the Xylariaceae fungus and the biological activity

Compound	Structure	Activity	Reference
carneic acid A	70	antimicrobial	Quang, *et al*., 2006a
carneic acid B	71	antimicrobial	”
cohaerin A	72	-	Quang, *et al*., 2005b
cohaerin B	73	-	”
cohaerin C	74	antioxidant and antimicrobial	Quang, *et al*., 2006b
cohaerin D	75	antioxidant and antimicrobial	”
cohaerin E	76	antioxidant and antimicrobial	”
cohaerin F	77	antioxidant and antimicrobial	”

Table 59 (continued)

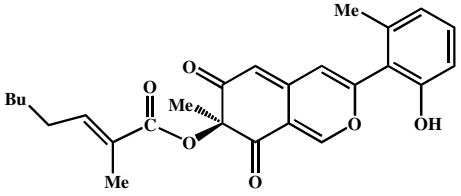
Compound	Structure	Activity	Reference
daldinin C	78	antioxidant	Quang, *et al*., 2004
daldinin E	79	antioxidant	”
daldinin F	80	antioxidant	”
isoorchracein	81	-	Anderson, *et al*., 1983
Ramulosin	82	antimicrobial	Anderson, *et al*., 1983, Takano, *et al*., 1992
Mellein	83	phytotoxic and antifungal	Anderson, *et al*., 1983, Cabras, *et al*., 2006
4-hydroxy-isoochracein	84	-	Anderson, *et al*., 1983
4,4′,5,5′-tetrahydroxy-1,1′-binaphthalene	85	antioxidant	Quang, *et al*., 2004
sassafrin A	86	antimicrobial	Quang, *et al*.,2005a
sassafrin B	87	antimicrobial	”
sassafrin C	88	antimicrobial	”
sassafrin D	89	antimicrobial	”
07H239-A	90	cytotoxic	McDonald, *et al*., 2004

Structures of the metabolites from the Xylariaceae fungus

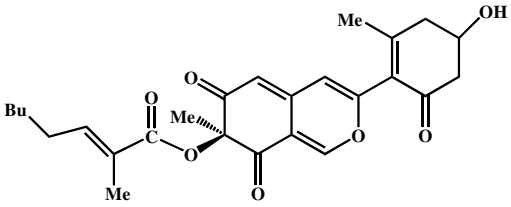


70 : R = H : carneic acid A

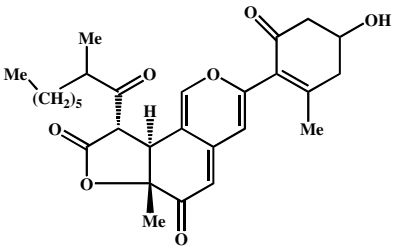
71 : R = OH : carneic acid B



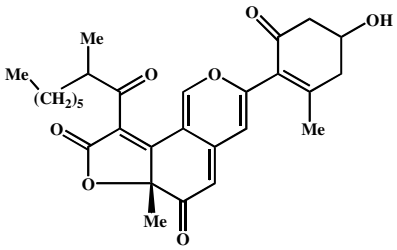
72 : cohaerin A



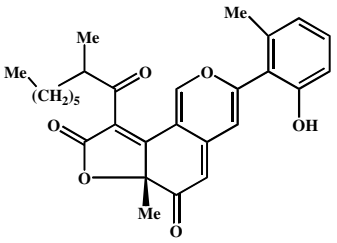
73 : cohaerin B



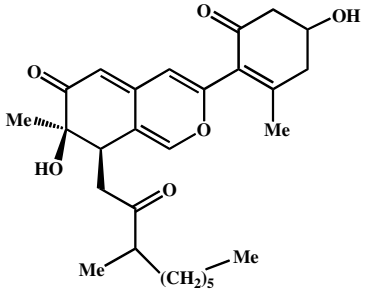
74 : cohaerin C



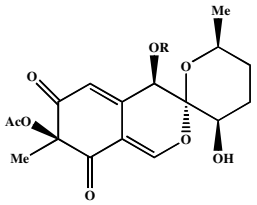
75 : cohaerin D

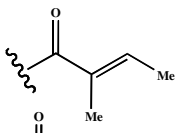


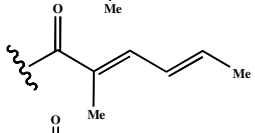
76 : cohaerin E

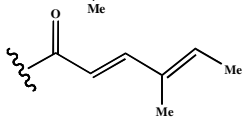


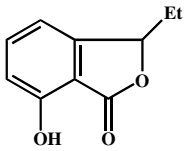
77 : cohaerin F



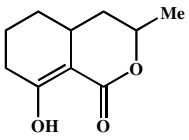
78 : R =  : daldinin C

79 : R =  : daldinin E

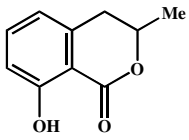
80 : R =  : daldinin F



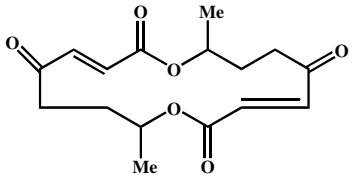
81 : isoorchracein



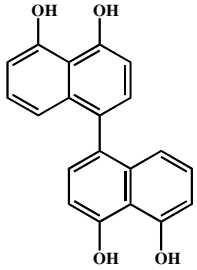
82 : ramulosin



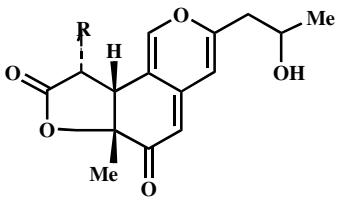
83 : mellein

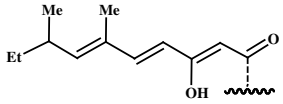


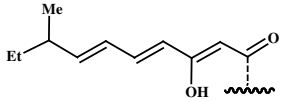
84 : 4-hydroxyisoochracein

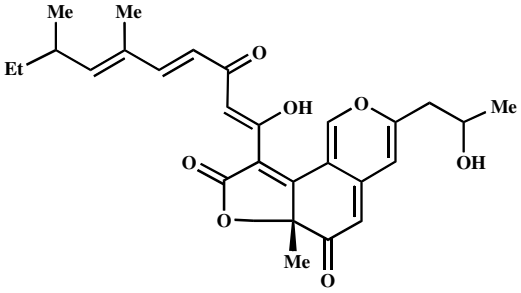


85 : 4,4′,5,5′-tetrahydroxy-1,1′-binaphthalene

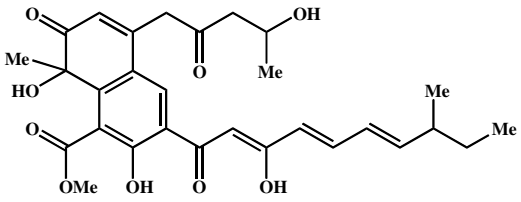


86 : R =  : sassafrin A

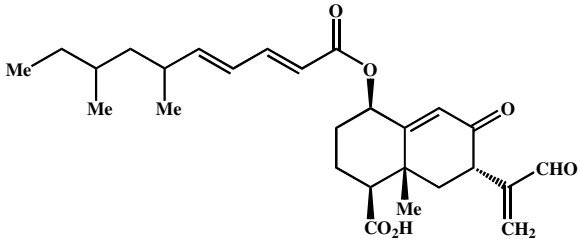
87 : R =  : sassafrin B



88 : sassafrin C



89 : sassafrin D



90 : 07H239-A

CHAPTER 4.2

EXPERIMENTAL

4.2.1 Fermentation and extraction

The fermentation and extraction were performed using the same procedure as those of Xylariaceae PSU-A80. The crude EtOAc extracts from the culture broth and mycelia were obtained in 1.0 g and 1.0 g, respectively, both as a brown gum.

4.2.2 Purification of the broth extract

The broth extract of the fungus Xylariaceae PSU-A80 (1.0 g) was chromatographed on Sephadex LH-20 column chromatography using 50% methanol-dichloromethane to afford forty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford six fractions, as shown in **Table 60**.

Table 60 Fractions obtained from the broth extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	41.0	Dark-brown gum
B	581.8	Dark-brown gum
C	235.3	Dark-brown gum
D	71.9	Dark-brown gum
E	40.0	Brown gum mixed with brown solid
F	30.0	Brown gum mixed with brown solid

Fraction A Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated none of UV-active

spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Fraction B Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated four spots with the R_f values of 0.37, 0.44, 0.51 and 0.72 and two spots near the baseline under UV-S. Further separation by column chromatography over silica gel using a gradient system of methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 61**.

Table 61 Subfractions obtained from Fraction B by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B1	100% CH_2Cl_2	1.4	Yellow gum
B2	1% MeOH/CH_2Cl_2	358.3	Dark-brown gum
B3	1-2% MeOH/CH_2Cl_2	107.2	Dark-brown gum
B4	3% MeOH/CH_2Cl_2-100% MeOH	106.4	Dark-brown gum

Subfraction B1 Chromatogram characteristics on normal phase TLC with 30% dichloromethane-petroleum ether as a mobile phase demonstrated none of UV-active spots. Because of the minute quantity, it was not further investigated.

Subfraction B2 Chromatogram characteristics on normal phase TLC with 30% dichloromethane-petroleum ether as a mobile phase demonstrated six spots with the R_f values of 0.16, 0.18, 0.23, 0.32, 0.36 and 0.59 and two spots near the baseline under UV-S and two other spots with the R_f values of 0.45 and 0.52 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel was performed using 20% dichloromethane-petroleum ether and gradually enriched with dichloromethane and then methanol until pure methanol. Fractions, which contained the similar

components, were combined and evaporated to dryness under reduced pressure to give ten subfractions, as shown in **Table 62**.

Table 62 Subfractions obtained from Subfraction B2 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B21	20-30% CH_2Cl_2/Petrol	15.9	Colorless gum
B22	30% CH_2Cl_2/Petrol	3.6	Pale brown gum
B23	30-40% CH_2Cl_2/Petrol	5.1	Colorless gum
B24	50% CH_2Cl_2/Petrol	14.3	Brown gum
B25	60-90% CH_2Cl_2/Petrol	34.8	Pale brown gum
B26	100% CH_2Cl_2-1% MeOH/CH_2Cl_2	45.5	Brown gum
B27	2% MeOH/CH_2Cl_2	30.0	Brown gum
B28	3-5% MeOH/CH_2Cl_2	75.8	Brown gum
B29	7-10% MeOH/CH_2Cl_2	40.0	Brown gum
B210	10% MeOH/CH_2Cl_2-100%MeOH	30.7	Brown gum mixed with pale brown solid

Subfraction B21 (US17) Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.73 under UV-S.

UV (MeOH) λ_{max} nm (log ε)	279 (3.22)
FT-IR (neat) ν_{cm-1}	3419 (OH stretching), 2928 (C-H stretching) 1641 (C=C stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	9.32 (*s*, 1H), 7.41 (*d*, *J* = 7.8 Hz, 1H), 7.35 (*t*, *J* = 8.1 Hz, 1H), 7.30 (*t*, *J* = 7.8 Hz, 1H), 7.29 (*dd*, *J* = 8.1, 1.8 Hz, 1H), 6.87 (*dd*, *J* = 8.1, 1.8 Hz, 1H), 6.76 (*d*, *J* = 7.8 Hz, 1H), 4.05 (*s*, 3H)

^{13}C NMR ($CDCl_3$) (δ ppm)		156.17, 154.50, 136.76, 127.72, 125.60, 121.87,
(75 MHz)		118.87, 115.00, 110.42, 103.91, 56.10
DEPT (135°) ($CDCl_3$)	CH :	127.72, 125.60, 121.87, 118.87, 110.42, 103.91
	CH_3 :	56.10

Subfraction B22 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B23 (US18) Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.50 under UV-S.

$[\alpha]_D^{28}$	-79.20 (c = 0.25, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)	249 (3.72), 326 (3.49)
FT-IR (neat) ν_{cm-1}	3419 (OH stretching), 2925 (C-H stretching)
	1664 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm)	11.01 (*s*, 1H), 7.29 (*d*, *J* = 8.4 Hz, 1H), 6.82 (*d*,
(300 MHz)	*J* = 8.4 Hz, 1H), 4.69 (*m*, 1H), 2.95 (*dd*, *J* =
	16.5, 3.3 Hz, 1H), 2.72 (*dd*, *J* = 16.5, 11.4 Hz,
	1H), 2.20 (*s*, 3H), 1.50 (*d*, *J* = 6.5 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm)	170.33, 160.54, 137.92, 115.72, 108.11,
(75 MHz)	75.41, 31.94, 20.92, 18.07
DEPT (135°) ($CDCl_3$)	CH : 137.92, 115.72, 75.41
	CH_2 : 31.94
	CH_3 : 20.92, 18.07

Subfraction B24 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B25 Chromatogram characteristics on normal phase TLC with 100% dichloromethane (4 runs) as a mobile phase demonstrated three spots with the R_f values of 0.11, 0.29 and 0.45 and one spot near the baseline under UV-S. Further separation by column chromatography over silica gel was performed using 60% dichloromethane-petroleum ether and gradually enriched with dichloromethane and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 63**.

Table 63 Subfractions obtained from Subfraction B25 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B25a	60-70% CH_2Cl_2/Petrol	9.0	Brown gum
B25b	80-90% CH_2Cl_2/Petrol	12.5	Brown gum
B25c	100%CH_2Cl_2-100%MeOH	14.7	Brown gum

Subfraction B25a Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-petroleum ether as a mobile phase demonstrated two spots with the R_f values of 0.47 and 0.58 under UV-S. Further purification by precoated TLC with 15% ethyl acetate-petroleum ether (14 runs) as a mobile phase afforded three bands.

Band 1 was obtained as a yellow gum (1.1 mg). Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.29 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a yellow gum (0.9 mg). Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Band 3 (US19) was obtained as a colorless gum (0.9 mg). Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.33.

$[\alpha]_D^{28}$		+12.3 (c = 0.03, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)		258 (3.47), 319 (3.27)
FT-IR (neat) ν_{cm-1}		3387 (OH stretching), 2926 (C-H stretching) 1673 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		7.50 (*t*, *J* = 8.0 Hz, 1H), 7.03 (*dt*, *J* = 8.0, 0.5 Hz, 1H), 6.93 (*dd*, *J* = 8.0, 0.5 Hz, 1H), 4.93 (*dd*, *J* = 6.5, 3.0 Hz, 1H), 3.01 (*ddd*, *J* = 18.0, 8.0, 4.5 Hz, 1H), 2.66 (*ddd*, *J* = 18.0, 8.5, 5.0 Hz, 1H), 2.35 (*m*, 1H), 2.22 (*m*, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		204.10, 160.34, 145.85, 136.94, 117.83, 117.32, 115.20, 67.76, 34.55, 31.24
DEPT (135°) ($CDCl_3$)	CH :	136.94, 117.83, 117.32, 67.76
	CH_2 :	34.55, 31.24

Subfraction B25b Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-petroleum ether as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B25c Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-petroleum ether as a mobile phase demonstrated none of UV-active spots. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B26 Chromatogram characteristics on normal phase TLC with 100% dichloromethane (4 runs) as a mobile phase demonstrated three spots with the R_f values of 0.11, 0.25 and 0.34 under UV-S. Further separation by column chromatography over silica gel was performed using 20% ethyl acetate-petroleum

ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give ten subfractions, as shown in **Table 64**.

Table 64 Subfractions obtained from Subfraction B26 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B26a	20-30% EtOAc/Petrol	4.2	Yellow gum
B26b	30% EtOAc/Petrol	3.3	Yellow gum
B26c	40% EtOAc/Petrol	6.5	Brown gum
B26d	50% EtOAc/Petrol	5.9	Brown gum
B26e	60% EtOAc/Petrol	3.2	Brown gum
B26f	70% EtOAc/Petrol	5.5	Brown gum
B26g	70-80% EtOAc/Petrol	5.8	Pale yellow gum
B26h	90% EtOAc/Petrol	3.3	Brown gum
B26i	100% EtOAc	1.0	Pale yellow gum
B26j	1% MeOH/EtOAc-100% MeOH	4.0	Brown gum

Subfraction B26a Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B26b Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated one pale spot with the R_f value of 0.42 under UV-S. The ^{1}H NMR spectrum indicated that it contained many components. Therefore, it was not further investigated.

Subfraction B26c Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.41 under UV-S. Further purification by

precoated TLC with 2% ethyl acetate-dichloromethane (8 runs) as a mobile phase afforded three bands.

Band 1 was obtained as a colorless gum (0.8 mg). Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.66 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US3** which was isolated from *P. microspora* PSU-A70 (see Chapter 2)

Band 2 was obtained as a yellow gum (0.9 mg). Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-dichloromethane as a mobile phase demonstrated two pale spots with the R_f values of 0.34 and 0.41 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was obtained as a yellow gum (0.7 mg). Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-dichloromethane as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Therefore, it was not further studied.

Subfraction B26d Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.29 under UV-S. Further purification by precoated TLC with 2% ethyl acetate-dichloromethane (8 runs) as a mobile phase afforded three bands.

Band 1 was obtained as a yellow gum (0.7 mg). Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane as a mobile phase demonstrated one pale spot with the R_f value of 0.75 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 (US20) was obtained as a pale yellow gum (0.7 mg). Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.61 under UV-S.

$[\alpha]_D^{26}$		-70.4 (c = 0.04, CH_3OH)
UV (MeOH) λ_{max} nm (log ε)		223 (3.23), 251 (3.20), 265 (3.17), 289 (3.10), 389 (2.80)
FT-IR (neat) ν_{cm-1}		3774 (OH stretching), 2926 (C-H stretching) 1670 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		12.60 (*s*, 1H), 8.22 (*s*, 1H), 7.53 (*dd*, *J* = 8.5, 7.5, Hz, 1H), 7.36 (*dd*, *J* = 7.5, 0.5 Hz, 1H), 7.24 (*d*, *J* = 8.0 Hz, 1H), 6.92 (*dd*, *J* = 8.5, 0.5 Hz, 1H), 6.77 (*d*, *J* = 8.0 Hz, 1H), 5.67 (*t*, *J* = 4.5 Hz, 1H), 5.28 (*dd*, *J* = 10.0, 4.5 Hz, 1H), 4.05 (*dd*, *J* = 14.5, 5.5 Hz, 1H), 3.39 (*dd*, *J* = 16.5, 5.5 Hz, 1H), 2.80 (*dt*, *J* = 12.5, 4.5 Hz, 1H), 2.39 (*dd*, *J* = 16.5, 14.5 Hz, 1H), 2.02 (*m*, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		204.21, 163.45, 155.10, 142.36, 137.54, 137.02, 135.87, 135.40, 123.15, 117.69, 117.25, 116.33, 114.62, 72.96, 62.47, 48.91, 42.80. 36.25
DEPT (135°) ($CDCl_3$)	CH :	137.02, 123.15, 117.69, 116.33, 114.62, 72.96, 62.47, 48.91
	CH_2 :	42.80, 36.25
EIMS *m/z* (% relative intensity):		336 (6), 319 (27), 318 (100), 300 (50), 290 (28)

Band 3 was obtained as a yellow gum (1.3 mg). Chromatogram characteristics on normal phase TLC with 1% ethyl acetate-dichloromethane as a mobile phase demonstrated none of UV-active spots. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B26e Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated two spots with the R_f values of 0.32 and 0.36 under UV-S. Further purification by precoated TLC with 4% ethyl acetate-dichloromethane (8 runs) as a mobile phase afforded three bands.

Band 1 was obtained as a yellow gum (0.9 mg). Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.23 under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a yellow gum (1.0 mg). Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane as a mobile phase demonstrated one pale spot with the R_f value of 0.18 under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 3 was obtained as a yellow gum (1.2 mg). Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane as a mobile phase demonstrated none of UV-active spots. The ^1H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction B26f Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated two spots with the R_f values of 0.23 and 0.50 under UV-S. Further purification by precoated TLC with 4% ethyl acetate-dichloromethane (10 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a yellow gum (0.9 mg). Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.55 under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 (US21) was obtained as a pale yellow gum (1.0 mg). Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.50 under UV-S.

$[\alpha]_D^{26}$	-23.5 (c = 0.04, MeOH)
UV (MeOH) λ_{max} nm (log ε)	226 (2.10), 249 (2.03), 266 (1.92), 294 (1.93),

		388 (1.71)
FT-IR (neat) ν_{cm-1}		3774 (OH stretching), 2927 (C-H stretching) 1697 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)		12.60 (*s*, 1H), 8.00 (*s*, 1H), 7.53 (*t*, *J* = 8.0 Hz, 1H), 7.37 (*dd*, *J* = 8.0, 1.0 Hz, 1H), 7.24 (*d*, *J* = 8.0 Hz, 1H), 6.92 (*dd*, *J* = 8.0, 1.0 Hz, 1H), Hz, 1H), 6.78 (*d*, *J* = 8.0 Hz, 1H), Hz, 1H), 5.67 (*t*, *J* = 3.0 Hz, 1H), 5.19 (*dd*, *J* = 10.0, 4.0 Hz, 1H), 4.07 (*dd*, *J* = 14.5, 6.0 Hz, 1H), 3.64 (*s*, 1H), 3.40 (*dd*, *J* = 16.5, 5.5 Hz, 1H), 2.80 (*dt*, *J* = 13.0, 4.0 Hz, 1H), 2.39 (*dd*, *J* = 16.5, 14.0 Hz, 1H), 2.02 (*ddd*, *J* = 13.0, 10.5, 3.0 Hz, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		204.27, 163.45, 155.03, 142.40, 137.51, 137.02, 135.87, 135.37, 123.26, 117.72, 116.40, 114.61, 74.37, 62.44, 56.03, 48.94, 42.76, 35.60
DEPT (135°) ($CDCl_3$)	CH :	137.02, 123.26, 117.72, 116.40, 114.61, 74.37, 62.44, 48.94
	CH_2 :	42.76, 35.60
	CH_3 :	56.03
EIMS *m/z* (% relative intensity):		350 (28), 318 (100), 300 (62), 290 (34), 271 (29)

Subfraction B26g (US22) Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-dichloromethane (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.14 under UV-S.

$[\alpha]_D^{27}$	-33.3 (c = 0.03, MeOH)
UV (MeOH) λ_{max} nm (log ε)	220 (4.38), 293 (4.35)
FT-IR (neat) ν_{cm-1}	3419 (OH stretching), 2927 (C-H stretching) 1683 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm)	7.33 (*m*, 5H), 7.15 (*s*, 1H), 4.25 (*dd*, *J* = 9.3, 6.9

(300 MHz)		Hz, 1H), 3.65 (*m*, 1H), 2.90 (*m*, 3H), 2.42 (*m*, 1H), 2.22 (*m*, 1H), 2.06 (*m*, 1H), 1.98 (*m*, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		167.98, 161.42, 133.62, 133.58, 129.52, 128.57, 128.44, 128.07, 121.19, 58.85, 45.29, 34.72, 28.01, 22.84
DEPT (135°) ($CDCl_3$)	CH :	129.52, 128.57, 128.44, 121.19, 58.85
	CH_2 :	45.29, 28.01, 22.84
	CH_3 :	34.72

Subfraction B26h Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.11 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US17**.

Subfraction B26i (US23) Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-dichloromethane (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.11 under UV-S.

$[\alpha]_D^{26}$		-12.8 (c = 0.03, MeOH)
UV (MeOH) λ_{max} nm (log ε)		225 (3.85), 302 (3.70)
FT-IR (neat) ν_{cm-1}		3364 (OH stretching), 2926 (C-H stretching) 1680 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		7.76 (*s*, 1H), 7.43 (*t*, *J* = 7.5 Hz, 1H), 7.37 (*d*, *J* = 7.5 Hz, 1H), 7.32 (*t*, *J* = 7.5 Hz, 1H), 7.00 (*s*, 1H), 4.32 (*dd*, *J* = 10.0, 7.0 Hz, 1H), 3.82 (*dt*, *J* = 8.5 Hz, 1H), 3.65 (*m*, 1H), 2.48 (*m*, 1H), 2.12 (*m*, 1H), 2.03 (*m*, 1H), 1.97 (*m*, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		165.83, 157.86, 133.23, 129.39, 128.61, 128.45, 115.86, 59.18, 45.67, 28.94, 21.83
DEPT (135°) ($CDCl_3$)	CH :	129.39, 128.61, 128.45, 115.86, 59.18
	CH_2 :	45.67, 28.94, 21.83

Subfraction B26j Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B27 Chromatogram characteristics on normal phase TLC with 0.5% methanol-dichloromethane (3 runs) as a mobile phase demonstrated a long tail under UV-S. Further separation by column chromatography over silica gel was performed using 30% ethyl acetate-petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 65**.

Table 65 Subfractions obtained from Subfraction B27 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B27a	30-50% EtOAc/Petrol	9.1	Yellow gum
B27b	60-70% EtOAc/Petrol	53.0	Yellow gum
B27c	80% EtOAc/Petrol-100% EtOAc	13.7	Brown gum
B27d	1% MeOH/EtOAc-100% MeOH	12.4	Brown gum

Subfraction B27a Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B27b Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.42 under UV-S and other violet spots with the R_f values of 0.54 and 0.68 after dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel using 30%

ethyl acetate-petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 66**.

Table 66 Subfractions obtained from Subfraction B27b by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B27b1	30-50% EtOAc/Petrol	2.9	Yellow gum
B27b2	60-70% EtOAc/Petrol	16.9	Yellow gum
B27b3	80-90% EtOAc/Petrol	6.5	Brown gum
B27b4	100% EtOAc-100% MeOH	18.5	Brown gum

Subfraction B27b1 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B27b2 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.34 under UV-S and the other violet spot with the R_f value of 0.68 and one spot near the baseline after dipping in ASA reagent and subsequently heating the TLC plate. Further separation on column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford three subfractions, as shown in **Table 67**.

Table 67 Subfractions obtained from Subfraction B27b2 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B27b21	7.3	Pale brown gum
B27b22	6.1	Pale brown gum
B27b23	1.5	Brown gum

Subfraction B27b21 Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B27b22 Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) as a mobile phase demonstrated two pale spots with the R_f values of 0.34 and 0.49 under UV-S and many spots after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B27b23 Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) as a mobile phase demonstrated many spots near the baseline after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B27b3 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B27b4 Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B27c Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B27d Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (4 runs) as a mobile phase demonstrated a long tail under UV-S and the other violet spot with the R_f value of 0.34 after dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel was performed using 20% ethyl acetate-petroleum ether and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give two subfractions, as shown in **Table 68**.

Table 68 Subfractions obtained from Subfraction B27d by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B27d1	20-60% EtOAc/Petrol	3.3	Brown gum
B27d2	70% EtOAc/Petrol-100% MeOH	7.7	Dark-Brown gum

Subfraction B27d1 Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) and 50% ethyl acetate-petroleum ether (2 runs) as mobile phases demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B27d2 Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) and 50% ethyl acetate-petroleum ether (2 runs) as mobile phases demonstrated a long tail after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B28 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated four spots with the R_f values of 0.33, 0.37, 0.42 and 0.53 under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give six subfractions, as shown in **Table 69**.

Table 69 Subfractions obtained from Subfraction B28 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B28a	100% CH_2Cl_2-0.5% MeOH/CH_2Cl_2	6.9	Pale brown gum
B28b	1% MeOH/CH_2Cl_2	10.2	Pale brown gum
B28c	1-1.5% MeOH/CH_2Cl_2	20.6	Pale brown gum
B28d	2% MeOH/CH_2Cl_2	7.1	Pale brown gum
B28e	3% MeOH/CH_2Cl_2	18.6	Pale brown gum
B28f	4% MeOH/CH_2Cl_2-100% MeOH	10.6	Brown gum

Subfraction B28a Chromatogram characteristics on normal phase TLC with 100% dichloromethane (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B28b Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated two spots with the R_f values of 0.20 and 0.27 under UV-S. Further purification by precoated TLC with 4% ethyl acetate-dichloromethane (6 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a pale brown gum (1.2 mg). Its chromatogram characteristics on normal phase TLC with 4% ethyl acetate-

dichloromethane (2 runs) as a mobile phase demonstrated two pale spots with the R_f values of 0.25 and 0.54 under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 was obtained as a pale brown gum (1.3 mg). Its chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane (3 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.18 under UV-S. Further purification by precoated TLC with 70% ethyl acetate-hexane (3 runs) as a mobile phase afforded a pale brown gum (0.4 mg). Its chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane (3 runs) as a mobile phase demonstrated one spot under UV-S with the same R_f value as **US26**.

Subfraction B28c Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated two spots near the baseline under UV-S. Further separation by column chromatography over silica gel was performed using 4% ethyl acetate-dichloromethane and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 70**.

Table 70 Subfractions obtained from Subfraction B28c by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B28c1	4-80% EtOAc/CH_2Cl_2	6.8	Pale brown gum
B28c2	80% EtOAc/CH_2Cl_2-100% EtOAc	4.9	Brown gum
B28c3	1% MeOH/EtOAc-100% MeOH	7.9	Brown gum

Subfraction B28c1 Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase demonstrated no

definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B28c2 Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.18 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B28c3 Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B28d Chromatogram characteristics on normal phase TLC with 4% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B28e Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-dichloromethane (3 runs) as a mobile phase demonstrated three spots with the R_f values of 0.14, 0.25 and 0.66 under UV-S. Further separation by column chromatography over silica gel was performed using 20% ethyl acetate-dichloromethane and gradually enriched with dichloromethane and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 71**.

Table 71 Subfractions obtained from Subfraction B28e by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B28e1	20-80% EtOAc/CH_2Cl_2	2.5	Pale brown gum
B28e2	80% EtOAc/CH_2Cl_2-1% MeOH/EtOAc	5.4	Dark-brown gum
B28e3	2-10% MeOH/EtOAc	4.5	Dark-brown gum

Table 71 (continued)

Subfraction	Eluent	Weight (mg)	Physical appearance
B28e4	10% MeOH/EtOAc - 100% MeOH	5.8	Dark-brown gum

Subfraction B28e1 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated one pale spot with the R_f value of 0.38. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B28e2 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.19 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US24**.

Subfraction B28e3 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B28e4 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B28f Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-dichloromethane (3 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B29 Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.20 and one spot near the baseline under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar

components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 72**.

Table 72 Subfractions obtained from Subfraction B29 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B29a	5% MeOH/CH_2Cl_2	8.3	Brown gum
B29b	5% MeOH/CH_2Cl_2	7.5	Colorless
B29c	5-7% MeOH/CH_2Cl_2	6.1	Brown gum
B29d	8% MeOH/CH_2Cl_2-100% MeOH	16.5	Brown gum

Subfraction B29a Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B29b (US24) Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.19 under UV-S.

$[\alpha]_D^{28}$	-8.88 (c = 0.23, MeOH)
UV (MeOH) λ_{max} nm (log ε)	234 (4.06), 394 (3.49)
FT-IR (neat) ν_{cm-1}	3394 (OH stretching), 2928 (C-H stretching) 1640 (C=C stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)	6.09 (*d*, *J* = 11.1 Hz, 1H), 6.39 (*ddd*, *J* = 15.0, 1.5 Hz, 1H), 5.84 (*dq*, *J* = 15.0, 6.6 Hz, 1H), 4.34 (*d*, *J* = 11.7 Hz, 1H), 4.25 (*d*, *J* = 11.7 Hz, 1H), 4.02 (*d*, *J* = 6.3 Hz, 1H), 3.95 (*quintet*, *J* = 6.3 Hz, 1H), 1.81 (*dd*, *J* = 6.6, 1.5 Hz, 1H), 1.15 (*d*, *J* = 6.3 Hz, 1H)

^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		134.65, 133.34, 132.80, 126.07, 80.40, 70.11, 56.83, 18.78, 18.42
DEPT (135°) ($CDCl_3$)	CH :	133.34, 132.80, 126.07, 80.40, 70.11
	CH_2 :	56.83
	CH_3 :	18.78, 18.42

Subfraction B29c Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated one major spot with the R_f value of 0.19 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US24**.

Subfraction B29d Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B210 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B3 Chromatogram characteristics on normal phase TLC with 30% dichloromethane-petroleum ether as a mobile phase demonstrated four spots with the R_f values of 0.16, 0.18, 0.23 and 0.32 and two spots near the baseline under UV-S. Further separation by column chromatography over silica gel was performed using 20% dichloromethane-petroleum ether and gradually enriched with dichloromethane and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give seven subfractions, as shown in **Table 73**.

Table 73 Subfractions obtained from Subfraction B3 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B31	20-30% CH_2Cl_2/Petrol	9.6	Pale brown gum
B32	30-40% CH_2Cl_2/Petrol	13.1	Pale brown gum
B33	50% CH_2Cl_2/Petrol	20.2	Pale brown gum
B34	60-90% CH_2Cl_2/Petrol	6.2	Pale brown gum
B35	100% CH_2Cl_2-1% MeOH/CH_2Cl_2	1.7	Brown gum
B36	2-7% MeOH/CH_2Cl_2	24.1	Brown gum
B37	8% MeOH/CH_2Cl_2-100%MeOH	8.4	Brown gum

Subfraction B31 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B32 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B33 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated a long tail and one major spot near the baseline under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 74**.

Table 74 Subfractions obtained from Fraction B33 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B33a	1-1.5% MeOH/CH_2Cl_2	7.3	Dark-brown gum
B33b	2-70% MeOH/CH_2Cl_2	6.9	Dark-brown gum
B33c	80% MeOH/CH_2Cl_2-100% MeOH	2.7	Dark-brown gum

Subfraction B33a Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 2% methanol-dichloromethane as mobile phases demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B33b Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 2% methanol-dichloromethane as mobile phases demonstrated one major spot with the R_f value of 0.23 under UV-S. Further purification by precoated TLC with 2% methanol-dichloromethane (9 runs) as a mobile phase afforded a brown gum (1.2 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.23 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US24**.

Subfraction B33c Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 2% methanol-dichloromethane as mobile phases demonstrated one major spot with the R_f value of 0.23 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US24**.

Subfraction B34 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated a long tail and one major spot near the baseline under UV-S. Further purification by precoated TLC with 2% methanol-dichloromethane (7 runs) as a mobile phase afforded a brown gum (2.3 mg). Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated one spot with the

R_f value of 0.20 under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B35 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B36 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated a long tail and one major spot with the R_f value of 0.20 under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 75**.

Table 75 Subfractions obtained from Fraction B36 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B361	1-2% MeOH/CH_2Cl_2	6.2	Dark-brown gum
B362	3-50% MeOH/CH_2Cl_2	5.5	Dark-brown gum
B363	60% MeOH/CH_2Cl_2-100% MeOH	10.0	Dark-brown gum

Subfraction B361 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^1H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B362 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated many spots under UV-S. Because the ^1H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B363 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B37 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (6 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B4 Chromatogram characteristics on normal phase TLC with 1% dichloromethane-petroleum ether (6 runs) as a mobile phase demonstrated a long tail and two major spots with the R_f value of 0.20 and 0.32 under UV-S. Further separation by column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four subfractions, as shown in **Table 76**.

Table 76 Subfractions obtained from Subfraction B4 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B41	16.5	Brown gum
B42	15.0	Brown gum
B43	35.0	Brown gum mixed with brown solid
B44	31.5	Brown gum mixed with brown solid

Subfraction B41 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail and one major spot with the R_f value of 0.23 under UV-S. The ^{1}H NMR spectrum indicated that the major components might be a mixture of long chain hydrocarbons. No attempted investigation was carried out.

Subfraction B42 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B43 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B44 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Fraction C Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane as a mobile phase demonstrated four spots with the R_f values of 0.20, 0.36, 0.45 and 0.57 and two spots near the baseline under UV-S. Further separation by column chromatography over silica gel using a gradient system of methanol-dichloromethane was performed. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give seven subfractions, as shown in **Table 77**.

Table 77 Subfractions obtained from Fraction C by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
C1	1% MeOH/CH_2Cl_2	4.8	Pale brown gum
C2	1% MeOH/CH_2Cl_2	6.9	Pale brown gum
C3	1% MeOH/CH_2Cl_2	9.7	Pale brown gum
C4	1% MeOH/CH_2Cl_2	25.1	Brown gum mixed with brown solid
C5	1% MeOH/CH_2Cl_2	31.4	Brown gum mixed with brown solid

Table 77 (continued)

Subfraction	Eluent	Weight (mg)	Physical appearance
C6	2-40% MeOH/CH_2Cl_2	91.1	Brown gum mixed with brown solid
C7	50% MeOH/CH_2Cl_2-100% MeOH	29.5	Brown gum mixed with brown solid

Subfraction C1 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction C2 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated one major spot with the R_f value of 0.80 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US24**.

Subfraction C3 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction C4 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated four spots with the R_f values of 0.19, 0.23, 0.34 and 0.43 under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give three subfractions, as shown in **Table 78**.

Table 78 Subfractions obtained from Subfraction C4 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
C41	100% CH_2Cl_2	2.0	Brown gum
C42	1% MeOH/CH_2Cl_2	12.9	Brown gum
C43	2-100% MeOH/CH_2Cl_2	5.9	Brown gum

Subfraction C41 Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated one pale spot with the R_f value of 0.70 under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction C42 Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 1% methanol-dichloromethane (2 runs) as mobile phases demonstrated four spots with the R_f values of 0.36, 0.45, 0.54 and 0.70 under UV-S. Further purification by precoated TLC with 2% ethyl acetate-dichloromethane (8 runs) as a mobile phase afforded three bands.

Band 1 was obtained as a yellow gum (0.3 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 1% methanol-dichloromethane as mobile phases demonstrated one spot with the R_f value of 0.57 under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Band 2 was obtained as a yellow gum (3.0 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 1% methanol-dichloromethane as mobile phases demonstrated one spot with the R_f value of 0.27 under UV-S. The ^{1}H NMR spectrum suggested that it was **US19**.

Band 3 was obtained as a brown gum (6.0 mg). Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 1% methanol-dichloromethane as mobile phases demonstrated one major spot with the R_f value of 0.27 under UV-S. The ^{1}H NMR data indicated that it contained a mixture of **US17** and **US19**.

Subfraction C43 Chromatogram characteristics on normal phase TLC with 100% dichloromethane and 1% methanol-dichloromethane (2 runs) as mobile phases demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction C5 Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction C6 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give five subfractions, as shown in **Table 79**.

Table 79 Subfractions obtained from Subfraction C6 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
C61	1% MeOH/CH_2Cl_2	4.7	Brown gum
C62	1-10% MeOH/CH_2Cl_2	1.2	Colorless gum
C63	10-15% MeOH/CH_2Cl_2	7.3	Brown gum
C64	15-70% MeOH/CH_2Cl_2	15.0	Brown gum mixed with brown solid
C65	70% MeOH/CH_2Cl_2-100% MeOH	20.7	Brown gum mixed with brown solid

Subfraction C61 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction C62 (US25) Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (4 runs) as a mobile phase demonstrated one spot with the R_f value of 0.18 under UV-S.

UV (MeOH) λ_{max} nm (log ε)		257 (1.84)
FT-IR (neat) ν_{cm-1}		3373 (OH stretching), 2923 (C-H stretching) 1720 and 1689 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		7.47 (*d*, *J* = 11.5 Hz, 1H), 6.54 (*ddd*, *J* = 15.0, 11.5, 1.5 Hz, 1H), 6.32 (*dq*, *J* = 15.0, 6.9 Hz, 1H), 4.92 (*s*, 1H), 2.07 (*s*, 3H), 1.94 (*dd*, *J* = 6.9, 1.5 Hz, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		169.99, 168.63, 145.49, 142.44, 125.61, 120.95, 56.90, 19.89, 18.10
DEPT (135°) ($CDCl_3$)	CH :	145.49, 142.44, 125.61, 76.19
	CH_2 :	56.90
	CH_3 :	19.89, 18.10
EIMS *m/z* (% relative intensity):		184 (5), 141 (12), 125 (15), 124 (100), 123 (30), 95 (35), 81 (42)

Subfraction C63 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction C64 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction C65 Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction C7 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction D Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated a long tail under UV-S. Further separation on column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford two subfractions, as shown in **Table 80**.

Table 80 Subfractions obtained from Fraction D by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
D1	23.6	Brown gum mixed with brown solid
D2	37.6	Brown gum mixed with brown solid

Subfraction D1 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction D2 Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (4 runs) as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction E Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated no definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Fraction F Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane (2 runs) and 6% methanol-dichloromethane as mobile phases demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

4.2.3 Purification of the mycelial extract

The mycelial extract of the fungus Xylariaceae PSU-A80 (1.0 g) was chromatographed on Sephadex LH-20 column chromatography using 100% methanol to afford fifty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford four fractions, as shown in **Table 81**.

Table 81 Fractions obtained from the mycelial extract by column chromatography over Sephadex LH-20

Fraction	Weight (mg)	Physical appearance
A	100.2	Brown gum mixed with brown solid
B	750.0	Dark-brown gum
C	36.8	Dark-brown gum
D	11.5	Brown gum mixed with brown solid

Fraction A Chromatogram characteristics on normal phase TLC with 100% dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Fraction B Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane (2 runs) as a mobile phase demonstrated two spots with the R_f values of 0.14 and 0.36 and two spots near the baseline under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give nine subfractions, as shown in **Table 82**.

Table 82 Subfractions obtained from Fraction B by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B1	5% MeOH/CH_2Cl_2	15.0	Brown gum mixed with brown solid
B2	5% MeOH/CH_2Cl_2	13.4	Brown gum
B3	5% MeOH/CH_2Cl_2	11.5	Brown gum
B4	5% MeOH/CH_2Cl_2	150.0	Brown gum
B5	5% MeOH/CH_2Cl_2	65.8	Brown gum
B6	7% MeOH/CH_2Cl_2	18.1	Brown gum
B7	7% MeOH/CH_2Cl_2	25.5	Brown gum
B8	7% MeOH/CH_2Cl_2	134.7	Brown gum
B9	10% MeOH/CH_2Cl_2-100%MeOH	137.3	Brown gum mixed with brown solid

Subfraction B1 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated none of UV-active spots. The ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons. Thus, it was not further studied.

Subfraction B2 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B3 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.27 under UV-S and the other spot with the R_f value of 0.34 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by precoated TLC with 70% ethyl acetate-hexane (3 runs) as a mobile phase afforded two bands.

Band 1 was obtained as a yellow gum (1.1 mg). Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-hexane as a mobile phase demonstrated one spot with the R_f value of 0.29 under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Band 2 (US26) was obtained as a colorless gum (1.8 mg). Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-hexane displayed one spot with the R_f value of 0.20.

$[\alpha]_D^{25}$		+21.3 (c = 0.03, $CHCl_3$)
UV (MeOH) λ_{max} nm (log ε)		214 (3.99), 254 (3.64), 317 (3.34)
FT-IR (neat) ν_{cm-1}		3419 (OH stretching), 2934 (C-H stretching) 1663 (C=C stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		7.46 (*dd*, *J* = 9.0, 6.0 Hz, 1H), 7.11 (*d*, *J* = 6.0 Hz, 1H), 6.91 (*d*, *J* = 9.0 Hz, 1H), 4.83 (*dd*, *J* = 9.0, 6.0 Hz, 1H), 3.86 (*s*, 3H), 2.85 (*ddd*, *J* = 18.0, 9.0, 3.0 Hz, 1H), 2.52 (*ddd*, *J* = 18.0, 9.0, 3.0 Hz, 1H), 2.25 (*m*, 1H), 2.10 (*m*, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		197.00, 160.08, 120.10, 147.76, 134.69, 118.77, 111.97, 68.47, 56.14, 36.34, 31.33
DEPT (135°) ($CDCl_3$)	CH :	134.69, 118.77, 111.97, 68.47
	CH_2 :	36.34, 31.33
	CH_3 :	56.14
EIMS *m/z* (% relative intensity):		192 (100), 164 (61), 135 (31), 136 (23)

Subfraction B4 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one major spot with the R_f value of 0.30 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel was performed using 80% ethyl acetate-hexane and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 83**.

Table 83 Subfractions obtained from Subfraction B4 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B41	80% EtOAc/hexane	7.3	Brown gum
B42	100% EtOAc	16.7	Brown gum
B43	1-7% MeOH/EtOAc	74.5	Brown gum
B44	7% MeOH/EtOAc - 100%MeOH	15.0	Brown gum mixed with brown solid

Subfraction B41 Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-hexane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B42 Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-hexane as a mobile phase demonstrated one spot with the R_f value of 0.18 under UV-S and the other spot with the R_f value of 0.25 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further purification by column chromatography over reverse phase C_{18} silica gel was performed using 30% methanol-water and gradually enriched with methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give four subfractions, as shown in **Table 84**.

Table 84 Subfractions obtained from Subfraction B42 by column chromatography over reverse phase C_{18} silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B42a	30-50% MeOH/H_2O	10.9	Brown gum
B42b	60% MeOH/H_2O	1.3	Brown gum
B42c	70% MeOH/H_2O	0.5	Brown gum
B42d	70% MeOH/H_2O-100%MeOH	0.7	Brown gum mixed with brown solid

Subfraction B42a Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated two spots with the R_f values of 0.32 and 0.45 as violet spots after dipping in ASA reagent and subsequently heating the TLC plate. Further separation on column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 85**.

Table 85 Subfractions obtained from Subfraction B42a by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B42a1	1.1	Colorless gum
B42a2	2.4	Colorless gum
B42a3	3.5	Colorless gum
B42a4	3.5	Colorless gum
B42a5	2.4	Colorless gum

Subfraction B42a1 Chromatogram characteristics on normal phase TLC with 65% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B42a2 Chromatogram characteristics on normal phase TLC with 65% ethyl acetate-dichloromethane as a mobile phase demonstrated one major spot with the R_f value of 0.25 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further separation on column chromatography over Sephadex LH-20 using 50% methanol-dichloromethane was performed. All subfractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness under reduced pressure to afford two subfractions, as shown in **Table 86**.

Table 86 Subfractions obtained from Subfraction B42a2 by column chromatography over Sephadex LH-20

Subfraction	Weight (mg)	Physical appearance
B42a21	1.2	Colorless gum
B42a22	1.1	Colorless gum

Subfraction B42a21 Chromatogram characteristics on normal phase TLC with 60% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B42a22 Chromatogram characteristics on normal phase TLC with 60% ethyl acetate-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the presence of many compounds, it was not further investigated.

Subfraction B42a3 Chromatogram characteristics on normal phase TLC with 65% ethyl acetate-dichloromethane as a mobile phase demonstrated one

spot with the R_f value of 0.25 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B42a4 Chromatogram characteristics on normal phase TLC with 65% ethyl acetate-dichloromethane as a mobile phase demonstrated one spot with the R_f value of 0.25 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major compound was **US27**.

Subfraction B42a5 Chromatogram characteristics on normal phase TLC with 65% ethyl acetate-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B42b Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated one spot with the R_f value of 0.27 under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B42c Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated one pale spot with the R_f value of 0.43 under UV-S. The ^{1}H NMR spectrum indicated that the major compound was **US27**.

Subfraction B42d Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-dichloromethane (2 runs) as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B43 Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-hexane as a mobile phase demonstrated one spot with the R_f value of 0.20 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major compound was **US27**.

Subfraction B44 Chromatogram characteristics on normal phase TLC with 70% ethyl acetate-hexane as a mobile phase demonstrated a long tail under

UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B5 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated two spots with the R_f values of 0.14 and 0.20 and the other spot with the R_f value of 0.28 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. Further separation by column chromatography over silica gel was performed using 80% ethyl acetate-hexane and gradually enriched with ethyl acetate and then methanol until pure methanol. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give seven subfractions, as shown in **Table 87**.

Table 87 Subfractions obtained from Subfraction B5 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B51	80% EtOAc/hexane	1.7	Brown gum
B52	80% EtOAc/hexane	3.1	Colorless gum
B53	90% EtOAc/hexane	8.2	Brown gum
B54	90% EtOAc/hexane	10.6	Brown gum
B55	90% EtOAc/hexane	19.3	Colorless gum
B56	90% EtOAc/hexane-1% MeOH/EtOAc	74.5	Brown gum
B57	2% MeOH/EtOAc-100% MeOH	15.0	Brown gum mixed with brown solid

Subfraction B51 Chromatogram characteristics on normal phase TLC with 80% ethyl acetate-hexane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B52 Chromatogram characteristics on normal phase TLC with 80% ethyl acetate-hexane as a mobile phase demonstrated one spot with the

R_f value of 0.50 under UV-S. The ^{1}H NMR data indicated that it contained **US4** (see Chapter 2).

Subfraction B53 Chromatogram characteristics on normal phase TLC with 80% ethyl acetate-hexane as a mobile phase demonstrated one pale spot with the R_f value of 0.43 under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B54 Chromatogram characteristics on normal phase TLC with 80% ethyl acetate-hexane as a mobile phase demonstrated one spot with the R_f value of 0.32 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major compound was **US4**.

Subfraction B55 (US27) Chromatogram characteristics on normal phase TLC with 80% ethyl acetate-hexane as a mobile phase demonstrated one spot with the R_f value of 0.32 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate.

$[\alpha]_D^{27}$		+0.75 (c = 0.95, $CHCl_3$)
FT-IR (neat) ν_{cm-1}		3394 (OH stretching), 2930 (C-H stretching) 1734 (C=O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300 MHz)		4.86 (*dqd*, *J* = 11.1, 6.3, 3.3 Hz, 1H), 4.37 (*dddd*, *J* = 4.8, 3.6, 3.6, 3.3 Hz, 1H), 2.75 (*dd*, *J* = 17.7, 4.8 Hz, 1H), 2.61 (*ddd*, *J* = 17.7, 3.6, 1.5 Hz, 1H), 2.00 (*dm*, *J* = 14.4 Hz, 1H), 1.71 (*ddd*, *J* = 14.4, 11.1, 3.3 Hz, 1H), 1.39 (*d*, *J* = 6.3 Hz, 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)		171.15, 72.56, 62.54, 38.32, 37.51, 21.35
DEPT (135°) ($CDCl_3$)	CH :	72.56, 62.54
	CH_2 :	38.32, 37.51
	CH_3 :	21.35

Subfraction B56 Chromatogram characteristics on normal phase TLC with 80% ethyl acetate-hexane as a mobile phase demonstrated one spot with the R_f value of 0.32 as a violet spot after dipping in ASA reagent and subsequently heating the TLC plate. The ^{1}H NMR spectrum indicated that the major compound was **US27**.

Subfraction B57 Chromatogram characteristics on normal phase TLC with 80% ethyl acetate-hexane as a mobile phase demonstrated none of definite spots under UV-S. Because the ^{1}H NMR spectrum indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B6 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated one major spot with the R_f value of 0.16 under UV-S. The ^{1}H NMR data indicated that the major compound was **US24**.

Subfraction B7 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated many spots near the baseline under UV-S. The ^{1}H NMR spectral data indicated that it contained a mixture of **US24** and **US27**.

Subfraction B8 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated many spots near the baseline under UV-S. Further separation by column chromatography over silica gel was performed using a gradient system of methanol-dichloromethane. Fractions, which contained the similar components, were combined and evaporated to dryness under reduced pressure to give two subfractions, as shown in **Table 88**.

Table 88 Subfractions obtained from Fraction B8 by column chromatography over silica gel

Subfraction	Eluent	Weight (mg)	Physical appearance
B81	100% CH_2Cl_2-10% MeOH/CH_2Cl_2	40.0	Brown gum
B82	10% MeOH/CH_2Cl_2-100% MeOH	47.5	Brown gum mixed with brown solid

Subfraction B81 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated none of UV-active spots. Because the ^{1}H NMR data indicated the absence of olefinic and aromatic protons, it was not further studied.

Subfraction B82 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Subfraction B9 Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction C Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-dichloromethane and 50 % ethyl acetate-dichloromethane (2 runs) as mobile phases demonstrated a long tail under UV-S. Because the ^{1}H NMR spectrum showed signals at high field, it was not further investigated.

Fraction D Chromatogram characteristics on normal phase TLC with 4% methanol-dichloromethane as a mobile phase demonstrated none of definite spots under UV-S. Because of the minute quantity, it was not further investigated.

CHAPTER 4.3

RESULTS AND DISCUSSION

Two new compounds (**US20** and **US25**) were isolated from the broth extract while one new compound (**US26**) was obtained from the mycelial extract. In addition, investigation of the broth and mycelial extracts led to the isolation of ten known compounds (**US3-4**, **US17-19**, **US21-24** and **US27**). The structures were identified by analysis of spectroscopic data and comparison of these data with those previously reported.

4.3.1 Compound US17

Compound **US17** was obtained as a colorless gum. It exhibited an UV absorption band at 279 nm (**Figure 141**) while a hydroxyl absorption band was found at 3419 cm^{-1} in the IR spectrum (**Figure 142**). The ^{1}H NMR spectrum (**Figure 143**) (**Table 89**) displayed characteristic signals for one hydroxy proton [δ_H 9.32 (*s*)], six aromatic protons for two sets of 1,2,3-trisubstituted benzenes [7.41 (*d*, *J* = 7.8 Hz, 1H), 7.35 (*t*, *J* = 8.1 Hz, 1H), 7.30 (*t*, *J* = 7.8 Hz, 1H), 7.29 (*dd*, *J* = 8.1 and 1.8 Hz, 1H), 6.87 (*dd*, *J* = 8.1 and 1.8 Hz, 1H) and 6.76 (*d*, *J* = 7.8 Hz, 1H)] and one methoxyl group [δ_H 4.05 (*s*, 3H)]. The ^{13}C NMR spectrum (**Figure 145**) (**Table 89**) contained 10 aromatic carbon resonances of a naphthalene skeleton and one methoxy carbon at δ_C 56.10. The hydroxy proton at δ_H 9.32 was attributed to 1-OH on the basis of HMBC correlations with C-1 (δ_C 154.50), C-2 (δ_C 110.42) and C-8a (δ_C 115.00) (**Figure 147**) (**Table 90**). The aromatic proton resonating at δ_H 6.87 was assigned as H-2 because of its HMQC cross peak with C-2 (**Figure 146**) (**Table 89**). According to the ^{1}H-^{1}H coupling in the COSY spectrum (**Figure 148**) (**Table 90**), multiplicities and coupling constants, H-3 and H-4 resonated at δ_H 7.35 and 7.29, respectively. Signal enhancement of both 1-OH and H-7 upon irradiation of Me-9 in the NOEDIFF experiment (**Figure 149**) (**Table 90**) established the attachment of the methoxyl group

at C-9 and the chemical shift of H-7 at δ_H 6.76. Consequently, two remaining aromatic protons, resonating at δ_H 7.30 and 7.41, were attributed to H-6 and H-5, respectively. Therefore, **US17** was identified as 8-methoxy-1-naphthol (Thines, *et al.*, 1995).

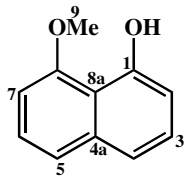


Table 89 The NMR data of compound US17 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	-	154.50 (C)
1-OH	9.32 (*s*)	-
2	6.87 (*dd*, 8.1, 1.8)	110.42 (CH)
3	7.35 (*t*, 8.1)	127.72 (CH)
4	7.29 (*dd*, 8.1, 1.8)	118.87 (CH)
4a	-	136.76 (C)
5	7.41 (*d*, 7.8)	121.87 (CH)
6	7.30 (*t*, 7.8)	125.60 (CH)
7	6.76 (*d*, 7.8)	103.91 (CH)
8	-	156.17 (C)
8a	-	115.00 (C)
9	4.05 (*s*)	56.10 (CH_3)

Table 90 The HMBC, COSY and NOE data of compound US17 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
1-OH	C-1, C-2, C-8a	-	Me-9
H-2	C-1, C-4, C-8a	H-3	1-OH
H-3	C-1, C-4a	H-2, H-4	-
H-4	C-2, C-4a, C-5	H-3	-
H-5	C-4, C-4a, C-7, C-8a	H-6	-
H-6	C-4a, C-5, C-7, C-8	H-5, H-7	-

Table 90 (continued)

Proton	HMBC correlations	COSY	NOE
1-OH	C-1, C-2, C-8a	-	Me-9
H-7	C-5, C-8, C-8a	H-6, H-9	H-6, Me-9
H-9	C-8	-	1-OH, H-7

4.3.2 Compound US18

Compound **US18** was obtained as a colorless gum. The UV (**Figure 150**) and IR (**Figure 151**) spectra were almost identical to those of **US3**. The ^{1}H NMR spectral data (**Figure 152**) (**Table 91**) were similar to those of **US3** except for the replacement of two-*meta*-coupled aromatic protons in **US3** with two-*ortho*-coupled aromatic protons (δ_H 7.29 and 6.82). The aromatic proton resonating at δ_H 6.82 was attributed to H-7 according to its HMBC correlations with C-1 (δ_C 170.33), C-5 (δ_C 108.11), C-8 (δ_C 160.54) and C-8a (δ_C 108.11) (**Figure 157**) (**Table 92**). The remaining aromatic proton was then assigned as H-6. In addition, the ^{1}H NMR spectrum displayed one singlet of a methyl group at δ_H 2.20. HMBC correlations of the methyl protons with C-4a (δ_C 137.92), C-5 (δ_C 108.11) and C-6 (δ_C 137.92) indicated the attachment of the methyl group at C-5. Comparison of the NMR data and specific rotation of **US18**: $[\alpha]_D$ –79.2 (c = 0.25, $CHCl_3$), with those of

methylmellein: $[\alpha]_D$ -118 (c = 0.60, $CHCl_3$), indicated that **US18** was (-)-5-methylmellein which was previously isolated from *Euphorbia fidjiana* (Cambie, *et al*., 1991).

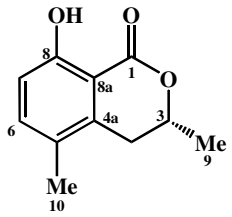


Table 91 The NMR data of compound US18 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	-	170.33 (C=O)
3	4.69 (*m*)	75.41 (CH)
4	a: 2.95 (*dd*, 16.5, 3.3)	31.94 (CH_2)
	b: 2.72 (*dd*, 16.5, 11.4)	
4a	-	137.92 (C)
5	-	108.11 (C)
6	7.29 (*d*, 8.4)	137.92 (CH)
7	6.82 (*d*, 8.4)	115.72 (CH)
8	-	160.54 (C)
8-OH	11.01 (*s*)	-
8a	-	108.11 (C)
9	1.50 (*d*, 6.5)	20.92 (CH_3)
10	2.20 (*s*)	18.07 (CH_3)

Table 92 The HMBC, COSY and NOE data of compound US18 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-1	-	-	-
H-3	C-4a, C-9	H_{ab}-4, H-9	H_{ab}-4, Me-9
H_a-4	C-3, C-4a, C-5, C-8, C-8a, C-9	H-3, H_b-4	H_b-4, Me-9, Me-10
H_b-4	C-3, C-4a, C-5, C-8, C-8a, C-9	H-3, H_a-4	H_a-4, Me-9, Me-10
H-6	C-4a, C-8, C-10	H-7	Me-10
H-7	C-1, C-5, C-8, C-8a	H-6	8-OH
8-OH	C-4a, C-7, C-8	-	-
H-9	C-1, C-3, C-4,	H-3	H-3, H_{ab}-4
H-10	C-4a, C-5, C-6	-	-

4.3.3 Compound US19

Compound **US19** was obtained as a colorless gum. It exhibited UV absorption bands at 258 and 319 nm (**Figure 159**) while hydroxyl and carbonyl absorption bands were found at 3387 and 1673 cm^{-1}, respectively, in the IR spectrum (**Figure 160**). The ^1H NMR spectrum (**Figure 161**) (**Table 93**) displayed characteristic signals for one chelated hydroxy proton at δ_H 12.43 (*s*), a 1,2,3-trisubstituted benzene [δ_H 7.50 (*t*, *J* = 8.0 Hz, 1H), 7.03 (*dt*, *J* = 8.0 and 0.5 Hz, 1H) and 6.93 (*dd*, *J* = 8.0 and 0.5 Hz, 1H)], one oxymethine proton [δ_H 4.93 (*dd*, *J* = 6.5 and 3.0 Hz, 1H)] and two sets of nonequivalent methylene protons [δ_H 3.01 (*ddd*, *J* = 18.0, 8.0 and 4.5 Hz, 1H), 2.66 (*ddd*, *J* = 18.0, 8.5 and 5.0 Hz, 1H) and δ_H 2.35 (*m*, 1H) and 2.22 (*m*, 1H)]. The ^{13}C NMR spectrum (**Figure 164**) (**Table 93**) showed one ketone carbonyl carbon (δ_C 204.10), three quaternary carbons (δ_C 160.34, 145.85 and 115.20), four methine cabons (δ_C 136.94, 117.83, 117.32 and 67.76) and two methylene carbons (δ_C 34.55 and 31.24). These data constructed a tetralone skeleton. The chelated hydroxyl group (δ_H 12.43) was placed at C-8 (δ_C 160.54), *peri* position to the ketone carbonyl group. This hydroxy proton gave HMBC correlations (**Figure 166**) (**Table 94**) with C-8 (δ_C 160.34), C-8a (δ_C 115.20) and C-7 (δ_C 117.83). The

aromatic proton at δ_H 6.93 was attributed to H-7 according to its HMQC cross peak with C-7. Thus, the remaining aromatic protons resonating at δ_H 7.50 and 7.03 were assigned as H-6 and H-5, respectively, on the basis of the multiplicity, coupling constant and HMBC correlations. In the ^{1}H-^{1}H COSY spectrum (**Figure 167**) (**Table 94**), the nonequivalent methylene protons (δ_H 2.35 and 2.22) gave cross peak with other nonequivalent methylene protons (δ_H 3.01 and 2.66) and the hydroxymethine proton (δ_H 4.93), thus constructing a -$CH_2CH_2CH(OH)$- unit. ^3J HMBC cross peaks of the inner methylene protons/C-1 (δ_C 204.10) and C-4a (δ_C 145.85) revealed that the methylene group was attached at C-3 and the hydroxymethine proton became H-4 of the tetralone unit. Therefore, **US19** was identified as isosclerone which was previously isolated from *Cytospora eucalypticola* (Kokubun, *et al*., 2003).

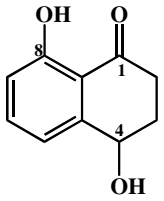


Table 93 The NMR data of compound US19 in $CDCl_3$

Table 94 The HMBC, COSY and NOE data of compound US19 in $CDCl_3$

Proton	HMBC correlations		COSY		NOE	
H_a-2	C-1, C-3, C-4		H_b-2, H_{ab}-3		H_b-2	
H_b-2	C-1, C-3, C-4	US19	H_a-2, H_{ab}-3		H_a-2	
H_a-3	C-1, C-2, C-4, C-4a	δ_H (*mult.*, J_{Hz})	δ_C (C-type)	δ_H (*mult.*, J_{Hz})	H_b-2, H_b-3, H-4	δ_C (C-type)
H_b-3	C-1, C-2, C-4, C-4a		204.10 (C=O)		H_a-2, H_{ab}-3, H-4	206.3 (C=O)
H-4	a: 3.01 (*ddd*, 18.0,		34.55 (CH_2)	a: 2.90 (*ddd*, 17.8,	H_a-2, H_{ab}-3,	36.1 (CH_2)
H-5	C-4, C-7, C-8a	8.0, 4.5)	H-6	7.6, 4.7)	H-5	
	b: 2.66 (*ddd*, 18.0,			b: 2.66 (*ddd*, 17.8,		-
	8.5, 5.0)			9.1, 4.8)		
3	a: 2.35 (*m*)		31.24 (CH_2)	a: 2.30 (*m*)		32.6 (CH_2)
	b: 2.22 (*m*)			b: 2.10 (*m*)		
4	4.93 (*dd*, 6.5, 3.0)		67.76 (CH)	4.84 (*dd*, 8.0, 3.8)		68.3 (CH)
4a	-		145.85 (C)	-		148.6 (C)
5	7.03 (*dt*, 8.0, 0.5)		117.32 (CH)	7.07 (*dm*, 7.6)		118.8 (CH)
6	7.50 *(t*, 8.0)		136.94 (CH)	7.50 (*dd*, 8.3, 7.6)		137.9 (CH)
7	6.93 (*dd*, 8.0, 0.5)		117.83 (CH)	6.84 (*dm*, 8.3)		117.6 (CH)
8	-		160.34 (C)	-		163.7 (C)
8-OH	12.43 (*s*)		-	-		-
8a	-		115.20 (C)	-		116.5 (C)

Table 94 (continued)

Proton	HMBC correlations	COSY	NOE
H-6	C-4a, C-8	H-5, H-7	H-6
H-7	C-5, C-8, 8a	H-6	H-5, H-7
8-OH	C-7, C-8, C-8a	-	H-6

4.3.4 Compound US20

Compound **US20** with the molecular formula $C_{20}H_{16}O_5$ from EIMS (*m/z* 336) (**Figure 178**) was isolated as a pale yellow gum. It exhibited UV absorption bands at 223, 251 265, 289 and 389 nm (**Figure 168**) while hydroxyl and carbonyl absorption bands were found at 3774 and 1670 cm^{-1}, respectively, in the IR spectrum (**Figure 169**). The ^1H NMR spectrum (**Figure 170**) (**Table 95**) contained signals of one hydrogen bonded hydroxyl group (δ_H 12.60, *s*, 1H), one phenolic hydroxyl group (δ_H 8.22, *s*), a 1,2,3-trisubstituted benzene [δ_H 7.53 (*dd*, *J* = 8.5 and 7.5 Hz, 1H), 7.36 (*dd*, *J* = 7.5 and 0.5 Hz, 1H) and 6.92 (*dd*, *J* = 8.5 and 0.5 Hz, 1H)], two *ortho*-coupled aromatic protons of a 1,2,3,4-tetrasubstituted benzene [δ_H 7.24 (*d*, *J* = 8.0 Hz, 1H) and 6.77 (*d*, *J* = 8.0 Hz, 1H)], two oxymethine protons [δ_H 5.67 (*t*, *J* = 4.5 Hz, 1H) and 5.28 (*dd*, *J* = 10.0 and 4.5 Hz, 1H)], one methine proton (δ_H 4.05 (*dd*, *J* = 14.5 and 5.5 Hz, 1H) and two sets of nonequivalent methylene protons [δ_H 3.39 (*dd*, *J* = 16.5 and 5.5 Hz, 1H), 2.39 (*dd*, *J* = 16.5 and 14.5 Hz, 1H)] and [δ_H 2.80 (*dt*, *J* = 12.5 and 4.5 Hz, 1H) and 2.02 (*m*, 1H)]. The presence of -CH(OH)CH_2CH(OH)- unit was realized due to the ^1H-^1H COSY correlations of H_{ab}-2 (δ_H 2.80 and 2.02) with H-1 (δ_H 5.67) and H-3 (δ_H 5.28) (**Figure 176**) (**Table 96**). In addition, this spectrum displayed ^1H-^1H cross peaks between H-6b (δ_H 4.05) and H_{ab}-7 (δ_H 3.39 and 2.39), thus constructing a -$CHCH_2$- unit. The chelated hydroxyl group was placed at C-9 (δ_C 163.45), *peri* position to a carbonyl group. This hydroxy proton gave ^3J HMBC correlations (**Figure 175**) (**Table 96**) with C-8a (δ_C 114.62) and C-10 (δ_C 117.69). The methine carbon (C-10) exhibited a HMQC cross peak (**Figure 174**) (**Table 95**) with the aromatic proton of the trisubstituted benzene resonating at δ_H 6.92. Consequently, two remaining aromatic protons resonating at δ_H 7.53 and 7.36 were attributed to H-11 and H-12, respectively. H-12 gave ^3J HMBC correlations with C-12b (δ_C 137.54) while H-6b of the -$CHCH_2$- unit showed HMBC cross peaks with C-12b and C-12c (δ_C 135.87). These results established a naphthalenone skeleton having an exocyclic double bond at C-12b. Nonequivalent methylene protons, H_{ab}-7, displayed HMBC cross peaks with C-6a (δ_C 112.00) of the 1,2,3,4-tetrasubstituted benzene and C-12b, thus connecting the tetrasubstituted benzene with C-6b of the naphthalenone. Signal enhancement of one of the *ortho*-aromatic protons (δ_H 7.24) upon irradiation of H_a-7 (**Figure 177**) (**Table 96**) established the attachment of this proton at C-6 (δ_C 123.15).

The remaining aromatic proton (δ_H 6.77) was then assigned as H-5. This assignment was supported by a HMBC correlation of H-5/C-6a. The attachment of a hydroxyl group at C-4 (δ_C 155.10) was established based on HMBC correlations between the *ortho*-aromatic protons and C-4 together with chemical shift of C-4. HMBC cross peaks of 4-OH (δ_C 8.22) of the tetrasubstituted benzene and H-3 (δ_H 5.28) of the -CH(OH)CH_2CH(OH)- unit with the same carbon, C-3a (δ_C 117.25), joined the dihydroxypropyl unit at C-3a of the tetrasubstituted benzene. According to the molecular formula $C_{20}H_{16}O_5$, **US20** possessed 13 degrees of unsaturation, thus joining C-12c with C-12d (δ_C 142.36) of the tetrasubstituted benzene and C-1 (δ_C 62.47) of the -CH(OH)CH_2CH(OH)- unit to from a pentacyclic skeketon. Irradiation of H-3 affected the intensity of H_a-2 and 4-OH signals, but did not enhance the H-1 signal (**Figure 177**) (**Table 96**), indicating *trans* relationship between H-1 and H-3. Since H-1 was coupled with H_{ab}-2 with a small coupling constant of 4.5 Hz, it was located at β-equatorial position. Both H-3 and H-6b were placed at α-axial and axial positions, respectively, as they were coupled with vicinal methylene protons with small and large coupling constants. Consequently, **US20** was identified as a new pentacyclic compound.

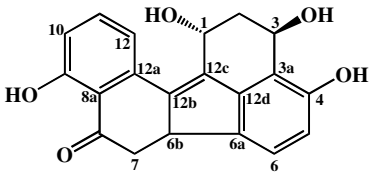


Table 95 The NMR data of compound US20 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	5.67 (*t*, 4.5)	62.47 (CH)
2	a: 2.80 (*dt*, 12.5, 4.5)	36.25 (CH_2)
	b: 2.02 (*m*)	
3	5.28 (*dd*, 10.0, 4.5)	72.96 (CH)
3a	-	117.25 (C)
4	-	155.10 (C)
4-OH	8.22 (*s*)	-
5	6.77 (*d*, 8.0)	114.62 (CH)
6	7.24 (*d*, 8.0)	123.15 (CH)
6a	-	135.40 (C)
6b	4.05 (*dd*, 14.5, 5.5)	48.91 (CH)
7	a: 3.39 (*dd*, 16.5, 5.5)	42.80 (CH_2)
	b: 2.39 (*dd*, 16.5, 14.5)	
8	-	204.21 (C=O)
8a	-	114.62 (C)
9	-	163.45 (C)
9-OH	12.60 (*s*)	-
10	6.92 (*dd*, 8.5, 0.5)	117.69 (CH)
11	7.53 (*dd*, 8.5, 7.5)	137.02 (CH)
12	7.36 (*dd*, 7.5, 0.5)	116.33 (CH)
12a	-	135.40 (C)
12b	-	137.54 (C)
12c	-	135.87 (C)
12d	-	142.36 (C)

Table 96 The HMBC, COSY and NOE data of compound US20 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-1	C-12d	H_{ab}-2	H_{ab}-2, H-12
H_a-2	C-3	H-1, H_b-2, H-3	H-1, H_b-2, H-3
H_b-2	C-3	H-1, H_a-2, H-3	H-1, H_a-2
H-3	C-3a	H_{ab}-2	H_a-2, 4-OH
4-OH	C-3a, C-4, C-5	-	H-3
H-5	C-4, C-6a	H-6	H-6
H-6	C-4, C-12d	H-5	H-5, H_a-7
H-6b	C-6a, C-7, C-12b, C-12c	H_{ab}-7	H_a-7
H_a-7	C-6a, C-6b, C-12b	H-6b, H_b-7	H-6, H-6b, H_b-7
H_b-7	C-6a, C-6b, C-12b	H-6b, H_a-7	H_a-7
9-OH	C-8a, C-9, C-10	-	-
H-10	C-8a, C-9, C-12	H-11	9-OH, H-11
H-11	C-9, C-12	H-10, H-12	H-10, H-12
H-12	C-8a, C-10, C-12b	H-11	H-1, H-11

4.3.4 Compound US21

Compound **US21** with the molecular formula $C_{21}H_{18}O_5$ from EIMS (*m/z* 350) (**Figure 188**) was isolated as a pale yellow gum. The UV (**Figure 179**) and IR (**Figure 180**) spectra were almost identical to those of **US20,** thus indicating that they possessed the same chromophore and functional groups. The ^{1}H NMR spectral data (**Figure 181**) (**Table 97**) were similar to those of **US20** except that one of the hydroxyl group in **US20** was replaced, in **US21**, by a methoxyl group (δ_H 3.64, *s*). A HMBC correlation of the methoxy protons with C-3 (δ_C 74.37) (**Figure 186**) (**Table 98**) established the location of the methoxyl group at C-3. In addition, the remaining HMBC correlations, NOE and ^{1}H-^{1}H COSY data (**Figure 187**) (**Table 98**) were almost identical to those of **US20.** Therefore, compound **US21** was identified as a methyl ether of **US20** (hypoxylonol B) which was previously isolation from *Hypoxylon truncatum* (Koyama, *et al.,* 2002).

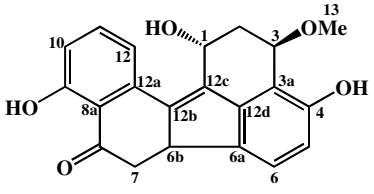


Table 97 The NMR data of compound US21 in $CDCl_3$

Position	US21		hypoxylonol B	
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	5.67 (*t*, 3.0)	62.44 (CH)	5.51 (*dddd*, 8.8, 6.4, 4.6,2.3)	63.6 (CH)
2	a: 2.80 (*dt*, 13.0, 4.0)	35.60 (CH_2)	a: 2.41 (*ddd*, 13.1, 6.0, 4.6)	39.80 (CH_2)
	b: 2.02 (*ddd*, 13.0, 10.5, 3.0)		b: 2.15 (*ddd*, 13.1, 8.8, 3.4)	
3	5.19 (*dd*, 10.0, 4.0)	74.37 (CH)	4.98 (*dd*, 6.0, 3.4)	73.10 (CH)
3a	-	116.40 (C)	-	119.10 (C)
4	-	155.03 (C)	-	155.20 (C)
4-OH	8.00 (*s*)	-	8.38 (*s*)	-
5	6.78 (*d*, 8.0)	114.61 (CH)	6.75 (*d*, 8.0)	114.30 (CH)
6	7.24 (*d*, 8.0)	123.26 (CH)	7.32 (*dd*, 8.0, 0.7)	124.10 (CH)
6a	-	135.37 (C)	-	137.00 (C)
6b	4.07 (*dd*, 14.5, 6.0)	48.94 (CH)	4.08 (*dddd*, 13.9, 5.5, 2.3, 0.7)	50.00 (CH)
7	a: 3.40 (*dd*, 16.5, 5.5)	42.76 (CH_2)	a: 3.39 (*dd*, 16.4, 5.5)	43.80 (CH_2)
	b: 2.39 (*dd*, 16.5, 14.0)		b: 2.29 (*dd*, 16.4, 13.9)	
8	-	204.27 (C=O)	-	206.10 (C=O)
8a	-	114.61 (C)	-	115.80 (C)
9	-	163.45 (C)	-	163.50 (C)
9-OH	12.60 (*s*)	-	12.59 (*s*)	-

Table 97 (continued)

Position	US21		hypoxylonol B	
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
10	6.92 (*dd*, 8.0, 1.0)	117.72 (CH)	6.83 (*dd*, 7.7, 1.7)	117.00 (CH)
11	7.53 (*t*, 8.0)	137.02 (CH)	7.54 (*t*, 7.7)	137.10 (CH)
12	7.37 (*dd*, 8.0, 1.0)	116.40 (CH)	7.51 (*dd*, 7.7, 1.7)	120.80 (CH)
12a	-	135.37 (C)	-	139.40 (C)
12b	-	137.51 (C)	-	138.00 (C)
12c	-	135.87 (C)	-	138.90 (C)
12d	-	142.40 (C)	-	145.30 (C)
13	3.64 (*s*)	56.03 (CH_3)	3.46 (*s*)	56.60 (CH_3)

Table 98 The HMBC, COSY and NOE data of compound US21 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-1	C-12d	H_{ab}-2	H_{ab}-2, H-12
H_a-2	C-1, C-3	H-1, H_b-2, H-3	H-1, H_b-2, H-3, Me-13
H_b-2	C-1, C-3	H-1, H_a-2, H-3	H-1, H_a-2
H-3	C-3a	H_{ab}-2	H_a-2, 4-OH, Me-13
4-OH	C-3a, C-4, C-5	-	H-3
H-5	C-4, C-6a	H-6	H-6
H-6	C-4, C-12d	H-5	H-5, H_a-7
H-6b	C-6a, C-7, C-8, C-12b, C-12c	H_{ab}-7	H_a-7
H_a-7	C-6b, C-8	H-6b, H_b-7	H-6, H-6b, H_b-7
H_b-7	C-6b, C-8	H-6b, H_a-7	H_a-7
9-OH	C-8a , C-9, C-10	-	-
H-10	C-8a, C-9, C-12	H-11	9-OH, H-11
H-11	C-9, C-12	H-10, H-12	H-10, H-12
H-12	C-8a, C-10, C-12b	H-11	H-1, H-11
H-13	C-3	-	H_a-2, H-3, 4-OH

4.3.6 Compound US22

Compound **US22** with the molecular formula $C_{15}H_{16}N_2O_2$ from EIMS (*m/z* 256) (**Figure 199**) was isolated as a pale yellow gum. It exhibited UV absorption bands at 220 and 293 nm (**Figure 189**) while hydroxyl and carbonyl absorption bands were found at 3419 and 1683 cm^{-1}, respectively, in the IR spectrum (**Figure 190**). The ^1H NMR spectrum (**Figure 191**) (**Table 99**) displayed characteristic signals for a monosubstituted benzene (δ_H 7.33, *m*, 5H), one olefinic proton (δ_H 7.15, *s*, 1H) of an α,β-unsaturated carbonyl unit, one methine proton (δ_H 4.25, *dd*, *J* = 9.3 and 6.9 Hz), two sets of nonequivalent methylene protons [(δ_H 2.42, *m*, 1H and 2.22, *m*, 1H) and (δ_H 2.06, *m*, 1H and 1.98, *m*, 1H)], one set of equivalent methylene protons (δ_H 3.65, *m*, 2H) and one methyl group (δ_H 2.90, *s*, 3H). The ^{13}C NMR spectrum (**Figure 197**) (**Table 99**) showed two amide carbonyl carbons (δ_C 167.98 and 161.42), two quaternary carbons (δ_C 133.62 and 133.58), three carbons for five aromatic methine carbons (δ_C 129.52, 128.57 and 128.44), one olefinic methine carbon (δ_C 121.19), one methine carbon (δ_C 58.85), three methylene carbons (δ_C 45.29, 28.01 and 22.84) and one methyl carbon (δ_C 34.72). In the ^1H-^1H COSY spectrum (**Figure 197**) (**Table 100**), the methylene protons (H_{ab}-8, δ_H 2.06 and 1.98) were coupled with two sets of methylene protons, (H_2-9, δ_H 3.65) and (H_{ab}-7, δ_H 2.42 and 2.22). H_{ab}-7 were further coupled with the methine proton (H-6, δ_H 4.25). These results indicated the presence of -$CH_2CH_2CH_2CH$- unit. HMBC cross peaks of H-6/C-1 (δ_C 167.98), H_2-9/C-4 (δ_C 161.42) and C-6 (δ_C 58.85) (**Figure 196**) (**Table 100**) established a pyrrolidine skeleton having two amide carbonyl functionalities attached at N-5 and C-6. A pyrrolo[1,2a]pyrazine-1,4-dione moiety with an exocyclic trisubstituted double bond at C-3 (δ_C 133.58) and the methyl group at N-2 were constructed on the basis of ^3J HMBC cross peaks of Me-17 (δ_H 2.90)/C-1 and C-3 and the olefinic H-10 (δ_H 7.15)/C-4. Consequently, the phenyl group was connected at C-10 (δ_C 121.19). This assigned location was confirmed by HMBC correlations of H-10 with C-12 and C-16 (δ_C 129.52) of the phenyl ring. Irradiation of H-10 enhanced signal intensity of Me-17 (**Figure 198**) (**Table 100**) established *E* configuration for the trisubstituted double bond. Therefore, compound **US22** was identified as a (*E*)-3-benzylidenehexahydro-2-methylpyrrolo[1,2-a]pyrazine-1,4-dione (Jin and liebscher, 1999).

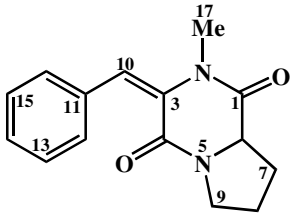


Table 99 The NMR data of compound US22 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	-	167.98 (C=O)
3	-	133.58 (C)
4	-	161.42 (C=O)
6	4.25 (*dd*, 9.3, 6.9)	58.85 (CH)
7	a: 2.42 (*m*)	28.01 (CH_2)
	b: 2.22 (*m*)	
8	a: 2.06 (*m*)	22.84 (CH_2)
	b: 1.98 (*m*)	
9	3.65 (*m*)	45.29 (CH_2)
10	7.15 (*s*)	121.19 (CH)
11	-	133.62 (C)
12, 16	7.33 (*m*)	129.52 (CH)
13,15	7.33 (*m*)	128.57 (CH)
14	7.33 (*m*)	128.44 (CH)
17	2.90 (*s*)	34.72 (CH_3)

Table 100 The HMBC, COSY and NOE data of compound US22 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-6	C-1, C-7, C-8	H_{ab}-7, H-9	H_a-7, H_{ab}-8a
H_a-7	C-1, C-6, C-8, C-9	H-6, H_b-7, H_{ab}-8	H-6, H_b-7, H_a-8
H_b-7	C-1, C-6, C-8, C-9	H-6, H_a-7, H_{ab}-8	H-6, H_a-7, H-9
H_a-8	C-6, C-7, C-9	H_{ab}-7, H_b-8, H-9	H_a-7, H_b-8, H-9
H_b-8	C-6, C-7, C-9	H_{ab}-7, H_a-8, H-9	H-6, H_a-7, H_a-8, H-9
H-9	C-4, C-6, C-7, C-8	H-6, H_{ab}-8	-
H-10	C-4, C-12, C-16	-	H-12, H-16, Me-17
H-12, 16	C-10, C-11	-	-
H-13, H-15	C-12, C-14, C-16	-	-
H-14	C-12, C-16	-	-
H-17	C-1, C-3	-	H-10

4.3.7 Compound US23

Compound **US23** was obtained as a pale yellow gum. The UV (**Figure 200**) and IR (**Figure 201**) spectra were almost identical to those of **US22**. The ^{1}H NMR spectral data (**Figure 202**) (**Table 101**) were similar to those of **US22** except that the methyl signal at δ_H 2.09 in **US22** was replaced, in **US23**, by a proton resonating at δ_H 7.76 (*s*). These indicated the replacement of the NMe group in **US22** with an NH group in **US23**. The disappearance of the methyl carbon resonance in **US23** supported this conclusion. Therefore, compound **US23** was identified as a (*E*)-3-benzylidenehexahydropyrrolo[1,2-a]pyrazine-1,4-dione (Jin, *et al*., 2000).

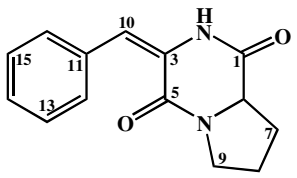


Table 101 The NMR data of compound US23 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	-	165.83 (C=O)
2-NH	7.76 (*s*)	-
3	-	133.23 (C)
4	-	157.86 (C=O)
6	4.32 (*dd*, 10.0, 7.0)	59.18 (CH)
7	a: 2.48 (*m*)	28.94 (CH_2)
	b: 2.03 (*m*)	
8	a: 2.12 (*m*)	21.83 (CH_2)
	b: 1.97 (*m*)	
9	a: 3.82 (*dt*, 8.5, 1.0)	45.67 (CH_2)
	b: 3.65 (*m*)	
10	7.00 (*s*)	115.86 (CH)
11	-	133.23 (C)
12, 16	7.37 (*d*, 7.5)	128.45 (CH)
13, 15	7.43 (*t*, 7.5)	129.39 (CH)
14	7.32 (*t*, 7.5)	128.61 (CH)

Table 102 The HMBC, COSY and NOE data of compound US23 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-6	C-1, C-7	H_{ab}-7	H_a-7
H_a-7	C-1, C-6, C-8, C-9	H-6, H_b-7, H_{ab}-8	H-6, H_b-7
H_b-7		H-6, H_a-7, H_{ab}-8	H-6, H_a-7
H_a-8	C-7, C-9	H_{ab}-7, H_b-8, H_{ab}-9	H_a-7
H_b-8		H_{ab}-7, H_a-8, H_{ab}-9	H_a-7, H_a-9
H_a-9	C-6, C-7, C-8	H_{ab}-8, H_b-9	-
H_b-9		H_{ab}-8, H-9[a]	-
H-10	C-5, C-12, C-16	-	H-12, H-16

Table 102 (continued)

Proton	HMBC correlations	COSY	NOE
H-12, H- 16	C-10, C-14	-	H-10
H-13, H-15	C-11	-	-
H-14	C-12, C-16	-	-

4.3.8 Compound US24

Compound **US24** was obtained as a colorless gum. It exhibited a hydroxyl absorption band at 3394 cm^{-1} in the IR spectrum (**Figure 210**). The ^1H NMR spectrum (**Figure 211**) (**Table 103**) contained signals for three olefinic protons [δ_H 6.39 (*ddd*, *J* = 15.0, 11.1 and 1.5 Hz), 6.09 (*d*, *J* = 11.1 Hz) and 5.84 (*dq*, *J* = 15.0 and 6.6 Hz)], two oxymethine protons [δ_H 4.02 (*d*, *J* = 6.3 Hz) and 3.95 (*quintet*, *J* = 6.3 Hz)], nonequivalent methylene protons of a hydroxymethyl group [δ_H 4.34 (*d*, *J* = 11.7 Hz) and 4.25 (*d*, *J* = 11.7 Hz)] and two methyl groups [δ_H 1.81 (*dd*, *J* = 6.6 and 1.5 Hz) and 1.15 (*d*, *J* = 6.3 Hz)]. The ^{13}C NMR spectrum (**Figure 214**) (**Table 103**)

showed one quaternary carbon (δ_C 134.65), three olefinic methine carbons (δ_C 133.34, 132.80 and 126.07), two oxymethine carbons (δ_C 80.40 and 70.11), one oxymethylene carbon (δ_C 56.83) and two methyl carbons (δ_C 18.78 and 18.42). The ^{1}H and ^{13}C NMR data indicated the presence of one trisubstituted double bond and one *trans*-disubstituted double bond. In the COSY spectrum (**Figure 217**) (**Table 104**), one of the *trans*-olefinic protons (δ_H 5.84) was coupled with the vinylic methyl protons, Me-8 (δ_H 1.81), while the other one (δ_H 6.39) was coupled with the olefinic proton (δ_H 6.09) of the trisubstituted alkene. These results established a *trans*-1,3-pentadienyl unit. Furthermore, the ^{1}H-^{1}H COSY correlations between one of the oxymethine protons (δ_H 3.95) with the methyl protons (δ_H 1.15) and the other oxymethine proton (δ_H 4.02) constructed 1,2-dihydroxypropyl unit. ^{3}J HMBC correlations of the hydroxymethyl protons (H_{ab}-9, δ_H 4.34 and 4.25) with C-5 (δ_C 132.80) of the diene moiety and C-3 (δ_C 80.40) of the dihydroxypropyl moiety (**Figure 216**) (**Table 104**) indicated that **US24** was (*E*,*E*)-4-hydroxymethyl-4,6-octadien-2,3-diol (Fuchser and Zeeck, 1997). Irradiation of H-6 (δ_H 6.39) enhanced signals of Me-8 and H_{ab}-9 (**Figure 218**) (**Table 104**), thus supporting the *E*-configuration of the trisubstituted double bond.

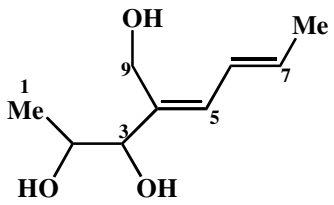


Table 103 The NMR data of compound US24 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	1.15 (*d*, 6.3)	18.78 (CH_3)
2	3.95 (*quintet*, 6.3)	70.11 (CH)
3	4.02 (*d*, 6.3)	80.40 (CH)
4	-	134.65 (C)
5	6.09 (*d*, 11.1)	132.80 (CH)
6	6.39 (*ddd*, 15.0, 11.1, 1.5)	126.07 (CH)
7	5.84 (*dq*, 15.0, 6.6)	133.34 (CH)
8	1.81 (*dd*, 6.6, 1.5)	18.42 (CH_3)
9	a: 4.34 (*d*, 11.7)	56.83 (CH_2)
	b: 4.25 (*d*, 11.7)	

Table 104 The HMBC, COSY and NOE data of compound US24 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-1	C-2, C-3	H-2	H-2, H-3, H-5
H-2	C-1, C-3, C-4	H-1, H-3	H-1, H-5
H-3	C-1, C-2, C-4, C-5, C-9	H-2	H-1, H-5, H_{ab}-9
H-5	C-3, C-4, C-6, C-7, C-9	H-6	H-1, H-3, H-7
H-6	C-5, C-8	H-5, H-7, Me-8	Me-8, H_{ab}-9
H-7	C-5, C-6	H-6, Me-8	H-5, Me-8

Table 104 (continued)

Proton	HMBC correlations	COSY	NOE
H-8	C-6, C-7	H-6, H-7	H-6, H-7
H_a-9	C-3, C-4, C-5	H_b-9	H-3
H_b-9		H_a-9	-

4.3.9 Compound US25

Compound **US25** with the molecular formula $C_9H_{12}O_4$ from EIMS (*m/z* 184) (**Figure 228**) was isolated as a yellow gum. It exhibited UV absorption band at 257 nm (**Figure 219**) while hydroxyl and two carbonyl absorption bands were found at 3373, 1720 and 1689 cm^{-1}, respectively, in the IR spectrum (**Figure 220**). The ^1H NMR spectrum (**Figure 221**) (**Table 105**) contained signals of *trans*-1,3-pentadienyl unit [δ_H 7.47 (*d*, *J* = 11.5 Hz), 6.54 (*ddd*, *J* = 15.0, 11.5 and 1.5 Hz), 6.32 (*dq*, *J* = 15.0 and 6.9 Hz) and (δ_H 1.94, *dd*, *J* = 6.9 and 1.5 Hz)], one oxymethylene proton (δ_H 4.92, *s*) and one methyl group (δ_H 2.07, *s*). The ^{13}C NMR spectrum (**Figure 224**) (**Table 105**) showed two carbonyl carbons (δ_C 169.99 and 168.63), one quaternary carbon (δ_C 120.95), three olefinic methine carbons (δ_C 145.49, 142.44 and 125.61), one oxymethylene carbon (δ_C 56.90) and two methyl carbons (δ_C 19.89 and 18.10). ^3J HMBC correlations of H-3 (δ_H 7.47)/C-1 (δ_C 169.99) and C-7 (δ_C 56.90) (**Figure 226**) (**Table 106**) constructed a diene carboxylic acid carrying the oxymethylene group at C-2. The remaining methyl group was connected with the

ester carbonyl carbon (δ_C 168.63) which gave a HMBC correlation with H_2-7 (δ_H 4.92). These results together with the chemical shift of H_2-7 established the substituent at C-7 to be an acetoxyl group. Consequently, **US25** was identified as a new dienoic acid.

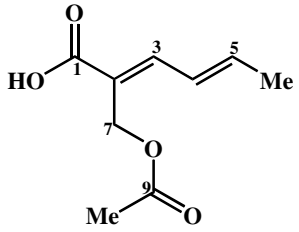


Table 105 The NMR data of compound US25 in $CDCl_3$

Position	δ_H (*mult.*, J_{Hz})	δ_C (C-type)
1	-	169.99 (C=O)
2	-	120.95 (C)
3	7.47 (*d*, 11.5)	145.49 (CH)
4	6.54 (*ddd*, 15.0, 11.5, 1.5)	125.61 (CH)
5	6.32 (*dq*, 15.0, 6.9)	142.44 (CH)
6	1.94 (*dd*, 6.9, 1.5)	18.10 (CH_3)
7	4.92 (*s*)	56.90 (CH_2)
9	-	168.63 (C=O)
10	2.07 (*s*)	19.89 (CH_3)

Table 106 The HMBC, COSY and NOE data of compound US25 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H-3	C-1, C-2, C-5, C-7	H-4	H-5
H-4	C-3	H-3, H-5, Me-6	Me-6, H-7
H-5	C-3, C-6	H-4, Me-6	H-3, Me-6
H-6	C-4, C-5	H-4, H-5	H-4, H-5
H-7	C-1, C-2, C-3	-	H-4
H-10	C-9	-	-

4.3.10 Compound US26

Compound **US26** with the molecular formula $C_{11}H_{12}O_3$ from EIMS (*m/z* 192) (**Figure 239**) was obtained as a colorless gum. The UV (**Figure 229**) and IR (**Figure 230**) spectra were similar to those of **US19**. The ^{1}H NMR spectral data (**Figure 231**) (**Table 107**) were similar to those of **US19** except that the chelated hydroxyl proton at δ_H 12.43 (*s*) in **US19** was replaced, in **US26**, by a methoxyl group (δ_H 3.86, *s*). A HMBC correlation of the methoxy protons with C-8 (δ_C 160.08) (**Figure 236**) (**Table 108**) and signal enhancement of these protons after irradiation of H-7 (δ_H 6.91) (**Figure 238**) (**Table 108**) supported the assignment. In addition, the remaining HMBC correlations, NOE and ^{1}H-^{1}H COSY data (**Figure 237**) (**Table 108**) were almost identical to those of **US19.** Therefore, compound **US26** was identified as a methyl ether of **US19**, a new tetralone.

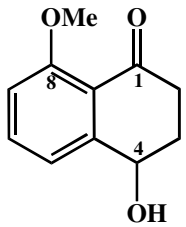


Table 107 The NMR data of compound US26 in $CDCl_3$

Position	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
1	-	197.00 (C=O)
2	a: 2.85 (*ddd*, 18.0, 9.0, 3.0)	36.34 (CH_2)
	b: 2.52 (*ddd*, 18.0, 9.0, 3.0)	
3	a: 2.25 (*m*)	31.33 (CH_2)
	b: 2.10 (*m*)	
4	4.83 (*dd*, 9.0, 6.0)	68.47 (CH)
4a	-	147.76 (C)
5	7.11 (*d*, 6.0)	118.77 (CH)
6	7.46 (*dd*, 9.0, 6.0)	134.69 (CH)
7	6.91 (*d*, 9.0)	111.97 (CH)
8	-	160.08 (C)
8a	-	120.10 (C)
9	3.86 (*s*)	56.14 (CH_3)

Table 108 The HMBC, COSY and NOE data of compound US26 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H_a-2	C-1, C-3, C-4	H_b-2, H_{ab}-3a	H_b-2, H_b-3
H_b-2	C-1, C-3, C-4	H_a-2, H_{ab}-3	H_a-2, H_b-3
H_a-3	C-1, C-2, C-4, C-4a	H_{ab}-2, H_b-3, H-4	H-4
H_b-3	C-1, C-2, C-4, C-4a	H_{ab}-2, H_a-3, H-4	H_{ab}-2, H-4
H-4	C-2, C-3, C-4a, C-5, C-8a	H_{ab}-3	H_{ab}-3, H-5
H-5	C-4, C-7, C-8a	H-6	H-4, H-6
H-6	C-4a, C-8	H-5, H_{ab}-7	H-5, H_{ab}-7
H-7	C-5, C-8a	H-6	H-6, Me-9
H-9	C-8	-	H-7

4.3.11 Compound US27

Compound **US27** was obtained as a colorless gum. It exhibited hydroxyl and carbonyl absorption bands at 3394 and 1734 cm^{-1}, respectively, in the IR spectrum (**Figure 244**). The ^1H NMR spectrum (**Figure 242**) (**Table 109**) contained signals of two oxymethine protons [δ_H 4.86 (*dqd*, *J* = 11.1, 6.3 and 3.3 Hz, 1H) and 4.37 (*dddd*, *J* = 4.8, 3.6, 3.6 and 3.3 Hz, 1H)], two sets of nonequivalent methylene protons [(δ_H 2.75, *dd*, *J* = 17.7 and 4.8 Hz, 1H and 2.61, *ddd*, *J* = 17.7, 3.6 and 1.5 Hz, 1H) and (δ_H 2.00, *dm*, *J* = 14.4 Hz, 1H and 1.71, *ddd*, *J* = 14.4, 11.1 and 3.3 Hz,1H)] and one methyl group (δ_H 1.39, *d*, *J* = 6.3 Hz, 1H). The ^{13}C NMR spectrum (**Figure 245**) (**Table 109**) showed one carbonyl carbon (δ_C 171.15), two oxymethine carbons (δ_C 72.56 and 62.54), two methylene cabons (δ_C 38.32 and 37.51) and one methyl carbon (δ_C 21.35). In the COSY spectrum (**Figure 248**) (**Table 110**), ^1H-^1H coupling was observed between H_{ab}-3 (δ_H 2.75 and 2.61) and H-4 (δ_H 4.37),

between H-4 and H_{ab}-5 (δ_H 2.00 and 1.71) and between H_{ab}-5 and H-6 (δ_H 4.86). The chemical shifts of C-2 (δ_C 171.15) and C-6 (δ_C 72.56) and HMBC cross peaks (**Figure 247**) (**Table 110**) of H_{ab}-3, H-4 and H-6 with C-2 constructed a lactone skeleton. Moreover, HMBC cross peaks of Me-7 (δ_H 1.39) with C-5 (δ_C 37.51) and C-6 revealed the attachment of the methyl group at C-6 of the lactone skeleton. Since H-4 was coupled with H_{ab}-3 and H_{ab}-5 with small coupling constants of 4.8, 3.6, 3.6 and 3.3 Hz, it was assigned at equatorial position. The large coupling constant (J = 11.1 Hz) between H_b-5 and H-6 established the location of H-6 at axial position. Consequently, **US27** was identified as *trans*-4-hydroxy-6-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one, which was previously isolated from *Daldinia* sp. (Buchanan, *et al*., 1996).

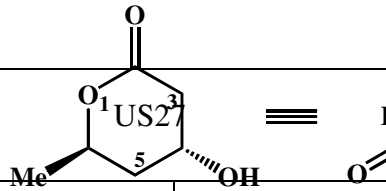
Position	 US27 ≡ *trans*-4-Hydroxy-6-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one			
	δ_H (*mult., J_{Hz}*)	δ_C (C-type)	δ_H (*mult., J_{Hz}*)	δ_C (C-type)
2	-	171.15 (C=O)	-	171.51 (C=O)
3	a: 2.75 (*dd*, 17.7, 4.8) b: 2.61 (*ddd*, 17.7, 3.6, 1.5)	38.32 (CH_2)	a: 2.68 (*dd*, 18.4, 4.9) b: 2.61 (*ddd*, 18.4, 3.6, 1.7)	38.31 (CH_2)
4	4.37 (*dddd*, 4.8, 3.6, 3.6, 3.3)	62.54 (CH)	4.35 (*dddd*, 4.9,3.8, 3.6, 3.3)	62.42 (CH)
5	a: 2.00 (*dm*, 14.4) b: 1.71 (*ddd*, 14.4, 11.1, 3.3)	37.51 (CH_2)	a: 2.00 (*dddd*, 14.5, 3.8, 3.2, 1.7) b: 1.70 (*ddd*, 14.5, 11.3, 3.3)	37.42 (CH_2)
6	4.86 (*dqd*, 11.1, 6.3, 3.3)	72.56 (CH)	4.85 (*ddq*, 11.1, 6.5, 3.1)	72.74 (CH)
7	1.39 (*d*, 6.3)	21.35 (CH_3)	1.39 (*d*, 6.4)	21.38 (CH_3)

Table 109 The NMR data of compound US27 in $CDCl_3$

Table 110 The HMBC, COSY and NOE data of compound US27 in $CDCl_3$

Proton	HMBC correlations	COSY	NOE
H_a-3	C-2, C-4, C-5	H_b-3, H-4	-
H_b-3	C-2, C-4, C-5	H_a-3, H-4, H_a-5	-
H-4	C-2, C-3, C-5, C-6	H_{ab}-3, H_{ab}-5	-
H_a-5	C-3, C-4, C-6, C-7	H-4, H_b-5, H-6	H-4, H_b-5, H-6, H-7
H_b-5	C-3, C-4, C-6, C-7	H-4, H_a-5, H-6	H-4, H_a-5, H-7
H-6	C-2, C-4, C-5, C-7	H_{ab}-5, H-7	-
H-7	C-5, C-6	H-6	-

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