Final Report

The study of bioactive compounds from *Dalbergia candenatensis*, *Alstonia scholaris* and *Alstonia macrophylla*

By Sarot Cheenpracha

June 2013

Contract No. MRG5480028

Final Report

The study of bioactive compounds from *Dalbergia candenatensis*, *Alstonia scholaris* and *Alstonia macrophylla*

Researcher	Institute
1. Sarot Cheenpracha	University of Phayao
2. Chatchanok Karalai	Prince of Songkla University

Project Granted by the Thailand Research Fund

Abstract

Project Code : MRG5480028

Project Title : The study of bioactive compounds from *Dalbergia candenatensis*, *Alstonia scholaris* and *Alstonia macrophylla*

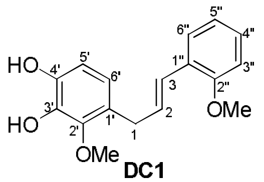
Investigator : Sarot Cheenpracha
E-mail Address : cheenpracha@gmail.com

Project Period : June 15th, 2011 – June 14th, 2013

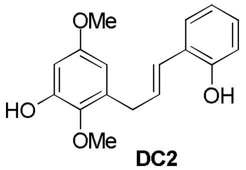
Abstract:

Investigation of chemical constituents of *Dalbergia candenatensis*, *Alstonia scholaris* and *Alstonia macrophylla* led to the isolation of 26 compounds. Nine compounds were isolated from the air-dried heartwood of *D. candenatensis* including five new phenolic compounds (DC1-DC5) and four known compounds (DC6-DC9). The other new indole alkaloids (AM1, AM2 and AM4) were isolated from the bark of *A. macrophylla* together with eight known componds (AM3, AM5-AM11), whereas six known compounds (ASB1-ASB5, ASL1) were isolated from the bark and leaves of *A. scholaris.* Structures of new compounds were characterized by spectroscopic methods including UV, IR, 1D- and 2D-NMR and MS whereas all known compounds were characterized by NMR and comparison of their spectral data with reported values. Alstonisine (AM9) exhibited antiplasmodial activity against *Plasmodium falciparum*, with an IC_{50} of 7.6 μM. Candenatenin K (DC5) exhibited potent activity against DPPH radical scavenging with IC_{50} value of 25.7 μM, whereas candenatenin H (DC2) showed cytotoxicity against NCI-H187 cell line with IC_{50} value of 14.8 μM.

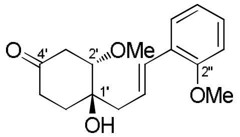
Keywords : *Dalbergia candenatensis*, *Alstonia scholaris*, *Alstonia macrophylla*, antiplasmodial activity, cytotoxicity, indole alkaloid



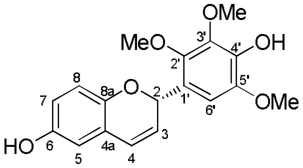
DC1



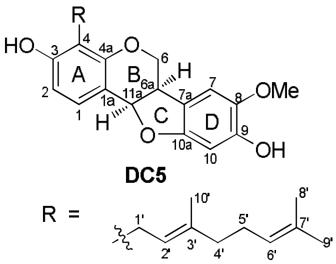
DC2



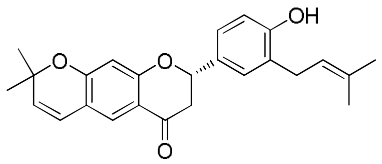
DC3



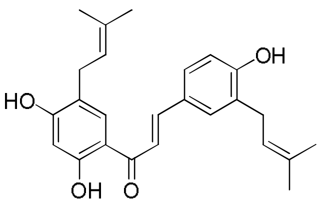
DC4



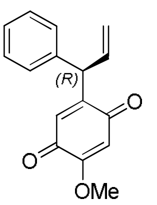
DC5



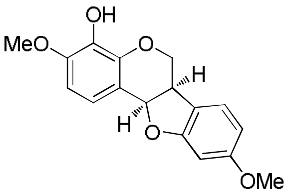
DC6



DC7

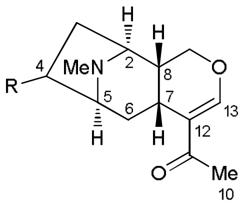


DC8



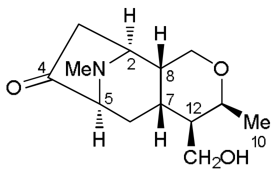
DC9

Structure of isolated compounds from *D. candenatensis*

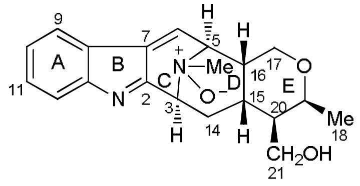


AM1; R = =O

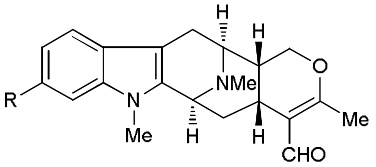
AM3; R = α-OH



AM2

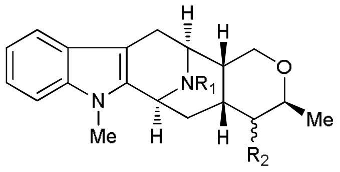


AM4



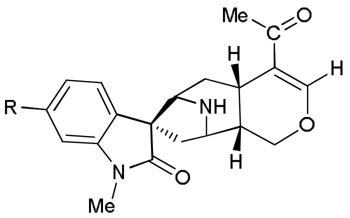
AM5; R = H

AM6; R = OMe



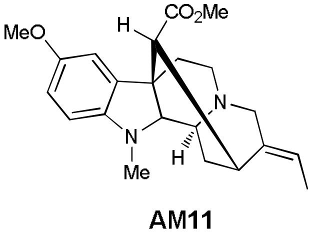
AM7; R$_1$ = H, R$_2$ = β-CHO

AM8; R$_1$ = Me, R$_2$ = α-CH$_2$OH



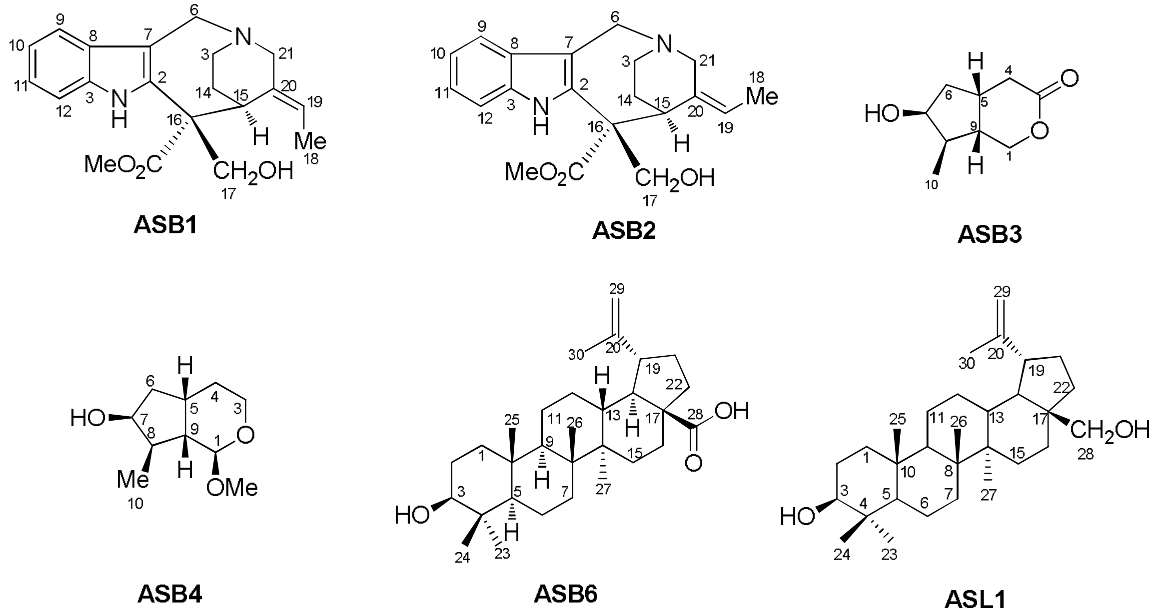
AM9;   R = H

AM10; R = OMe



AM11

Structure of isolated compounds from *A. macrophylla*



ASB1 ASB2 ASB3

ASB4 ASB6 ASL1

Structure of isolated compounds from *A. scholaris*

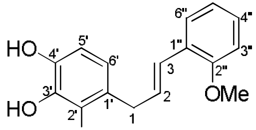
บทคัดย่อ

รหัสโครงการ : MRG5480028
ชื่อโครงการ : The study of bioactive compounds from *Dalbergia candenatensis*, *Alstonia scholaris* and *Alstonia macrophylla*
ชื่อนักวิจัย : Sarot Cheenpracha
E-mail Address : cheenpracha@gmail.com
ระยะเวลาโครงการ : 15 **มิถุนายน** 2554 – 14 **มิถุนายน** 2556

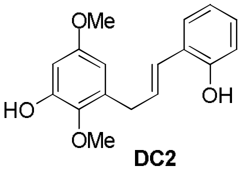
บทคัดย่อ:

การศึกษาองค์ประกอบทางเคมีของต้นสักขี ตีนเป็ดขาว และทุ้งฟ้า สามารถแยกสารประกอบได้ทั้งหมด 26 สาร ประกอบด้วยสารประกอบ 9 สาร ที่แยกได้จากลำต้นสักขี ซึ่งเป็นสารใหม่ 5 สาร (DC1-DC5) และสารที่มีการรายงานมาแล้ว 4 สาร (DC6-DC9) สารใหม่กลุ่มอินโดลอัลคาลอยด์อีก 3 สาร (AM1, AM2 และ AM4) ได้ถูกแยกจากเปลือกลำต้นของทุ้งฟ้าและสารที่มีการรายงานมาแล้วจำนวน 8 สาร (AM3, AM5-AM11) ขณะที่ในส่วนเปลือกลำต้นและใบของตีนเป็ดขาวสามารถแยกสารที่มีการรายงานมาแล้วจำนวน 6 สาร (ASB1-ASB5, ASL1) การวิเคราะห์โครงสร้างของสารประกอบใหม่อาศัยข้อมูลทางสเปกโทสโกปี เช่น UV, IR, 1D- และ 2D-NMR และข้อมูลของ MS ในขณะที่สารที่มีการรายงานมาแล้วอาศัยการเปรียบเทียบข้อมูล NMR ที่ได้มีการรายงานมาแล้ว สารประกอบ alstonisine (AM9) แสดงฤทธิ์การยับยั้งเชื้อ *Plasmodium falciparum* ด้วย IC_{50} = 7.6 μM นอกจากนี้ สารประกอบ candenatenin K (DC5) แสดงฤทธิ์ยับยั้ง DPPH ในระดับที่ดีมากด้วย IC_{50} = 25.7 μM และสารประกอบ candenatenin H (DC2) แสดงการยับยั้งเซลล์มะเร็ง NCI-H187 ด้วย IC_{50} = 14.8 μM

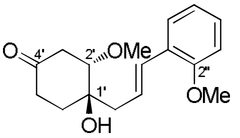
คำสำคัญ : *Dalbergia candenatensis*, *Alstonia scholaris*, *Alstonia macrophylla,* antiplasmodial activity, cytotoxicity, indole alkaloid



DC1



DC2



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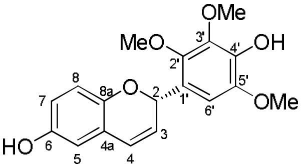

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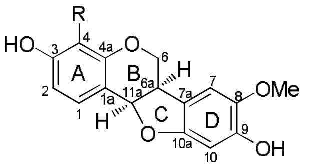
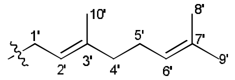
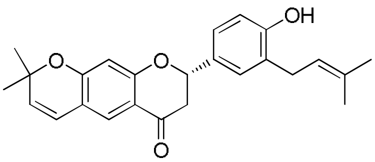


DC5

R = 



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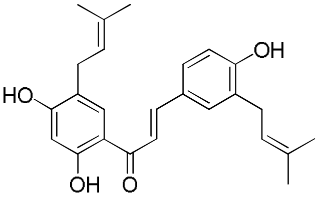
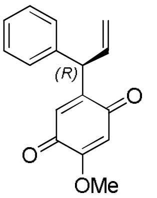


DC7



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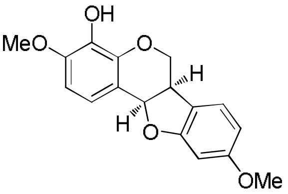
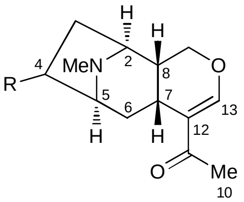


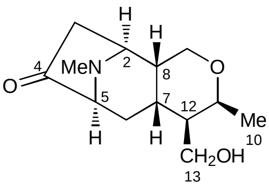
DC9

โครงสร้างของสารประกอบที่แยกได้จากต้นสักขี



AM1; R = =O

AM3; R = α-OH



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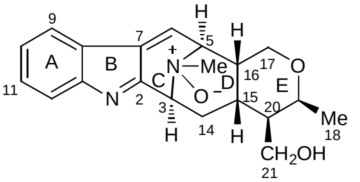

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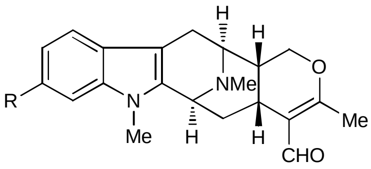
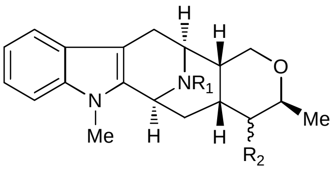


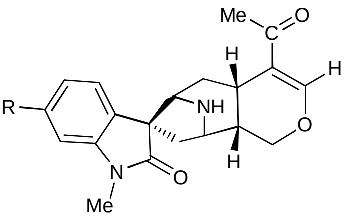
AM5; R = H

AM6; R = OMe



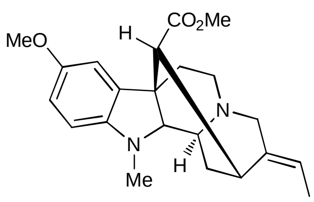
AM7; R_1 = H, R_2 = β-CHO

AM8; R_1 = Me, R_2 = α-CH_2OH



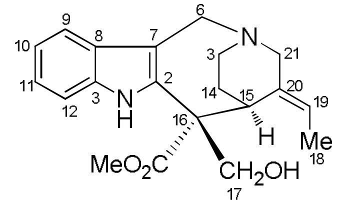
AM9; R = H

AM10; R = OMe

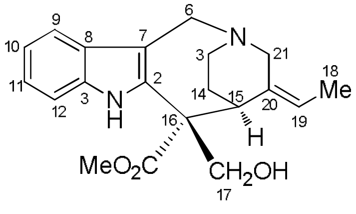


AM11

โครงสร้างของสารประกอบที่แยกได้จากทุ้งฟ้า



ASB1



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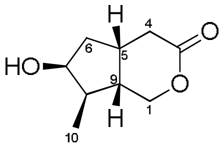

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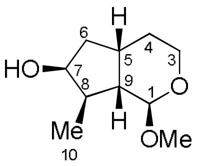

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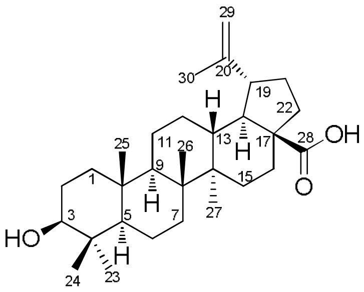

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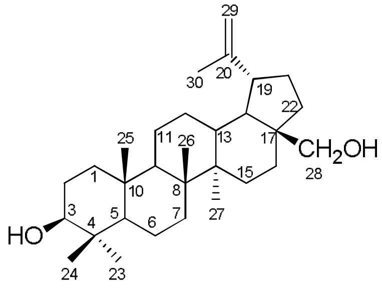


ASL1

โครงสร้างของสารประกอบที่แยกได้จากตีนเป็ดขาว

CONTENT

Executive summary

Project Code : MRG5480028
Project Title : The study of bioactive compounds from *Dalbergia candenatensis*, *Alstonia scholaris* and *Alstonia macrophylla*
Investigator : Sarot Cheenpracha
E-mail Address : cheenpracha@gmail.com
Project Period : June 15th, 2011 – June 14th, 2013

Introduction

Natural products have served as the source and inspiration for a large fraction of the current pharmacopoeia. Although estimates vary depending on the definition of what is considered a natural product-derived drug, it is safe to say that between 25% and 50% of currently marketed drugs owe their origins to natural products. While it is common for natural product scientists to claim that natural products form the basis for many of the drugs currently in commercial use or in development.

Drug discovery can build on several sources; however, the Natural Products Research Laboratories focus on bioactivity-directed isolation and characterization of lead natural product principles from single medicinal herbs and formulations. The subsequent preclinical optimization of a lead compound is a cyclical process of obtaining bioassay screening results, analyzing activity data, designing new target compounds, and synthesizing new analogues.

Due to the wide use in Thai traditional medicine and their alleged pharmaceutical activities of plants, we are interested in studying the chemical constituents and biological activity of *Dalbergia candenatensis*, *Alstonia macrophylla*, and *Alstonia scholaris*. This research project will contribute significantly to scientific basis of traditional medicine and its possible use as therapeutic agents. The results of this project would have potential application in improving the well-being of Thai people.

Experiment section

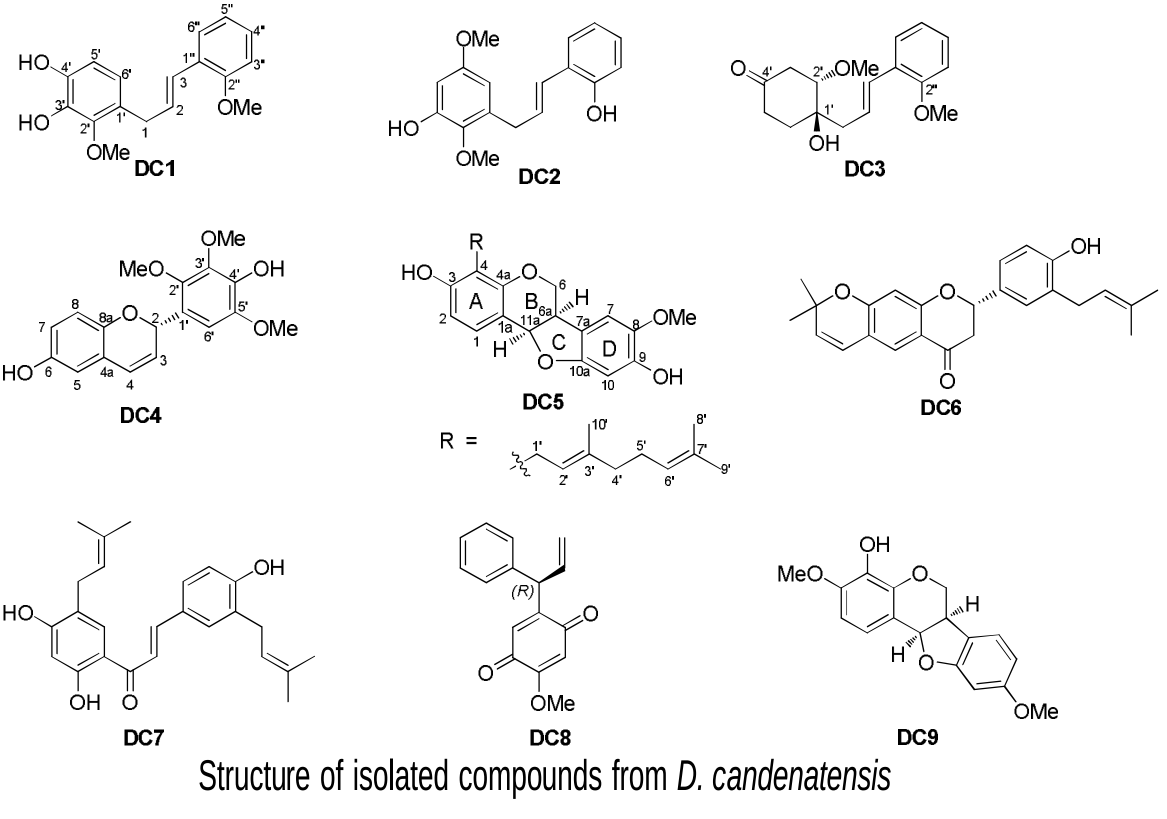
The dried and chopped heartwood of *D. candenatensis* was extracted with acetone evaporated to afford a dark brown gum which was further subjected to repeated CC to give pure compounds.

The bark of *A. macrophylla* was extracted with MeOH, twice at room temperature, and the solvent will evaporate to give the extracts. The residues are dissolved in 1% HCl and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 (aq.) to pH 8-9 and extracted with EtOAc to give the alkaloidal fractions. The crude base will evaluate for biological activities and separate by SiO_2 open column chromatography.

The bark and leaves of *A. scholaris* were carried out in the usual manner which has been described in the detail above.

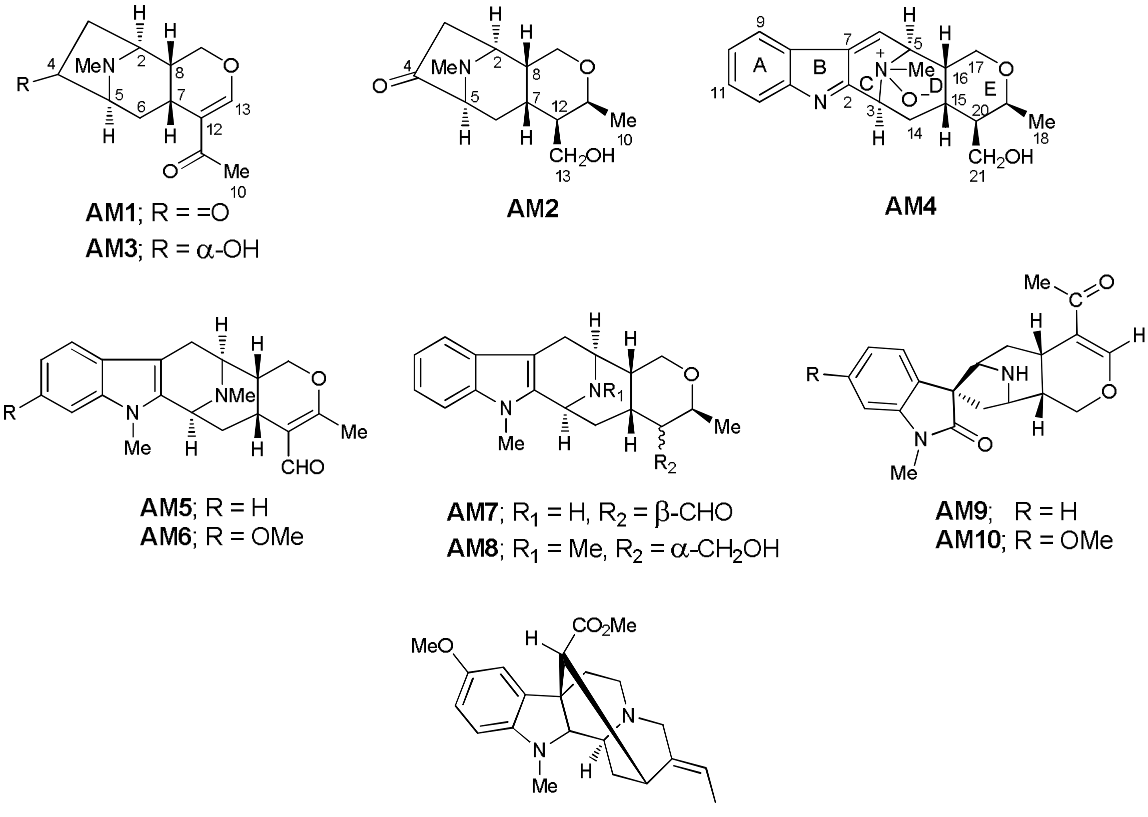
Results and discussion

The acetone extract of air-dried heartwood of *D. candenatensis* was separately fractionated by a combination of silica gel CC to obtain five new compounds (DC1-DC5), together with four known structures, dinklagin A (DC6), stipulin (DC7), (*R*)-4-methoxydalbergione (DC8) and melilotocarpan A (DC9).



Structure of isolated compounds from *D. candenatensis*

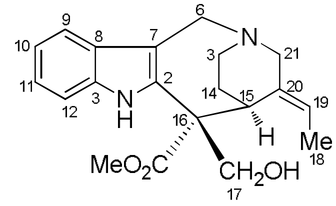
The bark of *A. macrophylla* was extracted with MeOH and partitioned between EtOAc and 1% HCl. The aqueous layer was adjusted to pH 8-9 with saturated Na_2CO_3; then the water soluble materials were extracted in fresh EtOAc. The EtOAc-soluble material was purified by silica gel column chromatography to afford two new unusual nitrogenous derivatives (AM1, AM2) and a new indole alkaloid (AM4) from the bark of *A. macrophylla*, in addition to eight known alkaloids: angustimaline (AM3), alstonerinal (AM5), alstophyllal (AM6), talcarpine (AM7), macrocarpine B (AM8), alstonisine (AM9), N_b-demethylalstophylline oxindole (AM10) and 10-methoxycathafoline (AM11).



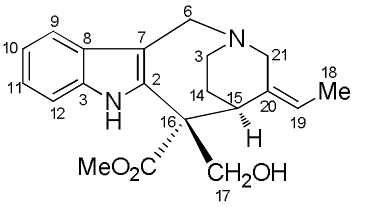
AM1; R = =O
AM3; R = α-OH
AM2
AM4
AM5; R = H
AM6; R = OMe
AM7; R_1 = H, R_2 = β-CHO
AM8; R_1 = Me, R_2 = α-CH_2OH
AM9; R = H
AM10; R = OMe
AM11

Structure of isolated compounds from *A. macrophylla*

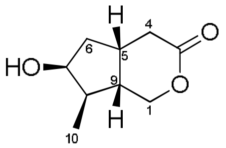
The alkaloidal extracts from the bark and leaves of *A. scholaris* were separated by a combination of silica gel CC to obtain two known alkaloids, 19,20-*E*-vallesamine (ASB1) and 19,20-*Z*-vallesamine (ASB2), two noriridoids, isoboonein (ASB3) and scholarein D (ASB4) and two triterpenoids, betulinic acid (ASB5) and betulin (ASL1).



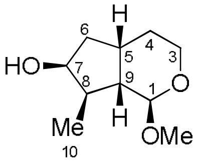
ASB1



ASB2



ASB3



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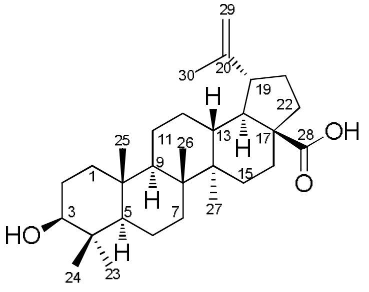
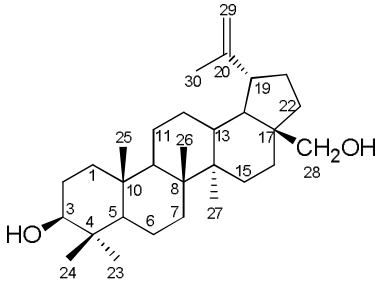


ASB6



ASL1

Structure of isolated compounds from *A. scholaris*

All structures were established by conventional ^{1}H and ^{13}C NMR spectral methods assisted by the performance of 2D NMR techniques, COSY, HMQC, HMBC, and NOESY, thus leading to the total assignments of the ^{1}H and ^{13}C NMR spectra of the new compounds. The known compounds were identified on the basis of spectral comparison with published data.

Alstonisine exhibited antiplasmodial activity against *Plasmodium falciparum*, with an IC_{50} of 7.6 μM. Candenatenin K exhibited potent activity against DPPH radical scavenging with IC_{50} value of 25.7 μM, whereas candenatenin H showed cytotoxicity against NCI-H187 cell line with IC_{50} value of 14.8 μM.

Objectives

1. Extraction and isolation chemical constituents from *Dalbergia candenatensis*, *Alstonia macrophylla*, and *Alstonia scholaris*.

2. Elucidation of isolated compounds from the plant materials.

3. Study of biological activity of crude extract and pure compounds e.g. anticancer, antimalarial, and acetylchlorinesterase activities.

Research methodology

General Experimental Procedures

The optical rotation values were determined with a Bellingham & Stanley ADP400 polarimeter. The UV spectra were recorded with a PerkinElmer UV−vis spectrophotometer. The IR spectra were recorded with a PerkinElmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker FTNMR Ultra Shield spectrometers. Chemical shifts are reported in parts per million (δ) in $CDCl_3$ with TMS as an internal reference. The TOFMS data were obtained from a Micromass LCT and MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

Plant Materials

The heartwood of *D. candenatensis* was collected in April 2010 in Sikhao district, Trang Province, Southern Thailand. Botanical identification was achieved through comparison with a voucher specimen number SC10 in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand by Prof. Puangpen Sirirugsa.

The bark of *A. macrophylla* was collected in April 2011 in Wangwisat District, Trang Province, Southern Thailand. Botanical identification was achieved through comparison with a voucher specimen, number QSBG-2009-983, in the Herbarium Collection of Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand, by Prof. Puangpen Sirirugsa.

The bark and leaves of *A. macrophylla* were collected in June 2011 in Nan Province. Botanical identification was achieved through comparison with a voucher specimen, number QSBG-2009-1262, in the Herbarium Collection of Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand by Prof. Puangpen Sirirugsa.

Extraction and Isolation

1. *D. candenatensis*

The dried and chopped heartwood of *D. candenatensis* (2.5 kg) was extracted successively with CH_2Cl_2 and acetone (3 x 5 L, 5 days) at room temperature. Filtration and subsequent evaporation of the combined acetone extract to dryness in vacuo afforded a dark brown gum (7.31 g) which was subjected to silica gel QCC with a gradient of hexanes–EtOAc (100:0 to 0:100, v/v) to give 9 fractions (A–I). Fraction C (51.8 mg) was purified by CC with CH_2Cl_2–hexanes (3:7, v/v) to give **DC6** (3.0 mg) and **DC8** (5.4 mg). Fraction E (1.53 g) was separated by QCC eluting with CH_2Cl_2–hexanes (1:1, v/v) to give seven subfractions (E1–E7). Subfraction E2 (40.5 mg) was subjected to CC with acetone–hexanes (1:4, v/v) to afford **DC2** (20.2 mg), whereas **DC5** (15.0 mg) was derived from subfraction E3 (36.3 mg) with the same procedure. Subfraction E6 (92.5 mg) was purified by CC with EtOAc–CH_2Cl_2 (1:49, v/v) to give **DC7** (50.0 mg). Fraction G (3.11 g) was separated by QCC with acetone–hexanes (3:17, v/v) to give five subfractions (G1–G5). Subfraction G3 (135.0 mg) was purified by CC with EtOAc–CH_2Cl_2 (3:97, v/v) to give **DC3** (7.3 mg). Subfraction G5 (100.3 mg) was purified by CC with $CHCl_3$–hexanes (9:1, v/v) to give **DC9** (2.0 mg). Fraction H (110.0 mg) was separated by CC with EtOAc–hexanes (3:97, v/v) to give **DC1** (3.3 mg) and **DC4** (2.3 mg).

*Candenatenin G (**DC1**)*: Viscous oil; UV (MeOH) (log $\mathcal{E}$) : 210 (4.09), 285 (3.31) nm; IR (neat): 3385, 2924, 1659, 1550,1463, 1376, 1245, 1166 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-EI-MS: $[M]^+$ *m/z* 286.1200 (calcd. for $C_{17}H_{18}O_4$, 286.1200).

*Candenatenin H (**DC2**)*: Viscous oil; UV (MeOH) (log $\mathcal{E}$) : 210 (4.07), 240 (3.49), 291 (3.31) nm; IR (neat): 3418, 3004, 2926, 1602, 1223 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; HR-EI-MS: $[M]^+$ *m/z* 286.1199 (calcd. for $C_{17}H_{18}O_4$, 286.1200).

*Candenatenin I (**DC3**)*: Viscous oil; $[\alpha]_{\mathrm{D}}^{26}$ –62.5 (*c* 0.30, $CHCl_3$); UV (MeOH)(log ε) : 210 (4.30), 253 (4.09), 300 (3.65) nm; IR (neat): 3426, 2928, 1709, 1598, 1243 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; HR-EI-MS: $[M]^+$ *m/z* 290.1513 (calcd. for $C_{17}H_{22}O_4$, 290.1499).

*Candenatenin J (**DC4**)*: Viscous oil; $[\alpha]_{D}^{27}$ -24.4 (c 0.09, MeOH).; UV (MeOH) (log ε) : 211 (3.80), 265 (3.26) nm; CD (CH_2Cl_2, *c* 0.20): $\Delta\varepsilon_{246}$ +0.4, $\Delta\varepsilon_{280}$ -1.4; IR (neat): 3425, 1612, 1273 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 4; HR-EI-MS $[M]^+$ *m/z* 330.3329 (calcd. for $C_{18}H_{18}O_6$: 330.3319).

*Candenatenin K (**DC5**)*: Viscous oil; $[\alpha]_{\mathrm{D}}^{26}$ –99.7 (*c* 0.30, $CHCl_3$); UV (MeOH) (log ε) : 210 (4.18), 298 (3.30) nm; IR (neat): 3418, 2925, 1613, 1217 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 5; HR-EI-MS: $[M]^+$ *m/z* 422.2098 (calcd. for $C_{26}H_{30}O_5$, 422.2088).

2. *A. macrophylla*

Dried and chopped bark of *A. macrophylla* (2.0 kg) was extracted with MeOH (2 × 5 L, 5 days) at room temperature, and the solvent was evaporated under vacuum. The residue was dissolved in 1% HCl (2 L) and partitioned with EtOAc (2 × 3 L). The aqueous layer was treated with saturated aqueous Na_2CO_3 to adjust the pH to 8-9 and extracted with EtOAc (2 × 3 L) to give an alkaloidal fraction (6.0 g). The alkaloidal fraction was purified by QCC with a gradient of hexanes-EtOAc-MeOH (100:0:0-0:0:100, v/v) to give nine fractions (A-I). Fraction C (1.5 g) was further subjected to silica gel QCC to provide nine subfractions (C1-C9). Subfraction C6 (395.7 mg) was separated by CC and eluted with acetone-CH_2Cl_2 (1:25, v/v) to give eight subfractions (C6a-C6f). Compounds **AM1** (5.1 mg), **AM5** (6.2 mg), and **AM8** (28.9 mg) were isolated from subfraction C6d (72.3 mg) by CC and elution with MeOH-CH_2Cl_2 (1:99, v/v). Recrystallization of subfraction C6 (298.4 mg) with acetone-hexanes (1:4, v/v) led to the isolation of compound **AM9** (20.3 mg). Subfraction C8 (93.5 mg) was subjected to CC and eluted with acetone-CH_2Cl_2 (1:9, v/v) to afford compounds **AM4** (2.0 mg) and **AM11** (3.4 mg). Fraction E (441.3 mg) was purified by CC with acetone-CH_2Cl_2 (1:5, v/v) followed by CC with acetone-hexanes (1:4, v/v) to give compounds **AM2** (4.8 mg) and **AM10** (5.1 mg). Compounds **AM6** (2.6 mg) and **AM7** (5.9 mg) were derived from fraction F (119.8 mg), eluting with MeOH-CH_2Cl_2 (1:49, v/v), while compound **AM3** (11.5 mg) was isolated from fraction G (2.06 g) by repeated CC with MeOH-CH_2Cl_2 (1:19, v/v).

Alstoniaphylline A ***(AM1)*****:** Light yellow gum; $[\alpha]_{\mathrm{D}}^{26}$ +50.6 (*c* 0.013, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.09), 257 (3.31) nm; IR ($CHCl_3$) ν_{max} 2926, 1743, 1651, 1618, 1195 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 10; APCI-TOFMS *m/z* 236.1287 $[M+H]^+$ (calcd for $C_{13}H_{18}NO_3$, 236.1281).

Alstoniaphylline B ***(AM2)*****:** Light yellow gum; $[\alpha]_{\mathrm{D}}^{26}$ +20.8 (*c* 0.010, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 221(3.64) nm; IR ($CHCl_3$) ν_{max} 3405, 2931, 1743, 1616, 1467, 1122 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 11; ESI-TOFMS *m/z* 240.1591 $[M+H]^+$ (calcd for $C_{13}H_{22}NO_3$, 240.1600).

Alstoniaphylline C ***(AM4)*****:** Colorless oil; $[\alpha]_{\mathrm{D}}^{26}$ +133.3 (*c* 0.020, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.10), 250 (3.96), 289 (3.88), 320 (3.78) nm; IR ($CHCl_3$) ν_{max} 3397, 2926, 1684, 1637, 1602, 1466, 1127 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 12; ESI-TOFMS *m/z* 341.1853 $[M+H)^+$ (calcd for $C_{20}H_{24}N_2O_3$, 341.1865).

3. *A. scholaris*

The extraction of alkaloids from the bark and leaves of *A. scholaris* was carried out in the usual manner which has been described in the detail above. Essentially, the ground bark (3.0 kg) and leave (6.0 kg) were exhaustively extracted MeOH (2 x 5 L) at ambient temperature. The MeOH extracts were then concentrated under reduced pressure, partitioned into dilute 1% HCl, basified with saturated aqueous Na_2CO_3 and liberated alkaloids were then taken into EtOAc to give a basic fraction containing a mixture of alkaloids.

The alkaloidal extract from the bark (20.1 g) was purified by QCC over silica gel with hexanes and increasing polarities with acetone and methanol, respectively to afford

11 fractions (F1-F11). Upon standing at room temperature, fraction F3 gave a pure compound **ASB5** (5.4 mg). Fraction F3 (480.2 mg) was further performed by CC eluting hexanes and increasing polarity with acetone to give 17 subfractions (F3a-F3q). Subfraction F3h (172.1 mg) was further separated by CC using 70% CH_2Cl_2-hexanes to give compounds **ASB3** (5.1 mg) and **ASB4** (4.2 mg). Fraction F9 (2.26 g) was subjected to CC eluting hexanes and increasing polarity of acetone to provide 11 subfractions (F9a-F9k). Subfraction F9e (228.0 mg) was further purified by CC with 3% MeOH-CH_2Cl_2 and followed by repeated CC with acetone-CH_2Cl_2-MeOH (3:94:3, v/v) to give compounds **ASB1** (29.6 mg) and **ASB2** (10.0 mg).

The alkaloidal extract (50.0 g) from the leaves was separated by QQC eluting with hexanes and increasing polarities with EtOAc and MeOH, respectively, to afford nine fractions (L1-L9). All fractions were tested to detect the presence of alkaloids using Dragendroff's reagent. The result showed that the alkaloids were not observed. Fraction L6 (1.98 g) was separated by CC with hexanes and increasing polarity with acetone and followed by repeated CC with 5% acetone-hexanes to give compound **ASL1** (5.6 mg).

Antimalarial Assay

Antimalarial activity against the parasite *P. falciparum* (K1, multidrug resistant) was evaluated using the method of Trager and Jensen (1976). Quantitative assessment of antimalarial activity *in vitro* was performed using the microculture radioisotope technique, based on the method described by Desjardins et al (2009). The concentrations of the compounds leading to a 50% reduction in parasite growth (IC_{50}) were determined by measuring the uptake of [^{3}H]-hypoxanthine by *P. falciparum* in vitro. The standard antimalarial compound was dihydroartemisinin (IC_{50} = 1.41 *n*M).

Cytotoxic Assay

The cytotoxicity of the compounds to the oral cavity cancer (KB) cell line was assessed using the resazurin microplate assay (REMA), which is a modified method of the fluorescent dye-uptake assay for measuring mammalian cell cytotoxicity (Brien et al., 2000). Ellipticine was used as standard, reference cytotoxic substances (IC_{50} = 4.87 μM).

Radical scavenging activity

The DPPH radical-scavenging activity was determined using the method proposed by Wojdylo et al. (2007). Briefly, 1 ml of 15 μM methanolic solution of DPPH was mixed with 1 ml of sample solution. The mixture was then vortexed vigorously and left for 45 min at room temperature in the dark. The absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. All determinations were performed in triplicate.

Acetylcholine esterase assay

Acetylcholine esterase inhibitory activity was measured by the spectrophotometric method developed by Ellman et al. (1961) and Shahwar et al. (2010). Acetylthiocholine iodide was used as substrate in the assay. The reaction mixture contained 1500 μl of Tris-HCl buffer (pH 8.0; 5.0 mM), 1000 μl of DTNB (3 mM DTNB), 200 μl of test compound solution or inhibitor and 200 μl of acetyl cholinesterase solution (type VI-S, EC 3.1.1.7), which were mixed and incubated for 15 min (25 °C). The reaction was initiated by the addition of 200 μl acetylthiocholine iodide (1.5 mM). The hydrolysis of acetylthiocholine iodide was monitored at 412 nm after 30 min. Galanthamine was used as positive control. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = (E - S)/ E \times 100$$

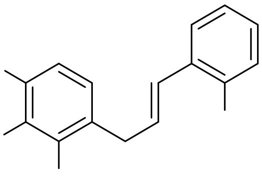
Where; E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

Results and discussion

1. Chemical constituents from *D. candenatensis*

The acetone extract of air-dried heartwood of *D. candenatensis* was separately fractionated by a combination of silica gel CC to obtain five new compounds (**DC1**-**DC5**), together with four known structures, dinklagin A (**DC6**) (Ngadjui et al., 2002), stipulin (**DC7**) (Ngameni et al., 2004), (*R*)-4-methoxydalbergione (**DC8**) (Chan et al., 1997) and melilotocarpan A (**DC9**) (Belofsky et al., 2006). All structures were established by conventional ^{1}H and ^{13}C NMR spectral methods assisted by the performance of 2D NMR techniques, COSY, HMQC, HMBC, and NOESY, thus leading to the total assignments of the ^{1}H and ^{13}C NMR spectra of the new compounds. The known compounds were identified on the basis of spectral comparison with published data.

1.1 Candenatenin G (DC1)



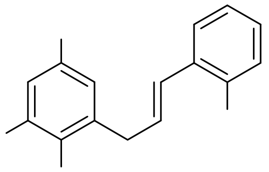
Compound **DC1** was obtained as a colorless viscous oil, and its molecular formula was determined as $C_{17}H_{18}O_4$ by HR-EI-MS at $[M]^+$ *m/z* 286.1200 (calcd. 286.1200). The IR spectrum showed a stretching frequency of hydroxy (3385 cm^{-1}) and aromatic (1550 cm^{-1}) functionalities. The UV spectrum displayed absorption bands at λ_{max} 210 and 285 nm, suggesting the presence of a conjugated aromatic chromophore. The ^{1}H NMR spectroscopic data (Table 1) of **DC1** showed a presence of two methoxyl groups at δ3.84 (6H, *s*, 2′-OMe, 2″-OMe), of a 1,2-disubstituted benzene at δ7.41 (1H, *dd*, J = 7.5, 1.8 Hz, H-6″), 7.19 (1H, *td*, J = 7.5, 1.8 Hz, H-4″), 6.90 (1H, *br d*, J = 7.5 Hz, H-3″), 6.87 (1H, *br t*, J = 7.5 Hz, H-5″), of a 1,2,3,4-tetrasubstituted benzene at δ

6.69 (1H, *d*, *J* = 8.4 Hz, H-6′) and 6.66 (1H, *d*, *J* = 8.4 Hz, H-5′) and of $–CH_2CH=CH–$ moiety at δ 6.78 (1H, *br d*, *J* = 15.9 Hz, H-3), 6.31 (1H, *dt*, *J* = 15.9, 6.6 Hz, H-2) and 3.52 (2H, *dd*, *J* = 6.6, 1.5 Hz, H_2-1). These data indicated that the structure of **DC1** was closely related to that of candenatenin A (Cheenpracha et al., 2009), except for the arrangements of OMe and OH groups in rings A and B. Only a methoxyl group was located on ring A at C-2′ and another methoxyl group was placed at C-2″ due to the ^3J HMBC correlation between methoxyl protons (δ 3.84) to C-2′ (δ 145.5) and C-2″ (δ 156.4), respectively. Accordingly, compound **DC1** was assigned as candenatenin G. The completed assignment of ^{13}C NMR data was shown in Table 1.

Table 1 ^{1}H and ^{13}C NMR data (300 MHz) of **DC1** in $CDCl_3$

No.	Candenatenin G **(1)**	
	δ_C	δ_H (*mult.*, *J* in Hz)
1	33.1	3.52 (*dd*, 6.6, 1.5)
2	129.6	6.31 (*dt*, 15.9, 6.6)
3	125.8	6.78 (*br d*, 15.9)
1′	124.9	
2′	145.5	
3′	136.3	
4′	143.0	
5′	111.3	6.66 (*d*, 8.4)
6′	121.0	6.69 (*d*, 8.4)
1″	126.5	
2″	156.4	
3″	120.6	6.90 (*br d*, 7.5)
4″	128.1	7.19 (*td*, 7.5, 1.8)
5″	110.8	6.87 (*br t*, 7.5)
6″	126.6	7.41 (*dd*, 7.5, 1.8)
2′-OMe	55.5	3.84 (*s*)
2″-OMe	61.5	3.84 (*s*)

1.2 Candenatenin H (DC2)

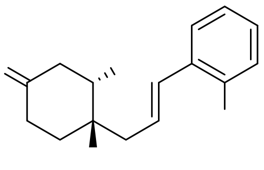


Compound **DC2** was isolated as a colorless viscous oil with the molecular formula $C_{17}H_{18}O_4$, determined from HR-EI-MS ($[M]^+$ *m/z* 286.1199, calcd. 286.1200). The ^1H NMR data (Table 2) showed signal patterns similar to those of **DC1**, due to two methoxyls and coupled ABX_2 protons. Differences included the appearance of the 1,2,3,5-tetrasubstituted aromatic protons at δ 6.52 (1H, *s*, H-6′) and 6.47 (1H, *s*, H-4′). Locations of two methoxyl and two hydroxy groups on **DC2** were clarified by HMBC spectrum of methylene protons H_2-1 with C-1′, C-2′ and C-6′, of 2′-OMe with C-2′, of H-6′ with C-1, C-2′, C-3′ and C-5′, and of H-3 with C-1 and C-2″, indicating the location of two methoxyl at C-2′, C-5′ and two hydroxy groups at C-3′, C-2″. The cross-peaks between H_2-1/2′-OMe and H_2-1/H-6′/5′-OMe were also observed in the NOESY experiments, confirming the assignment of the OMe at C-2′ and C-5′. In the ^1H NMR data, the appearance of ABX_2-type proton signals at δ 6.34 (1H, *br d*, *J* = 15.4 Hz, H-3), 5.93 (1H, *dt*, *J* = 15.4, 7.8 Hz, H-2) and 3.25 (2H, *dd*, *J* = 7.8, 1.5 Hz, H_2-1) with the coupling constant value of 15.4 Hz were signified for *trans*-configuration of –$CH_2CH=CH$– unit. Thus, compound **DC2** was named as candenatenin H.

Table 2 ^{1}H and ^{13}C NMR data (300 MHz) of **DC2** in $CDCl_3$

No.	Candenatenin H **(DC 2)**	
	δ_C	δ_H (*mult.*, *J* in Hz)
1	29.8	3.25 (*dd*, 7.8, 1.5)
2	135.4	5.93 (*dt*, 15.4, 7.8)
3	123.3	6.34 (*br d*, 15.4)
1′	119.0	
2′	151.8	
3′	144.7	
4′	99.4	6.47 (*s*)
5′	140.2	
6′	113.2	6.52 (*s*)
1″	123.7	
2″	152.7	
3″	115.4	6.85 (*br d*, 7.5)
4″	128.7	7.12 (*td*, 7.5, 1.5)
5″	120.2	6.83 (*br t*, 7.5)
6″	129.8	7.06 (*br d*, 7.5)
2′-OMe	56.0	3.67 (*s*)
5′-OMe	56.7	3.73 (*s*)
3′-OH		5.49 (*s*)

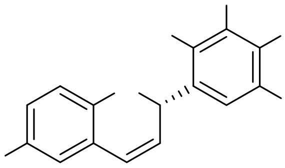
1.3 Candenatenin H (DC3)



Compound **3**, $C_{17}H_{22}O_4$ ($[M]^+$ *m/z* 290.1513, calcd. 290.1499), was obtained as a colorless viscous oil. Its IR spectrum showed a carbonyl stretching frequency (1709 cm^{-1}). The ^1H NMR signals of **DC3** (Table 3) on ring B as well as the $–CH_2CH=CH–$ moiety were similar to those of **DC1**. However, the main differences are all signals in A ring. Compound **DC3** exhibited the presence of cyclohexanone moiety at δ 3.43 (1H, *dd*, *J* = 4.8, 3.0 Hz, H-2′), 2.80 (1H, *dd*, *J* = 14.7, 3.0 Hz, H-3′a), 2.60 (1H, *m*, H-5′a), 2.50 (1H, *m*, H-3′b), 2.17 (1H, *m*, H-5′b), 2.07 (1H, *m*, H-6′a) and 1.74 (1H, *m*, H-6′b). The remaining methoxyl proton signal at δ 3.30 was located at C-2′ because of the HMBC correlations of the oxymethine proton H-2′ (δ 3.43) with the carbon at δ 56.9 (OMe).

The relative configuration of **DC3** was determined on the basis of the coupling constants. The small *J* values of H-2′ (*J* = 4.8 and 3.0 Hz) as well as cross-peaks between 2′-OMe with H_2-1 (δ 2.59 and 2.41) in NOESY spectrum indicated that H-2′ should be equatorially oriented and the OH and OMe groups were *trans* orientation. Therefore, compound **DC3** was named as candenatenin I. Since the isolation process was carried out in MeOH, candenatenin I (**DC3**) could be an artifact via 1,4-Michael addition of MeOH to candenatenin B (Cheenpracha et al., 2009).

1.4 Candenatenin J (DC4)



Compound **DC4** was obtained as a colorless viscous oil and had a molecular formula $C_{18}H_{18}O_6$ as deduced from the HR-EI-MS data ($[M]^+$ *m/z* 330.3329, calcd. 330.3319). The IR spectrum showed absorption bands at 3425 (OH) and 1612 (aromatic ring) cm^{-1}. The UV absorption bands at λ_{max} 211 and 265 nm suggested the presence of a conjugated aromatic chromophore.

Table 3 ^{1}H and ^{13}C NMR data (300 MHz) of **DC3** in $CDCl_3$

No.	Candenatenin I **(DC3)**	
	δ_C	δ_H (*mult.*, *J* in Hz)
1	41.6	2.41 (*dd*, 14.1, 7.8)
		2.59 (*m*)
2	124.6	6.17 (*dt*, 15.9, 7.8)
3	130.2	6.79 (*d*, 15.9)
1′	72.0	
2′	83.6	3.43 (*dd*, 4.8, 3.0)
3′	40.4	2.80 (*dd*, 14.7, 3.0)
		2.50 (*m*)
4′	210.6	
5′	37.0	2.60 (*m*)
		2.17 (*m*)
6′	32.7	2.07 (*m*)
		1.74 (*m*)
1″	126.0	
2″	156.5	
3″	110.9	6.80 (*d*, 7.5)
4″	128.7	7.16 (*br t*, 7.5)
5″	120.7	6.85 (*t*, 7.5)
6″	126.7	7.36 (*dd*, 7.5, 1.5)
2′-OMe	56.9	3.30 (*s*)
2″-OMe	55.4	3.77 (*s*)

The ^{1}H-NMR data (Table 4) displayed the characteristic of flav-3-ene (Cheenpracha et al., 2009; Deodhar et al., 2007) at δ 6.46 (1H, *dd*, *J* = 10.2, 2.0 Hz, H-4), 6.15 (1H, *dd*, *J* = 3.5, 2.0 Hz, H-2) and 5.75 (1H, *dd*, *J* = 10.2, 3.5 Hz, H-3). The ^{1}H NMR data of **DC4** also showed two sets of aromatic proton signals. The first set was a 1,2,4-trisubstituted benzene ring which resonated at δ 6.63 (1H, *d*, *J* = 8.5 Hz, H-8), 6.57 (1H, *dd*, *J* = 8.5, 3.0 Hz, H-7), and 6.52 (1H, *d*, *J* = 3.0 Hz, H-5). The other set was a penta-substituted benzene ring which showed a singlet aromatic proton at δ 6.80 (1H, *s*, H-6′). In addition, three OMe signals at δ 3.94 (3H, *s*), 3.93 (3H, *s*) and 3.88 (3H, *s*) were also observed in the ^{1}H NMR spectrum which were attached to C-3′, C-2′ and C-5′, respectively, on the basis of HMBC correlations. The positions C-4′ and C-6 were attached to OH groups due to the lower field of ^{13}C NMR spectral data at δ 140.2 and 149.6, respectively. The absolute configuration at C-2 was estimated as *S* from the CD spectrum, which showed a negative Cotton effect at 280 nm (Cheenpracha et al., 2009; Garo et al.,1996). Thus, compound **DC4** was named as candenatenin J.

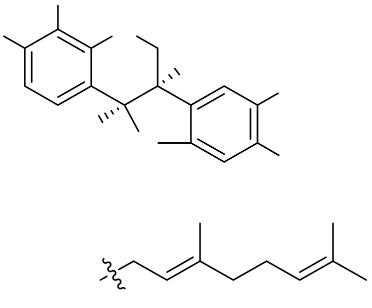
Table 4 NMR data (500 MHz, $CDCl_3$) of **DC4**[a]

No.	Candenatenin J **(DC4)**		
	δ_C	δ_H (*mult.*, *J* in Hz)	HMBC (^{1}H→ ^{13}C)
2	71.2	6.15 (*dd*, 3.5, 2.0)	3, 4, 1′, 2′, 6′
3	125.7	5.75 (*dd*,10.2, 3.5)	2, 4, 1′
4	123.9	6.46 (*dd*, 10.2, 2.0)	2, 5, 8a
4a	122.1		
5	113.1	6.52 (*d*, 3.0)	4, 6, 7, 8a
6	149.6		
7	115.7	6.57 (*dd*, 8.5, 3.0)	5, 6, 8, 8a
8	116.6	6.63 (*d*, 8.5)	6, 7, 8a
8a	147.1		
1′	128.3		
2′	146.1		
3′	145.2		
4′	140.2		
5′	146.0		
6′	108.7	6.80 (*s*)	2, 1′, 2′, 4′

2′-OMe	60.8	3.93 (*s*)	2′
3′-OMe	61.2	3.94 (*s*)	3′
5′-OMe	61.9	3.88 (*s*)	5′

[a] Assignments were made using HMQC and HMBC data.

1.5 Candenatenin K (DC5)



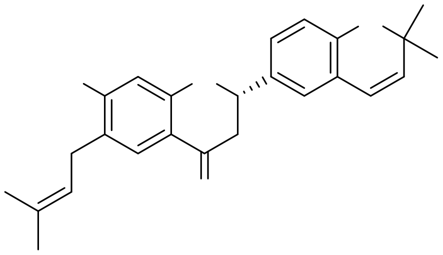
Compound **DC5** was isolated as a colorless viscous oil. Its molecular formula was established as $C_{26}H_{30}O_5$ by HR-EI-MS at *m/z* 422.2098 $[M]^+$ (calcd. 422.2088), suggesting the presence of 12 degrees of unsaturations and supported by NMR data (Table 5). The ^{1}H NMR spectrum showed the characteristic of a pterocarpan structure due to the splitting pattern of the protons at δ 5.39 (1H, *d*, *J* = 6.9 Hz, H-11a), 4.20 (1H, *dd*, *J* = 11.1, 4.5 Hz, H-6α), 3.45 (1H, *t*, *J* = 11.1 Hz, H-6β), and 3.41 (1H, *ddd*, *J* = 11.1, 6.9, 4.5 Hz, H-6a), related to the oxymethylene protons of the heterocyclic ring B, and the bridging protons of rings B and C (H-6a and H-11a) (Belofsky et al., 2006; Woo et al., 2011). The latter proton signals were assigned as *cis*-orientation by NOESY experiments. The signals of three methyl singlets [δ 1.72 (3H, *s*, H_3-10′), 1.59 (3H, *s*, H_3-8′), and 1.51 (3H, *s*, H_3-9′)], two olefinic protons [δ 5.15 (1H, *br t*, *J* = 7.2 Hz, H-2′) and 4.97 (1H, *m*, H-6′)], and three methylenes [δ 3.35 (2H, *m*, H_2-1′), 2.00 (2H, *m*, H_2-5′), and 1.96 (2H, *m*, H_2-4′)] were recognized to geranyl moiety (Woo et al., 2011). In addition, the remaining of two singlet signals of aromatic protons at δ 6.72 (1H, *s*, H-7), and 6.43 (1H, *s*, H-10), and two *ortho*-coupled doublets centered at δ 7.19 (1H, *d*, *J* = 8.4 Hz, H-1), and 6.49 (1H, *d*, *J* = 8.4 Hz, H-2) gave clear evidence of a 3,4,8,9-tetrasubstitution pattern for the pterocarpan moiety (Belofsky et al., 2006). The HMBC correlations (Table 5) of the OMe protons at δ 3.79 with C-8 and of an aromatic proton at δ 6.72 (H-7) with C-6a, C-8, C-9 and C-10a established the attachment of the OMe and OH groups at C-8 and C-9, respectively. Furthermore, the methylene protons at δ 3.35 (H_2-1′) showed HMBC connectivity to C-3, C-4, and C-4a, confirming the location of substituents on the A ring in the NOESY spectrum. This spectral evidence finalized the complete structure of **DC5** which was named as candenatenin K.

Table 5 NMR data (500 MHz, $CDCl_3$) of **DC5**

No.	Candenatenin K **(DC5)**		
	δ_C	δ_H (*mult.*, *J* in Hz)	HMBC ($^1H \rightarrow {}^{13}C$)
1	129.3	7.19 (*d*, 8.4)	11a, 3, 4a
1a	112.6		
2	110.0	6.49 (*d*, 8.4)	1a, 3, 4
3	155.9		
4	114.9		
4a	153.8		
6	66.8	4.20 (*dd,* 11.1, 4.5) 3.45 (*t*, 11.1)	6a, 11a, 4a, 7a
6a	40.3	3.41 (*ddd*, 11.1, 6.9, 4.5)	6, 4a, 7a
7	107.9	6.72 (*s*)	6a, 7a, 8, 9, 10a
7a	117.1		
8	141.1		
9	146.7		
10	98.1	6.43 (*s*)	7a, 8,9, 10a
10a	154.0		
11a	78.8	5.39 (*d*, 6.9)	6, 6a, 1, 1a, 4a
1′	22.3	3.35 (*m*)	3, 4, 4a, 2′
2′	121.5	5.15 (*br t*, 7.2)	1′, 4′, 10′
3′	138.4		
4′	39.7	1.96 (*m*)	2′, 3′, 5′, 6′, 10′
5′	26.4	2.00 (*m*)	3′, 4′, 6′, 7′
6′	123.9	4.97 (*m*)	
7′	131.9		

8′	25.6	1.59 (*s*)	6′, 7′, 9′
9′	17.7	1.51 (*s*)	6′, 7′, 8′
10′	16.2	1.72 (*s*)	2′, 3′, 4′
8-OMe	57.0	3.79 (*s*)	8

1.6 Dinklagin A (DC6)



Compound **DC6** was isolated as a colorless viscous oil. The ^{1}H NMR (Table 6) spectroscopic displayed the characteristic of flavanone moiety at δ 5.32 (*dd*, *J* = 13.5, 2.9 Hz), 3.02 (*dd*, *J* = 17.0, 13.5 Hz) and 2.76 (*dd*, *J* = 17.0, 2.9 Hz). Two sets of aromatic proton signals were observed in the ^{1}H NMR spectrum. The first set was a 1,2,4,5-tetrasubstituted benzene ring which resonated at δ 7.69 (*s*) and 6.43 (*s*). The other set was a 1,3,5-trisubstituted benzene ring which showed the aromatic protons at δ 7.02 (*d*, *J* = 2.3 Hz), 7.18 (*dd*, *J* = 7.5, 2.3 Hz) and 6.80 (*d*, *J* = 7.5 Hz). In addition, the presence of a isoprene unit resonated at δ 5.30 (*m*), 3.32 (*brd*, *J* = 7.6 Hz) and 1.74 (*s*) and a chromene unit at δ 6.34 (*d*, *J* = 10.0 Hz), 5.64 (*d*, *J* = 10.0 Hz) and 1.44 (*s*). From above data, compound **DC6** was assigned as dinklagin A (Ngadjui et al., 2002)

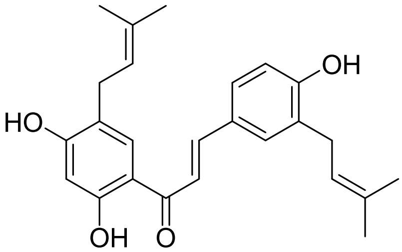
Table 6 Comparison of ^{1}H NMR data (500 MHz, $CDCl_3$) of **DC6** and dinklagin A

No.	Dinklagin A δ_H (*mult.*, *J* in Hz)	**DC6** δ_H (*mult.*, *J* in Hz)
2	5.35 (*dd*, 13.4, 2.8)	5.32 (*dd*, 13.5, 2.9)
3	3.00 (*dd*, 16.8, 13.3)	3.02 (*dd*, 17.0, 13.5)
	2.78 (*dd*, 16.8, 2.8)	2.76 (*dd*, 17.0, 2.9)
5	7.71 (*s*)	7.69 (*s*)
8	6.45 (*s*)	6.43 (*s*)
2′	7.09 (*d*, 2.1)	7.02 (*d*, 2.3)
5′	6.82 (*d*, 8.3)	6.80 (*d*, 7.5)
6′	7.20 (*dd*, 8.3, 2.1)	7.18 (*dd*, 7.5, 2.3)

Table 6 (continued)

No.	Dinklagin A δ_H (*mult*., *J* in Hz)	**DC6** δ_H (*mult*., *J* in Hz)
1″	3.34 (*d*, 7.1)	3.32 (*brd*, 7.6)
2″	5.30 (*brt*, 7.1)	5.30 (*m*)
4″	1.79 (*s*)	1.74 (s)
5″	1.79 (*s*)	1.74 (*s*)
4‴	6.35 (*d*, 9.9)	6.34 (*d*, 10.0)
5‴	5.67 (*d*, 9.8)	5.64 (*d*, 10.0)
6‴(Me)$_2$	1.46 (*s*)	1.44 (*s*)
7-OH	6.02 (*brs*)	5.89 (*s*)

1.7 Stipulin (DC7)

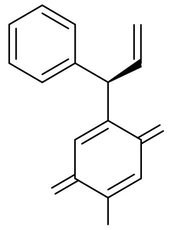


Compound **DC7** was obtained as a colorless viscous oil and its ^{1}H NMR data (Table 7) showed the characteristic of chalcone group at δ 7.73 (*d*, *J* = 15.6 Hz) and 7.33 (*d*, *J* = 15.6 Hz). The geometry of double bond was indicated as *trans*-orientation. The aromatic protons at δ 7.54 (*s*), 6.34 (*s*), 7.33 (*m*) and 7.33 (*m*), 6.77 (*d*, *J* = 8.1 Hz) were assigned as 1,2,4,5-tetrasubstituted and 1,2,4-trisubstituted benzene rings, respectively. In addition, the ^{1}H NMR spectroscopic displayed the presence of two isoprene units at δ 5.25 (2H, *br t*, *J* = 7.2 Hz), 3.31 (2H, *d*, *J* = 7.2 Hz), 3.25 (2H, *d*, *J* = 7.2 Hz) and 1.71 (12H, s). From above data, compound **DC7** was named as stipulin (Ngameni et al., 2004).

Table 7 Comparison of ^{1}H NMR data (500 MHz, $CDCl_3$) of stipulin and **DC7**

No.	Stipulin δ (*mult.*, *J* in Hz)	**DC7** δ (*mult.*, *J* in Hz)
2	7.42 (*brs*)	7.54 (*s*)
5	6.87 (*d*, 8.3)	6.34 (*s*)
6	7.44 (*d*, 8.3)	7.33 (*m*)
3′	6.43 (*brs*)	6.77 (*d*, 8.1)
6′	7.65 (*s*)	7.33 (*m*)
α	7.46 (*d*, 15.4)	7.33 (*d*, 15.6)
β	7.84 (*d*, 15.4)	7.73 (*d*, 15.6)
1″	3.42 (*d*, 7.1)	3.25 (*d*, 7.2)
2″	5.35 (*t*, 7.1)	5.25 (*br t*, 7.2)
4″	1.82 (*s*)	1.71 (*s*)
5″	1.82 (*s*)	1.71 (*s*)
1‴	3.37 (*d*, 7.1)	3.31 (*d*, 7.2)
2‴	5.35 (*t*, 7.1)	5.25 (*br t*, 7.2)
4‴	1.82 (*s*)	1.71 (*s*)
5‴	1.82 (*s*)	1.71 (*s*)
2′-OH	13.34 (*s*)	13.34 (*s*)

1.8 (*R*)-4-Methoxydalbergione (DC8)

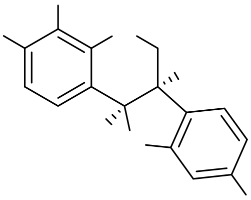


Compound **DC8** was isolated as a colorless viscous oil and its ^{1}H NMR data (Table 8) displayed the presence of monosubstituted benzene ring at δ 7.32 (2H, *t*, *J* = 8.4 Hz), 7.27 (1H, *t*, *J* = 8.4 Hz), and 7.19 (2H, *d*, *J* = 8.4 Hz), of monosubstituted alkene at δ 6.10 (1H, *ddd*, *J* = 16.8, 10.2, 6.8 Hz), 5.28 (1H, *d*, *J* = 10.2 Hz), 5.00 (1H, *dd*, *J* = 16.8, 1.2 Hz) and 4.93 (1H, *d*, *J* = 6.8 Hz) and of methoxyl group at δ 3.81 (*s*). Finally, the ^{1}H and ^{13}C NMR (Table 10) displayed the presence of a benzoquinone at δ 6.49 (*d*, *J* = 0.9 Hz) and 5.91 (*s*). Comparison of ^{13}C NMR data of **DC8** and (*R*)-4-methoxydalbergione was indicated similarity. Thus, compound **DC8** was named as (*R*)-4-methoxydalbergione (Chan et al., 1997).

Table 8 Comparison of NMR data of (*R*)-4-methoxydalbergione and **DC8**

No.	(*R*)-4-Methoxydalbergione	**DC8**	
	δ_C	δ_C	δ_H (*mult.*, *J* in Hz)
1	151.0	151.3	
2	186.8	186.5	
3	108.4	108.6	5.91 (*s*)
4	159. 7	159.6	
5	182.5	182.5	
6	132.1	132.1	6.49 (*d*, 0.9)
1′	141.1	141.0	
2′	129.3	129.7	7.19 (*d*, 8.4)
3′	129.4	129.3	7.32 (*t*, 8.4)
4′	127.6	127.6	7.27 (*t*, 8.4)
5′	129.4	129.6	7.32 (*t*, 8.4)
6′	129.3	129.9	7.19 (*d*, 8.4)
H_A	48.1	48.1	4.93 (*d*, 6.8)
H_X	138.9	138.3	6.10 (*ddd* ,16.8, 10.2, 6.8)
CH_2	117.9	117.8	5.28 (*d*, 10.2)
			5.00 (*dd*, 16.8, 1.2)
OMe	56.6	56.4	3.81 (*s*)

1.9 Melilotocarpan A (DC9)



Compound **DC9** was isolated as a colorless viscous oil. The ^{1}H NMR spectrum (Table 9) showed the characteristic of a pterocarpan structure due to the splitting pattern of the protons at δ 5.51 (1H, *d*, *J* = 6.5 Hz, H-4), 4.36 (1H, *dd*, *J* = 10.5, 4.8 Hz, H-2β), 3.64 (1H, *d*, *J* = 10.5 Hz, H-2α), and 3.57 (1H, *dd*, *J* = 6.5, 4.8 Hz, H-3), related to the oxymethylene protons of the heterocyclic ring B, and the bridging protons of rings B and C (H-3 and H-4) (Belofsky et al., 2006; Woo et al., 2011). The latter proton signals were assigned as *cis*-orientation by NOESY experiments. In addition, the remaining of two doublet signals of aromatic protons at δ 7.08 (1H, *d*, *J* = 8.5 Hz, H-5), and 6.61 (1H, *d*, *J* = 8.5 Hz, H-6), and 1,3,4-trisubstituted benzene ring at δ 6.99 (1H, *d*, *J* = 8.5 Hz, H-6′), 6.39 (1H, *dd*, *J* = 8.5, 2.3 Hz, H-5′) and 6.37 (1H, *brs,* H-3′). Comparison NMR data of compound **DC9** and melilotocarpan A showed the same patterns. Thus, compound **DC9** was named as melilotocarpan A (Belofsky et al., 2006).

Table 9 Comparison of NMR data of melilotocarpan A and **DC9**

No.	Melilotocarpan A		**DC9**	
	δ_C	δ_H (*mult*., *J* in Hz)	δ_C	δ_H (*mult*., *J* in Hz)
2	66.9	3.64 (*d*, 10.5)	67.0	3.59 (*d*, 10.2)
		4.36 (*dd*, 10.5, 4.8)		4.30 (*dd*, 10.2, 3.4)
3	39.6	3.57 (*dd*, 6.5,4.8)	39.7	3.54 (*m*)
4	78.7	5.51 (*d*, 6.5)	78.5	5.48 (*d*, 6.5)
5	126.3	7.19 (*d*, 8.4)	124.8	7.08 (*d*, 8.5)
6	109.2	6.71 (*d*, 8.4)	106.5	6.61 (*d*, 8.5)

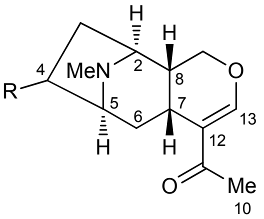
Table 9 (continued)

No.	Melilotocarpan A		**DC9**	
	δ_C	δ_H (*mult*., *J* in Hz)	δ_C	δ_H (*mult*., *J* in Hz)
7	135.1		134.1	
8	149.9		147.4	
9	148.7		143.4	
10	113.4		114.1	
1′	119.1		118.9	
2′	160.9		160.7	
3′	106.7	6.47 (*d*, 2.2)	105.5	6.37 (*brs*)
4′	161.4		161.3	
5′	97.1	6.47 (*dd*, 8.8, 2.2)	97.0	6.39 (*dd*, 8.5, 2.3)
6′	125.0	7.15 (*d*, 8.8)	121.1	6.99 (*d*, 8.5)
7-OMe	61.4	3.92 (*s*)	56.3	3.85 (*s*)
4′-OMe	55.7	3.78 (*s*)	55.5	3.70 (*s*)

2 Chemical constituents from *A. macrophylla*

The bark of *A. macrophylla* was extracted with MeOH and partitioned between EtOAc and 1% HCl. The aqueous layer was adjusted to pH 8-9 with saturated Na_2CO_3; then the water soluble materials were extracted in fresh EtOAc. The EtOAc-soluble material was purified by silica gel column chromatography to afford two new unusual nitrogenous derivatives (**AM1**, **AM2**) and a new indole alkaloid (**AM4**) from the bark of *A. macrophylla*, in addition to eight known alkaloids: angustimaline (**AM3**) (Kam et al., 1997), alstonerinal (**AM5**) (Kam, Iek, and Choo, 1999), alstophyllal (**AM6**) (Kam, and Choo, 2004), talcarpine (**AM7**) (Wong, Lim, and Chuah, 1996), macrocarpine B (**AM8**) (Kam, Choo, and Komiyama, 2004), alstonisine (**AM9**) (Wong, Lim, and Chuah, 1996), N_b-demethylalstophylline oxindole (**AM10**) (Wong, Lim, and Chuah, 1996) and 10-methoxycathafoline (**AM11**) (Kam, Iek, and Choo, 1999). The known compounds were identified by comparison of their physical and spectroscopic data with published values.

2.1 Alstoniaphylline A (AM1) and angustimaline (AM3)



AM1; R = =O

AM3; R = -OH

Alstoniaphylline A (**AM1**) was isolated as a light yellow gum. Its molecular formula, $C_{13}H_{18}NO_3$, was determined by the pseudomolecular ion peak at *m/z* 236.1287 $[M+H]^+$ (calcd. for 236.1281) in the APCI-TOFMS. The IR spectrum displayed absorption bands for a ketone carbonyl (1743 cm^{-1}) and conjugated carbonyl (1651 cm^{-1}) stretching, and the UV absorption maxima at λ_{max} 210 and 257 nm supported the presence of an α,β-unsaturated ketone in the structure. The structure of **AM1** was established conclusively by ^1H and ^{13}C NMR (Table 10). The ^{13}C NMR and DEPT spectroscopic data showed the presence of 13 carbons attributable to two methyls, [δ 42.4 (*N*-Me) and 25.1 (C-10)], three methylenes, of which one is an oxymethylene carbon [δ 67.0 (C-9), 37.2 (C-3) and 32.3 (C-6)], five methines, of which one is an olefinic [δ 157.4 (C-13), 69.1 (C-5), 60.3 (C-2), 36.9 (C-8) and 23.4 (C-7),] and three quaternary carbons, of which two are ketone and conjugated ketone carbonlys [δ 219.0 (C-4), 195.5 (C-11) and 120.8 (C-12)]. The ^1H NMR data (Table 10) displayed the presence of a lower field signal at δ 7.57 (1H, *s*, H-13) attributable to a vinylic proton associated with a vinyl ether function, an *N*-methyl singlet signal at δ 2.31 (3H, *s*, *N*-Me) and an acetyl group at δ 2.19 (3H, *s*, H-10). These data was in good agreement with the E-ring of a typical type-B macroline such as angustimaline (**AM3**) which was previously isolated from *A. angustifolia*. However, the oxymethine carbon signal at C-4 in **AM3** was replaced with a keto group (δ 219.0) in **AM1**. The connectivities of H-2/H_2-3, H-2/H-8, H-8/H_2-9, H-7/H_2-6 and H_2-6/H-5 in the COSY spectrum led to the assignments of the characteristic of macroline compounds and correspond to the C(3)–C(2)–C(8)–C(9) and C(5)–C(6)-C(7) fragments, respectively. The molecule can therefore be assembled accordingly and requires only insertion of a ketone function to complete the structure of alstoniaphylline A as shown in **AM1**, which is in perfect agreement with the HMBC data.

The HMBC cross-peaks of the methine proton [δ 3.44 (H-2)] with the carbons at δ 219.0 (C-4), 67.0 (C-9), 36.9 (C-8) and 23.4 (C-7), of the methylene protons [δ 2.30, 1.63 (H_2-6)] with the carbons at δ 219.0 (C-4), 69.1 (C-5) and 23.4 (C-7), and of *N*-Me (δ 2.31) with the carbons at δ 69.1(C-5) and 60.3 (C-2) confirmed a ketone carbonyl at C-4. HMBC correlations for an olefinic proton [δ 7.57 (H-13)] with the carbons at δ 195.5 (C-11), 120.8 (C-12), 67.0 (C-9) and 23.4 (C-7), the methyl group [δ 2.19 (H_3-10)] with the carbons at δ 195.5 (C-11) and 120.8 (C-12), and the methylene protons [δ 4.48, 4.22 (H_2-9)] with the carbons at δ 157.4 (C-13), 36.9 (C-8) and 23.4 (C-7) indicated alstoniaphylline A possessed the non-indole portion of a type-B macroline alkaloid. The ring junction stereochemistry between rings C, D and E in **AM1** is assumed to follow the same pattern as known macroline compounds, i.e. angustimaline (**AM3**) and alstonerinal (**AM5**), on the basis of the chemical shifts and NOE data. The interactions between H-2/*N*-Me/*N*-5 and H-7/H-8 were observed for **1** in the NOE experiments, confirming the C/D and D/E ring junction stereochemistry as *cis*-orientation. This compound is probably derived from fragmentation of a macroline-type precursor. Therefore, the structure of **AM1** was assigned as alstoniaphylline A.

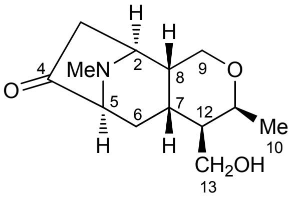
Table 10 NMR data of compounds **AM1** and **AM3**

No.	Alstoniaphylline A **(AM1)**		Angustimaline (**AM3**)
	δ_C, type	δ_H (*mult.J* in Hz)	δ_H (*mult. J* in Hz)
2	60.3, CH	3.44 (*d*, 7.8)	3.32 (*d*, 7.8)
3	37.2, CH_2	2.77 (*dd*, 17.5, 7.8)	2.30 (*m*)
		2.13 (*d*, 17.5)	2.05 (*m*)
4	219.0, C		4.11 (*m*)
5	69.1, CH	2.85 (*m*)	3.06 (*brs*)
6	32.3, CH_2	2.30 (*m*)	2.05 (*m*)
		1.63 (*m*)	1.43 (*m*)
7	23.4, CH	2.85 (*m*)	2.55 (*m*)

Table 10 (continued)

No.	Alstoniaphylline A **(AM1)**		Angustimaline (**AM3**)
	δ_C, type	δ_H (*mult.J* in Hz)	δ_H (*mult. J* in Hz)
8	36.9, CH	1.92 (*m*)	1.64 (*m*)
9	67.0, CH_2	4.48 (*t*, 11.6)	4.63 (*t*, 11.6)
		4.22 (*ddd*, 11.6, 2.4, 2.0)	4.32 (*ddd*, 11.6, 2.4, 2.0)
10	25.1, CH_3	2.19 (*s*)	2.18 (*s*)
11	195.5, C		
12	120.8, C		
13	157.4, CH	7.57 (s)	7.54 (*s*)
N-Me	42.4, CH_3	2.31 (s)	2.53 (*s*)

2.2 Alstoniaphylline B (AM2)



Alstoniaphylline B **(AM2)** was obtained as a light yellow gum. The molecular formula was established as $C_{13}H_{22}NO_3$ by ESI-TOFMS data, according to the $[M+H]^+$ ion peak at *m/z* 240.1591 (calcd. for 240.1600). The IR spectrum demonstrated the absorption bands of hydroxy (3405 cm^{-1}) and ketone carbonyl (1743 cm^{-1}) functionalities, indicative of a five-membered ring ketone. The ^{13}C NMR spectrum (Table 11) showed a total of 13 peaks in agreement with the molecular formula (two methyls, four methylenes, six methines and one quaternary carbon). Two methyl groups were indicated, an *N*Me (δ 2.31) and a Me-CH- (δ 1.20). The quaternary carbon resonance at *v* 220.0 is due to a ketone function.

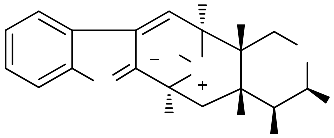
The ^1H and ^{13}C NMR spectral data (Table 11) revealed a non-indole portion resembling **AM1** in all respects, except for the absence of the vinylic proton at δ 7.57

(1H, *s*, H-13), one methine at δ 3.44 (1H, *m*, H-11) and one pair of methyl doublet protons at δ 1.20 (3H, *d*, *J* = 6.1 Hz, H_3-10). The presence of an acetyl group at δ 2.19 (3H, *s*, H-10) in the saturated E-ring of **AM2** replaced of the oxymethylene protons at δ 3.65 (1H, *dd*, *J* = 11.8, 5.0 Hz, H-13a) and 3.30 (1H, *m*, H-13b) in **AM1**. Analysis of the COSY, HMQC and HMBC spectra led to structure **AM2** for this compound. The HMBC correlations of H_3-10 (δ 1.20) with the carbons at δ 70.1 (C-11) and 46.6 (C-12), and of H_2-13 (δ 3.65 and 3.30) with the carbons at δ 70.1 (C-11), 46.6 (C-12) and 27.4 (C-7) suggested that H_3-10 should be attached to C-11. The relative stereochemistry of the substituents at C-11 and C-12 was readily confirmed from the observed NOE interaction between H_2-13/H-8, H-11/H-9a and H-11/H-6α. The structure of **AM2** was therefore elucidated as alstoniaphylline B, which retains all of the features of the non-indole portion of a type-A macroline compound (Ghedira et al., 1988).

Table 11 NMR data of compound **AM2**

No.	Alstoniaphylline B **(AM2)**	
	δ_C, type	δ_H (*mult., J* in Hz)
2	60.8, CH	3.44 (*m*)
3	37.0, CH_2	2.68 (*dd*, 18.7, 11.5), 2.00 (*dd*, 18.7, 3.7)
4	220.0, C	
5	69.2, CH	2.92 (*brs*)
6	26.7, CH_2	2.01 (*m*)
		1.77 (*m*)
7	27.4, CH	2.26 (*m*)
8	40.6, CH	1.87 (*m*)
9	67.1, CH_2	4.13 (*t*, 11.8), 3.80 (*dd*, 11.8, 5.4)
10	20.0, CH_3	1.20 (*d*, 6.1)
11	70.1, CH	3.44 (*m*)
12	46.6, CH	1.68 (*m*)
13	60.9, CH_2	3.65 (*dd*, 11.8, 5.0), 3.30 (*m*)
N-Me	42.2, CH_3	2.31 (*s*)

2.3 Alstoniaphylline C (AM4)



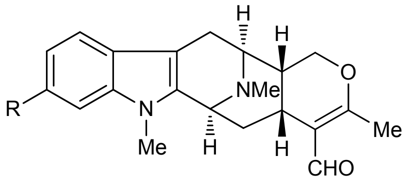
Alstoniaphylline C (**AM4**) was isolated as a colorless oil with the molecular formula $C_{20}H_{24}N_2O_3$, as determined by ESITOFMS ($[M + H]^+$, *m/z* 341.1853, calcd 341.1865). IR absorptions at 3397 cm^{-1} implied the presence of a hydroxy functionality. The UV spectrum showed absorption maxima at λ_{max} 210, 250, 289, and 320 nm, indicating the presence of a typical modified indolenine chromophore (Kam, and Choo, 2004). This was confirmed by the characteristic chemical shift of C-2 at δ 182.2 in the ^{13}C NMR spectrum due to the conjugated imino carbon (Feng et al., 2009).

The ^{1}H NMR spectroscopic data (Table 12) revealed the presence of a substituted indole moiety due to the signals of four aromatic protons [δ 7.69 (1H, *d*, *J* = 8.1 Hz, H-9), 7.45 (1H, *t*, *J* = 8.1Hz, H-11), 6.85 (1H, *t*, *J* = 8.1 Hz, H-10), and 6.75 (1H, *d*, *J* = 8.1 Hz, H-12)] and an E-ring from a typical type-A macroline (e.g., macrocarpine A (Kam, Choo, and Komiyama, 2004) and **AM2**) [δ 4.05 (1H, *t*, *J* = 11.8 Hz, H-17α), 3.82 (1H, *dd*, *J* = 11.8, 4.8 Hz, H-17β), 3.60 (1H, *dd*, *J* = 10.8, 4.8 Hz, H-21a), 3.51 (1H, *dd*, *J* = 10.8, 6.3 Hz, H-21b), 3.45 (1H, *m*, H-19), 2.18 (1H, *m*, H-15), 1.95 (1H, *br d*, *J* = 11.8 Hz, H-16), 1.62 (1H, *m*, H-20), and 1.21 (3H, *d*, *J* = 6.3 Hz, H_3-18)]. The full ^{1}H and ^{13}C NMR spectroscopic assignments for **AM4** based on 2D NMR experiments (Table 12) indicated that **AM4** had the same skeleton as macrocarpine A, except for the absence of methylene proton signals [δ 3.25 (1H, *dd*, *J* = 17, 7 Hz, H-6a), 2.47 (1H, *m*, H-6b)] and *N*(1)-Me [δ 3.62 (3H, s)]. A ^{1}H NMR resonance at δ 6.08 (1H, *d*, *J* = 5.8 Hz, H-6), which correlated with the methine proton at δ 3.27 (1H, *br d*, *J* = 5.8 Hz) in the ^{1}H–^{1}H COSY spectrum, was attributed to the C-6 vinylic proton. This finding was confirmed by a HMBC experiment, demonstrating correlations of H-6 with C-2, C-5, and C-7. In addition, alstoniaphylline C (**AM4**) possessed an N_4-oxido group, deduced from the downfield chemical shifts at δ 68.4 (C-3), 56.3 (C-5), and 41.7 (N_4-Me) compared to δ 54.8 (C-3), 53.6 (C-5), and 23.2 (N_4-Me) in **AM5** (Kam, Iek, and Choo, 1999). The relative configuration of **AM4** was readily confirmed from the observed NOE interactions and comparison with the known, published compound macrocarpine A.

Table 12 NMR Spectroscopic data (400 MHz, $CDCl_3$) for alstoniaphylline C (**AM4**)

No.	Alstoniaphylline C (**4**)	
	δ_C, type	δ_H (*mult., J* in Hz)
2	182.2, C	
3	68.4, CH	4.70 (*br s*)
4		
5	56.3, CH	3.27 (*br d*, 5.8)
6	107.8, CH	6.08 (*d*, 5.8)
7	137.2, C	
8	120.8, C	
9	125.3, CH	7.69 (*d*, 8.1)
10	118.8, CH	6.85 (*t*, 8.1)
11	136.6, CH	7.45 (*t*, 8.1)
12	107.4, CH	6.75 (*d*, 8.1)
13	152.0, C	
14	27.1, CH_2	2.21 (*dd*, 7.6, 2.8)
		1.84 (*dd*, 7.6, 1.8)
15	25.9, CH	2.18 (*m*)
16	39.7, CH	1.95 (*br d*, 11.8)
17	67.0, CH_2	4.05 (*t*, 11.8)
		3.82 (*dd*, 11.8, 4.8)
18	20.0, CH_3	1.21 (*d*, 6.3)
19	70.0, CH	3.45 (*m*)
20	46.9, CH	1.62 (*m*)
21	61.4, CH_2	3.60 (*dd*, 10.8, 4.8)
		3.51 (*dd*, 10.8, 6.3)
N-Me	41.7, CH_3	2.24 (*s*)

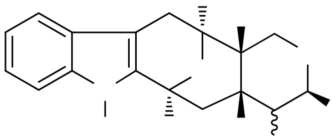
2.4 Alstonerinal (AM5) and alstophyllal (AM6)



AM5; R = H
AM6; R = OMe

Compounds **AM5** and **AM6** were isolated as a light yellowish oil and obtained of type-A macroline isomers. The ^{1}H NMR spectrum (Table 13) indicated a macroline indole derivative with aromatic methoxy substitution at C-11 for **AM6**. Other clear signals include those due to the characteristic *N*(1)-Me, *N*(4)-Me, and Me-18 groups. In the case of the ^{1}H NMR spectrum, the H-18 (methyl) and H-21 (aldehyde-H) signals are clearly distinguishable as before (Table 13), while the remaining hydrogen resonances are partially overlapped or coincident. Compound **AM5** displayed the presence of indole moiety at δ 7.50 (*d*, *J* = 8 Hz), 7.34 (*d*, *J* = 8 Hz), 7.34 (*d*, *J* = 8 Hz) and 7.11 (*t*, *J* = 8 Hz) and of a type-A macroline at δ 9.68 (*s*), 4.20 (*m*), 4.49 (*t*, *J* = 11 Hz), 3.90 (*m*), 3.35 (*dd* , *J* = 16, 7 Hz), 3.11 (*brt*, *J* = 7 Hz), 2.64 (*dt*, *J* = 12, 5 Hz), 2.53 (*d*, *J* = 16 Hz), 2.15 (*m*), 1.93 (*m*) and 1.85 (*m*). The ^{1}H NMR data of **AM6** showed similar to those of **AM5**, except for the appearance of a methoxyl group at C-11. From above data, compounds **AM5** and **AM6** were designed as alstonerinal and alstophyllal, respectively.

2.5 Talcarpine (AM7) and macrocarpine B (AM8)



Compounds **AM7** and **AM8** were obtained as a light yellowish oil. Examination of the ^{1}H NMR spectral data (Tables 14) revealed a macroline compound resembling talcarpine (Wong, Lim, and Chuah, 1996) and macrocarpine B (Kam, Choo, and Komiyama, 2004) in all respects, respectively. Characteristic ^{1}H NMR signals of **AM7** [δ 7.51 (*d, J* = 7 Hz) 7.31 (*d*, *J* = 7 Hz) 7.21 (*t*, *J* = 7 Hz) and 7.12 (*t*, *J* = 7 Hz)] indicated the presence of an indole moiety.

Table 13 Comparison of ^{1}H NMR data of compounds **AM5, AM6** and alstonerinal, alstophyllal

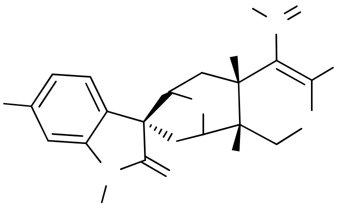
No.	**Alstonerinal**	**Alstophyllal**	**AM5**	**AM6**
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
3	3.86 (*t*, 3)	3.84 (*t*, 3)	3.90 (*m*)	3.84 (*t*, 3)
5	3.06 (*brd*, 7)	3.06 (*d*, 7)	3.11 (*brt*, 7)	3.06 (*d*, 7)
6	2.48 (d, 16)	2.45 (*d*, 16)	2.53 (d, 16)	2.45 (*d*, 16)
	3.31 (*dd* ,16, 7)	3.29 (*dd*, 16, 7)	3.35 (*dd* ,16, 7)	3.29 (*dd*, 16, 7)
9	7.44 (*brd*, 8)	7.34 (*d*, 8)	7.50 (*d*, 8)	7.34 (*d*, 8)
10	7.08 (*td*, 8, 1)	6.76 (*dd*, 8, 2)	7.11 (*t*, 8)	6.76 (*dd*, 8, 2)
11	7.19 (*td*, 8, 1)		7.22 (*t*, 8)	
12	7.31 (*brd*, 8)	6.81 (*d*, 2)	7.34 (*d*, 8)	6.81 (*d*, 2)
14	1.79 (*td*, 12, 3)	1.77 (*td*, 12, 4)	1.85 (*m*)	1.77 (*td*, 12, 4)
	2.12 (*ddd*, 12, 5, 3)	2.14 (*m*)	2.15 (*m*)	2.14 (*m*)
15	2.61 (*dt*, 12, 5)	2.61 (*m*)	2.64 (*dt*, 12, 5)	2.61 (*m*)
16	1.89 (*m*)	1.89 (*m*)	1.93 (*m*)	1.89 (*m*)
17	4.18 (*ddd*, 11, 4, 2)	4.19 (*ddd*, 11, 4, 2)	4.20 (*m*)	4.19 (*ddd*, 11, 4, 2)
	4.46 (*t*, 11)	4.46 (*t*, 11)	4.49 (*t*, 11)	4.46 (*t*, 11)
18	2.15 (*s*)	2.17 (*s*)	2.11 (*s*)	2.17 (*s*)
21	9.65 (*s*)	9.66 (*s*)	9.68 (*s*)	9.66 (*s*)
N(1)-Me	3.63 (*s*)	3.60 (*s*)	3.68 (*s*)	3.60 (*s*)
N(4)-Me	2.31 (*s*)	2.32 (*s*)	2.35 (*s*)	2.32 (*s*)
11-OMe		3.89 (*s*)		3.89 (*s*)

Comparison ^{1}H NMR spectroscopic of **AM7** and talcarpine (Wong, Lim, and Chuah, 1996) showed the same pattern signal. Thus, compound **AM7** was named as talcarpine. While compound **AM8** also exhibited the ^{1}H NMR signals similar to those of AM7 except that the addition signal of *N*(1)-Me and oxymethylene protons in **AM8** were observed. The disappear signal of formyl group indicated that the oxymethylene protons were place at C-21. Thus, compound **AM8** was assigned as macrocarpine B.

Table 14 Comparison of ^{1}H NMR data of compounds **AM7, AM8,** talcarpine and macrocarpine B

No.	**Talcarpine**	**Macrocarpine B**	**AM7**	**AM8**
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
3	3.92-4.05 (*m*)	3.98 (*t*, 3)	4.00 (*m*)	4.03 (*brs*)
5	2.89 (*d*, 7)	2.91 (*d*, 7)	2.93 (*d*, 7)	2.95 (*d*, 7)
6	3.24 (*dd,* 16, 7)	2.43 (*d*, 17)	3.29 (*dd,* 16, 7)	2.47 (*d*, 17)
		3.26 (*dd*, 17, 7)		3.29 (*dd*, 17, 7)
9	7.48 (*br d,* 7)	7.49 (*br d*, 8)	7.51 (*d,* 7)	7.52 (*br d*, 8)
10	7.09 (*br t*, 7)	7.10 (*td*, 8, 1)	7.12 (*t*, 7)	7.13 (*td*, 8, 1)
11	7.19 (*br t*, 7)	7.18 (*td*, 8, 1)	7.21 (*t*, 7)	7.21 (*td*, 8, 1)
12	7.28 (*br d*, 7)	7.29 (*br d*, 8)	7.31 (*d*, 7)	7.32 (*br d*, 8)
14	2.41-2.55 (*m*)	1.54 (*ddd*, 12, 4, 3)	2.51 (*m*)	1.57 (*m*, 12, 4, 3)
	1.40-1.48 (*m*)	2.29 (*m*)	1.47 (*m*)	2.31 (*m*)
15	2.15-2.24 (*m*)	1.97 (*dt*, 13, 4)	2.22 (*m*)	1.95 (*m*)
16	2.00-2.09 (*m*)	1.86 (*dt*, 11, 4)	2.08 (*m*)	1.86 (*dt*, 11, 4)
17	4.13 (*t*, 11)	3.73 (*dd*, 11, 4)	4.16 (*t*, 11)	3.76 (*dd*, 11, 4)
	3.89 (*dd,* 11, 5)	4.06 (*t*, 11)	3.92 (*dd,* 11, 5)	4.10 (*t*, 11)
18	1.30 (*d*, 6.8)	1.15 (*d*, 6)	1.32 (*d*, 6.8)	1.18 (*d*, 6)
19		3.49 (*m*)		3.52 (*m*)
20	1.78 (*br s*)	1.46 (*m*)	1.81 (*br s*)	1.50 (*m*)
21	9.94 (*d*, 3)	3.31 (*dd*, 11, 8)	9.97 (*d*, 3)	3.35 (*m*)
		3.49 (*m*)		3.52 (*m*)
N(1)-Me	3.62 (*s*)	3.62 (*s*)	3.65 (*s*)	3.65 (*s*)
N(4)-Me	2.32 (*s*)	2.30 (*s*)	2.35 (*s*)	2.35 (*s*)

2.6 Alstonisine (AM9) and N_b-Demethylalstophylline oxindole (AM10)



Two macroline oxindoles, **AM9** and **AM10**, corresponding to the type-B isomers were obtained. The ^{13}C NMR spectra for both **AM9** and **AM10** showed the oxindole lactam carbonyl resonance at δ 182.2 and the spirocyclic C-7 resonance at δ 57.3, which are characteristic of macroline oxindoles possessing the more common *R* configuration at the spirocenter C-7. The ^{1}H NMR spectra (Table 15) of both compounds showed many features in common with other macroline oxindoles, such as the *N*(1)-Me, the characteristic ring junction hydrogens, H-3 and H-5, COMe and vinyl-H. However, in contrast to other macroline indoles, the signal due to H-11 is absent, and the signal due to OMe has undergone a change in their coupling pattern. From above data, compounds **AM9** and **AM10** were assigned as alstonisine and N_b-demethyl alstophylline oxindole**.**

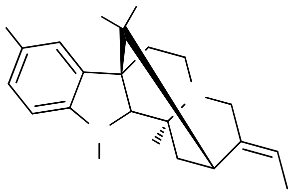
Table 15 Comparison of ^{1}H NMR data of compounds **AM9, AM10**, alstonisine and N_b-demethyl alstophylline oxindole

No.	**Alstonisine**	**N_b-Demethyl alstophylline oxindole**	**AM9**	**AM10**
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
3	3.15-3.20 (*m*)	3.10-3.22 (*m*)	3.20 (*m*)	3.16 (*m*)
5	3.68 (*d*, 7)	3.66 (*br d,* 7)	3.69 (*m*)	3.68 (*m*)

Table 15 (continued)

No.	**Alstonisine**	***N*$_b$-Demethyl alstophylline oxindole**	**AM9**	**AM10**
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
6	2.19 (*d*, 14) 2.52 (*dd*, 14, 7)	2.13 (*d*, 14) 2.50 (*dd*, 14, 7)	2.20 (*d*, 14.0) 2.53 (*m*)	2.17 (*d*, 14.0) 2.52 (*dd*, 14.0, 6.5)
9	6.86 (*dd,* 7, 1.5)	8.17 (*d*, 8)	6.89 (*br d*, 8.0)	8.19 (*d*, 8.0)
10	7.30 (*dd,* 7, 1.5)	6.80 (*dd*, 8, 2)	7.32 (*dd*, 8.0, 2.1)	6.82 (*d*, 8.0)
11	7.32 (*dd*, 7, 1.5)		7.34 (*dd*, 8.0, 2.1)	
12	8.25 (*dd,* 7, 1.5)	6.45 (*d*, 2)	8.27 (*brd*, 8.0)	6.47 (*brs*)
14	1.47-1.58 (*m*) 2.20-2.33 (*m*)	1.52 (*ddd*, 14, 12, 3.5) 2.17-2.30 (*m*)	1.59 (*m*) 2.30 (*m*)	1.56 (*m*) 2.23 (*m*)
15	3.35-3.41 (*m*)	3.31-3.42 (*m*)	3.40 (*m*)	3.38 (*m*)
16	1.90-2.00 (*m*)	1.90-2.07 (*m*)	2.00 (*m*)	1.98 (*m*)
17	4.26 (*ddd*, 11, 4, 1.5) 4.45 (*t*, 11)	4.25 (*ddd,* 11, 4, 1.5) 4.45 (*t*, 11)	4.29 (*m*) 4.47 (*t*, 12.0)	4.27 (*m*) 4.47 (*t*, 11.5)
18	2.24 (*s*)	2.24 (*s*)	2.26 (*s*)	2.26 (*s*)
21	7.62 (*s*)	7.62 (*s*)	7.64 (*s*)	7.64 (*s*)
N-Me	3.19 (*s*)	3.16 (*s*)	3.22 (*s*)	3.19 (*s*)
OMe		3.85 (*s*)		3.84 (*s*)

2.7 10-Methoxycathafoline (AM11)



Compounds **AM11** was isolated as a light yellow oil. The NMR spectral data (Table 16) indicated that **AM11** was an alkaloid of the akuammiline-type and corresponds to the 10-methoxy substituted derivative of cathafoline (Das, Cosson, and Lukacs, 1977), which was also present in the stem-bark extract. The ^{1}H NMR of **AM11** displayed the same chemical shift and spiting pattern to those of 10-methoxycathafoline as showed in Table 16. Thus, the known compound **AM11** was named as 10-methoxycathafoline

Table 16 ^{1}H NMR spectroscopic of **AM11** and 10-methoxycathafoline

No.	**10-Methoxycathafoline**	**AM11**
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
2	2.44 (*s*)	2.47 (*s*)
3	4.12 (*d*, 5)	4.12 (*d*, 5.0)
5	2.64 (*m*)	2.74 (*m*)
	3.88 (*td*, 14, 5)	3.81 (*m*)
6	1.45 (*dd*, 15, 5)	1.33 (*m*)
	3.07 (*ddd*, 15, 14, 5)	3.01 (*d*, 15.0)
9	6.61 (*d*, 1)	6.94 (*d*, 1.2)
11	6.63 (*dd*, 8, 1)	6.63 (*dd*, 8.0, 1.2)
12	6.53 (*d*, 8)	6.19 (*d*, 8.0)
14	1.61 (*dd*, 14, 3)	1.99 (*dd*, 14.2, 3.3)
	2.37 (*ddd*, 14, 5, 3)	2.31 (*m*)
15	3.60 (*brs*)	3.61 (*brs*)

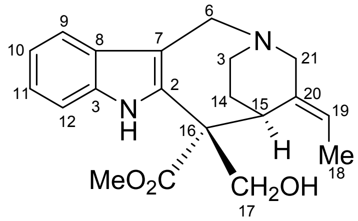
Table 16 (continued)

No.	10-Methoxycathafoline	AM11
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
16	2.94 (*d*, 4)	2.94 (*d*, 4)
18	1.50 (*dd*, 7, 3)	1.59 (*dd*, 7, 3)
19	5.41 (*brq*, 7)	5.40 (*brq*, 7)
21	2.94 (*d*, 16)	2.94 (*d*, 16)
	3.95 (*brd*, 16)	3.81 (*brd*, 16)
N(1)-Me	2.65 (*s*)	2.58 (*s*)
10-OMe	3.73 (*s*)	3.73 (*s*)
CO_2Me	3.77 (*s*)	3.79 (*s*)

3. Chemical constituents from *A. scholaris*

The alkaloidal extracts from the bark and leaves of *A. scholaris* were separated by a combination of silica gel CC to obtain two known alkaloids, 19,20-*E*-vallesamine (**ASB1)** (Atta-ur-Rahman et al., 1987) and 19,20-*Z*-vallesamine (**ASB2**) (Atta-ur-Rahman et al., 1987), two noriridoids, isoboonein (**ASB3)** (Feng et al., 2008) and scholarein D (**ASB4**) (Feng et al., 2008) and two triterpenoids, betulinic acid (**ASB5)** and betulin (**ASL1**).

3.1 19,20-*E*-Vallesamine (ASB1)



Compound **ASB1** was obtained as a colourless viscous oil. The ^{1}H-NMR (Table 17) showed one doublet at δ 1.78 (3H, *d*, *J* = 6.6 Hz) for the olefinic methyl group. An

AB double doublet at δ 5.20 (*d*, J = 16.0 Hz) and 4.15 (*d*, J = 16.0 Hz) were assigned to H-16α and 16β, respectively. The H-15 proton appeared as a multiplet centered at δ 3.76 while H-21α and H-21β protons resonated at δ 3.91 (*d*, J = 13.1 Hz) and 3.79 (*m*). The H_2-14 protons appeared as multiplets at δ 3.76. Another set of AB doublets resonated at δ 4.30 (*d*, J = 10.2 Hz) and 3.90 (*d*, J = 10.2 Hz) which were assigned to H-17a and H-17b protons. The ester methyl group appeared as a singlet at δ 3.79 while the olefinic proton resonated at δ 5.96 as a quartet (J = 6.6 Hz). On the basis of this data, the gross structure **ASB1** could be assigned to the alkaloid. The ^{13}C NMR spectrum revealed the presence of one methyl carbon, five methylene carbons and six methine carbons, in agreement with structure. The chemical shifts of **ASB1** were similar to those reported in the literature for vallesamine (Atta-ur-Rahman et al., 1987). The major difference appeared at the C-19 and C-20 carbons which were shifted and indicated a change in the stereochemistry at the C-19 and C-20 double bond. On the basis of this data the alkaloid was assigned the structure corresponding to 19,20-*E*-vallesamine.

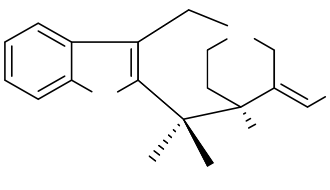
Table 17 ^{1}H and ^{13}C NMR data of **ASB1** and 19,20-*E*-vallesamine

No.	19,20-*E*-Vallesamine		**ASB1**	
	δ_C	δ_H (*mult., J* in Hz)	δ_C	δ_H (*mult., J* in Hz)
2	133.62		134.2	
3	47.41	2.96-2.85 (*m*)	47.3	3.27 (*m*)
				3.16 (*m*)
6	51.21	4.82 (*d*, 17.16)	48.5	5.20 (*d*, 16.0)
		4.09 (*d*, 17.13)		4.15 (*d*, 16.0)
7	109.15		101.6	
8	128.17		127.2	
9	118.35	7.17 (*brd*, 6.9)	117.8	7.53 (*d*, 8.0)
10	119.13	7.07 (*t*, 7.00)	120.3	7.15 (*t*, 8.0)
11	122.36	7.30 (*t*, 7.9)	123.3	7.22 (*t*, 8.0)
12	110.65	7.30 (*brd*, 7.9)	111.1	7.34 (*d*, 8.0)

Table 17 (continued)

No.	19,20-*E*-vallesamine		**ASB119A**	
	δ_C	δ_H (*mult., J* in Hz)	δ_C	δ_H (*mult., J* in Hz)
13	131.47		134.8	
14	23.81	2.33 (*m*) 1.89 (*m*)	20.4	2.28 (*m*)
15	36. 27	3.63 (*m*)	34.7	3.76 (*m*)
16	48.54		58.4	
17	70.11	4.19 (*d*, 10.86) 3.81 (*d*, 10.83)	71.0	4.30 (*d*, 10.2) 3.90 (*d*, 10.2)
18	14.04	1.74 (*d*, 6.93)	14.3	1.78 (*d*, 6.6)
19	124.01	5.56 (*q*, 6.6)	124.6	5.96 (*q*, 6.6)
20	132.40		130.6	
21	54.04	3.60 (*m*)	51.1	3.91 (*d*, 13.1) 3.79 (*m*)
N-H		9.50 (*brs*)		10.24 (*brs*)
$CO_2\underline{Me}$	52.81		55.0	3.79 (*s*)
$\underline{CO_2}Me$	175.20		173.0	

3.2 19,20-*Z*-vallesamine (ASB2)

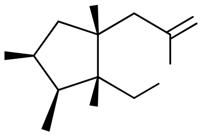


Compound **ASB2** was isolated as a light yellow oil and this compound was identified as alkaloid by ^{1}H and ^{13}C NMR spectral data. The ^{1}H and ^{13}C NMR spectra of **ASB2** (Table 18) were similar to those of **ASB1**. The difference was found at the C-19 and C-20 carbons which were shifted upfield at δ 128.7 and 127.4, respectively, which indicated a change in the stereochemistry at the C-19 and C-20 double bond. On the basis of this data the alkaloid was assigned the structure corresponding to 19,20-*Z*-vallesamine (Atta-ur-Rahman et al., 1987).

Table 18 ^{1}H and ^{13}C NMR data of **ASB2** and 19,20-*Z*-vallesamine

No.	19,20-*Z*-vallesamine		**ASB2**	
	δ_C	δ_H (*mult., J* in Hz)	δ_C	δ_H (*mult., J* in Hz)
2	134.36		134.0	
3	47.28	2.95-2.85 (*m*)	47.2	3.18 (*m*)
				3.11 (*m*)
6	49.15	4.93 (*d*, 16.44)	48.9	5.11 (*d*, 15.6)
		4.05 (*d*, 16.44)		4.15 (*d*, 15.6)
7	109.29		110.0	
8	128.21		128.8	
9	117.90	7.44 (*brd*, 7.8)	118.0	7.53 (*d*, 8.2)
10	119.55	7.12 (*t*, 7.08)	120.1	7.23 (*t*, 8.2)
11	122.56	7.03 (*t*, 7.6)	123.1	7.14 (*t*, 8.2)
12	111.04	7.27 (*brd*, 10.59)	111.1	7.35 (*d*, 8.2)
13	135.06		134.9	
14	22.22	2.19 (*m*)	22.8	2.30 (*m*)
		2.01 (*m*)		2.29 (m)
15	35.40	3.66 (*m*)	35.0	3.74 (*m*)
16	58.80		58.3	
17	70.00	4.20 (*d*, 10.20)	70.1	4.29 (*d*, 12.0)
		3.80 (*d*, 10.20)		3.92 (*d*, 12.0)
18	14.17	1.69 (*d*, 6.4)	14.2	1.78 (*d*, 6.9)
19	127.42	5.52 (*q*, 6.3)	128.7	5.67 (*q*, 6.9)
20	127.78		127.4	
21	52.36	3.60 (*m*)	52.2	3.81 (m)
N-H		10.22 (*brs*)		10.09 (*brs*)
$CO_2\underline{Me}$	52.94	3.70 (*s*)	53.3	3.78 (*s*)
$\underline{CO_2}Me$	174.11		173.7	

3.3 Isoboonein (ASB3)



Compound **ASB3** was obtained as a colorless oil. The ^{13}C NMR spectroscopic showed 9 spectral lines which were sorted by DEPT experiments as one methyl, three methylenes, four methines and one quaternary carbons. A closer analysis of the ^{13}C NMR data allowed the identification of a carbonyl group whose signals appeared at δ 172.3) identified for a lactone. The ^{1}H NMR data (Table 19) clearly exhibited the ABX spin at δ 4.34 (1H, *dd*, J = 12.0, 4.0 Hz), 4.15 (1H, *d*, J = 12.0 Hz) and 2.15 (1H, *m*) and ABC spin at δ 2.96 (1H, *m*), 2.66 (1H, *dd*, J = 15.5, 7.5 Hz) and 2.39 (1H, *dd*, J = 15.5, 4.0 Hz). In addition, the proton signal at δ 1.06 (3H, *d*, J = 6.0 Hz) was assigned to H_3-10 in which this proton correlated with the signal at δ 2.95 (1H, *ddq*, J = 10.0, 8.0, 4.0 Hz). The connectivity of $-CH_2(1)-CH(9)-CH(8)-CH(7)-CH_2(6)-CH(5)-CH_2(4)-$ confirmed by COSY cross-peaks. Thus on the basis of its spectroscopic data and comparison of the NMR chemical shifts with those reported in the literature (Bianco et al., 1994; Feng et al., 2008), compound **ASB3** was assigned as isoboonein.

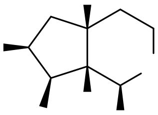
Table 19 ^{1}H and ^{13}C NMR data of **ASB3** and isoboonein

No.	Isoboonein		**ASB3**	
	δ_C	δ_H (*mult.*, J in Hz)	δ_C	δ_H (*mult.*, J in Hz)
1	67.5	4.32 (1H, *dd,* J = 12.0, 4.0) 4.16 (1H, *d,* J = 12.0)	68.5	4.34 (1H, *dd,* J = 12.0, 4.0) 4.15 (1H, *d,* J = 12.0)
3	172.3	-	172.3	-
4	31.6	2.66 (1H, *dd,* J = 15.5, 7.5) 2.39 (1H, *dd*, J = 15.5, 4.0)	32.6	2.66 (1H, *dd,* J = 15.5, 7.5) 2.39 (1H, *dd*, J = 15.5, 4.0)
5	33.5	2.95 (1H, *m*)	34.5	2.96 (1H, m)
6	40.6	2.06 (1H, *ddd,* J = 14.0, 8.0, 1.0) 1.43 (1H, *ddd,* J = 11.0, 3.5)	41.6	2.05 (1H, *ddd,* J = 14.0, 8.0, 1.0) 1.42 (1H, *ddd,* J = 11.0, 3.5)

Table 19 (continued)

No.	Isoboonein		ASB3	
	δ_C	δ_H (*mult., J* in Hz)	δ_C	δ_H (*mult., J* in Hz)
7	74.4	4.15 (1H, *m*)	75.5	4.15 (1H, *m*)
8	40.7	1.94 (1H*, ddq, J* = 10.0, 8.0, 4.0)	41.7	1.95 (1H*, ddq, J* = 10.0, 8.0, 4.0)
9	40.4	2.16 (1H, *m*)	41.4	2.15 (1H, *m*)
10	13.3	1.09 (1H, *d, J* = 8.0)	12.6	1.06 (1H, *d, J* = 6.0)

3.4 Scholarein D (ASB4)

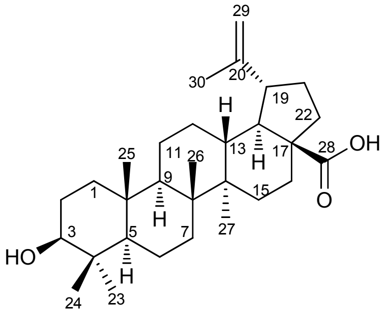


Compound **AMB4** was obtained as a colorless oil. The ^{1}H and ^{13}C NMR (Table 20) and comparison with the data of isoboonein established the structure of scholarein D. The ^{13}C NMR and DEPT spectra of **AMB4** displayed ten signals, corresponding to two CH_3, three CH_2, and five CH groups. Of these, three signals were assigned to oxygenated C-atoms at δ 99.6, 74.1 and 54.8. The ^{1}H-NMR spectrum displayed clear signals for a Me (δ 1.06 (*d*, *J* = 7.0 Hz) and a CH group (δ 4.56 (s)); signals at δ 4.25 (m), 3.75 (*td*, *J* = 12.0, 2.9 Hz) and 3.51 (*m*) were assigned to two oxygenated C-atoms according to the HMQC spectrum. The above data indicated the presence of a bicyclic noriridoid skeleton, which was confirmed by the ^{1}H-^{1}H-COSY cross-peaks. After careful analysis of the ^{1}H and ^{13}C NMR data of **AMB4**, we found that this compound was similar to those of scholarein D (Feng et al., 2008).

Table 20 ^{1}H and ^{13}C NMR data of **AMB4** and scholarein D

No.	Scholarein D		**AMB4**	
	δ_C	δ_H (*mult., J* in Hz)	δ_C	δ_H (*mult., J* in Hz)
1	99.4	4.54 (*brs*)	99.6	4.56 (*s*)
3	58.1	3.70 – 3.73 (*m*)	58.2	3.75 (*td*, 12.0, 2.9)
		3.49 – 3.52 (*m*)		3.51 (*m*)
4	28.7	1.47 – 1.50 (*m*)	28.8	1.53 (*m*)
		1.34 – 1.36 (*m*)		1.38 (*m*)
5	31.2	2.38 – 2.41 (*m*)	31.3	2.39 (*m*)
6	41.7	1.83 – 1.86 (*m*)	41.8	1.88 (*m*)
		1.73 – 1.76 (*m*)		1.76 (*m*)
7	74.0	4.23 – 4.26 (*m*)	74.1	4.25 (*m*)
8	38.2	2.04 – 2.08 (*m*)	38.3	2.09 (*m*)
9	46.5	1.71 – 1.74 (*m*)	46.6	1.73 (*m*)
10	13.0	1.04 (*d*, 7.0)	12.2	1.06 (*d*, 7.0)
OMe	54.7	3.37 (*s*)	54.8	3.40 (*s*)

3.5 Betulinic acid (ASB5)



Compound **ASB5** was obtained as a white solid, mp 279-280°C. It gave a purple vanillin-sulfuric acid test indicating a triterpene. The IR spectrum showed absorption bands of a hydroxy group at 3415 cm^{-1} and a carbonyl group at 1686 cm^{-1}.

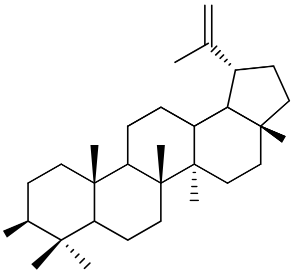
The ^{1}H and ^{13}C NMR spectral data of **ASB5** (Table 21) were similar to those of **ASL1**. The difference in the spectrum of **ASB5** was shown as disappearance of an oxymethylene protons at δ 3.82 (*d*, *J* = 10.5 Hz, H-28a) and 3.36 (*d*, *J* = 10.8 Hz, H-28b) in the ^{1}H NMR spectrum and the ^{13}C NMR spectrum displayed a signal of a carboxyl carbon at δ 179.8 instead of an oxy-methylene carbon, thus suggesting a carboxylic functionality at C-28. The location of the carboxyl group was confirmed by HMBC experiment. Thus on the basis of its spectroscopic data and comparison of the NMR chemical shifts with those reported in the literature (Kitajima, Shindo and Tanaka, 1990), (Table 21) compound **ASB3** was assigned as betulinic acid.

Table 21 ^{1}H and ^{13}C NMR data of **ASB1**[a] and betulinic acid

No.	Betulinic acid		**ASB5**	
	δ_C	δ_H (*mult., J* in Hz)	δ_C	δ_H (*mult., J* in Hz)
3	78.0	3.19 (*dd*, *J* = 10.8, 5.4)	79.0	3.20 (*dd*, *J* = 10.8, 5.4)
5	54.4	0.69 (*m*)[a]	55.3	0.69 (*m*)
20	149.4		149.4	
21	29.6	1.89 (*m*)[a]	30.5	1.89 (*m*)
22	36.0	1.40 (*m*), 1.93 (*m*)	37.0	1.40 (*m*), 1.90 (*m*)
23	27.0	0.97 (*s*)	27.9	0.99 (*s*)
24	14.3	0.75 (*s*)	15.3	0.77 (*s*)
25	15.1	0.82 (*s*)	16.1	0.84 (*s*)
26	15.0	0.94 (*s*)	16.0	0.96 (*s*)
27	13.7	0.98 (*s*)	14.6	1.00 (*s*)
28	179.6		179.8	
29	108.7	4.74 (*br s*), 4.16 (*br s*)	109.6	4.78 (*br s*), 4.63 (*br s*)
30	18.4	1.69 (*s*)	19.3	1.71 (*s*)

[a] Data from HMQC

3.6 Betulin (ASL1)



Compound **ASL1** was obtained as a white solid, mp 230-231°C. It gave a purple vanillin-sulfuric acid test indicating a triterpene. The spectral data was shown as only six singlet signals of methyl groups at δ 0.77, 0.84, 0.98, 1.00, 1.04 and 1.70. In addition, AB system of oxymethylene protons was shown at δ 3.82 (*d*, *J* = 10.5 Hz, H-28a) and 3.36 (*d*, *J* = 10.8 Hz, H-28b). This compound was established as betulin by comparison of its spectral data with those reported in the literature (Tinto et al., 1992) (Table 22). Compound **ASL1** was assigned as betulin.

Table 22 ^{1}H NMR data of compound **ASL1** and betulin

No.	**Betulin**	**ASL1**
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
3	3.19 (*dd*, 10.8, 5.1)	3.21 (*dd,* 10.8, 5.1)
5	0.68 (*m*)	0.72 (*m*)
19	2.38 (*m*)	2.41 (*m*)
21	1.91 (*m*)	1.95 (*m*)
23	0.97 (*s*)	0.98 (*s*)
24	0.76 (*s*)	0.77 (*s*)
25	0.82 (*s*)	0.84 (*s*)
26	1.02 (*s*)	1.04 (*s*)
27	0.98 (*s*)	1.00 (*s*)

Table 22 (continued)

No.	**Betulin**	**ASL1**
	δ_H (*mult., J* in Hz)	δ_H (*mult., J* in Hz)
28	3.80 (*dd*, 10.8, 1.5)	3.82 (*d*, 10.5)
	3.33 (*d,* 10.8)	3.36 (*d*, 10.5)
29	4.68 (*d,* 2.1)	4.71 (*s*, 2.1)
	4.58 (*m*)	4.61 (*s*)
30	1.68 (*s*)	1.70 (*s*)

4.4 Biological activities

In this research, we isolated several compounds from *D. candenatensis*, *A. scholaris*, and *A. macrophylla* including phenolic compounds, indole alkaloids and triterprenoids. These plants showed different biological activities. However, the bioactive tests were chosen according to literature report. As shown in Table 23, the effective dose of indole alkaloids, **AM1-AM8** and **AM10-AM11** for antimalaria, acetylcholine esterase inhibition suggested inactivity (IC_{50} >50 µg/mL), except **AM9** exhibited antiplasmodial activity against *Plasmodium falciparum*, with an IC_{50} of 7.6 µM. It should be noted that the alkaloid AM**10**, with a methoxyl group at C-11, was found to have a reduced anti-malarial activity, compared with **AM9**.

Candenatenin K (**DC5**) exhibited potent activity against DPPH radical scavenging with IC_{50} value of 25.7 µM, which higher than standard compound, ascorbic acid (IC_{50} of 44.4 µM). All isolated compounds were also evaluated for their cytotoxicity against oral cancer (KB), breast cancer (MCF7), and small cell lung cancer (NCI-H187) Unfortunately, only candenatenin H (**DC2**) showed significant cytotoxicity against NCI-H187 cell line with IC_{50} value of 14.8 µM. The others exhibited weakly or inactive cytotoxicity.

Table 23 Biological activities of crude extracts and selected pure compounds

Samples	DPPH scavenging Activity (μM)[a]	Anti-malaria (μM)[a]	Cytotoxicity (μM)[a]			Acetylcholine esterase inhibition (μM)[a]
			KB	MCF7	NCI-H187	
DC extract	20.31	NT[b]	22.10	11.50	12.35	NT
AM extract	NT	3.07	28.39	NA	39.47	NA
ASB extract	NA	NA[c]	NA	NA	NA	NA
ASL extract	NA	NA	23.19	NA	NA	NA
DC1	76.68	-	39.70	30.29	50.99	-
DC2	41.00	-	25.44	40.14	14.78	-
DC3	NA	-	NA	NA	55.49	-
DC4	NA	-	NA	27.23	66.68	-
DC5	25.70	-	51.42	24.04	39.67	-
DC7	NA	-	56.53	NA	83.22	-
DC8	NA	-	NA	26.20	39.24	-
DC9	NA	-	NA	NA	44.02	-
AM9		7.60			NA	
Ascorbic acid	44.4					
Doxorubicine			0.51		0.13	
Ellipticine			1.77	13.50	7.02	
Mefloquine		0.029				

[a]IC$_{50}$ of crude extract is reported in μg/mL, [b]NA = Not active at 50 μg/mL, [c]NT = Not tested, DC = *Dalbergia candenatensis* extract, AM = *Alstonia macrophylla* extract, ASB = *Alstonia scholaris* bark extract, ASL = *Alstonia scholaris* leaves extract.

Conclusion

Searching for bioactive compounds from Thai medicinal plants, *D. candenatensis*, *A. scholaris* and *A. macrophylla* led to the isolation of 8 new compounds including five new phenolic compounds, candenatenins G-K (**DC1**-**DC5**), two unusual nitrogenous derivatives, alstoniaphyllines A (**AM1**) and B (**AM2**), and a new indole alkaloid, alstoniaphylline C (**AM4**). Four known compounds (**DC6**-**DC9**) were isolated from the air-dried heartwood of *D. candenatensis* while eight known componds (**AM3**, **AM5**-**AM11**) and six known compounds (**ASB1**-**ASB5**, **ASL1**) were isolated from *A. macrophylla* and *A. scholaris*, respectively. Structures of new compounds were characterized by spectroscopic methods including UV, IR, 1D- and 2D-NMR and MS whereas all known compounds were characterized by NMR and comparison of their spectral data with reported values. Alstonisine (**AM9**) exhibited antiplasmodial activity against *Plasmodium falciparum*, with an IC_{50} of 7.6 μM. Candenatenin K (**DC5**) exhibited potent activity against DPPH radical scavenging with IC_{50} value of 25.7 μM, whereas candenatenin H (**DC2**) showed cytotoxicity against NCI-H187 cell line with IC_{50} value of 14.8 μM.

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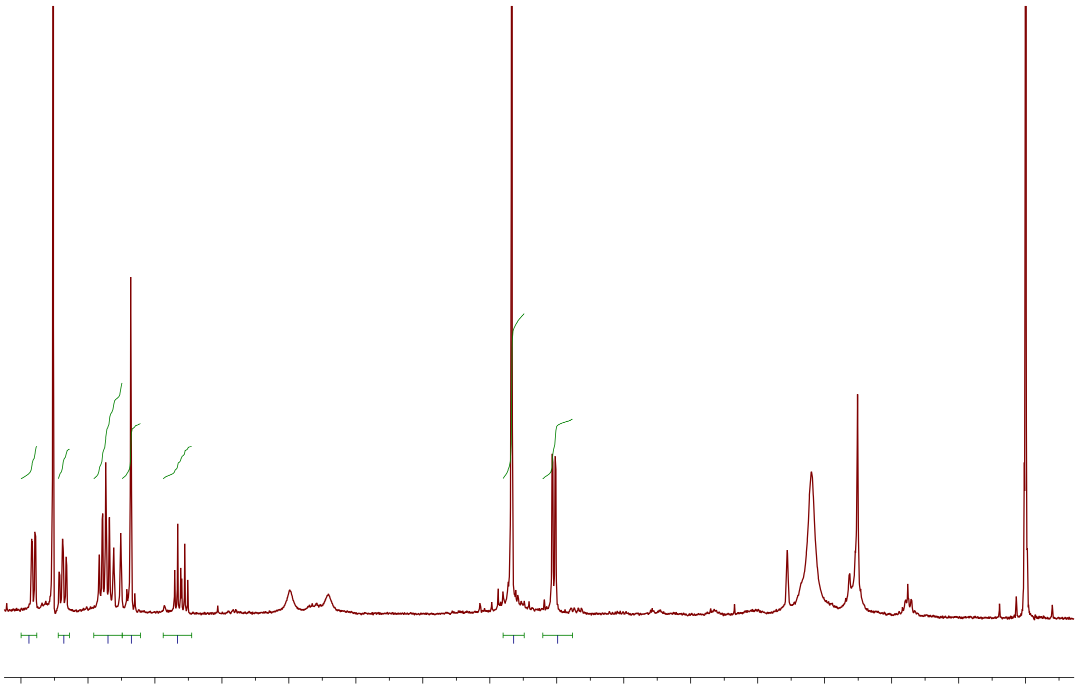
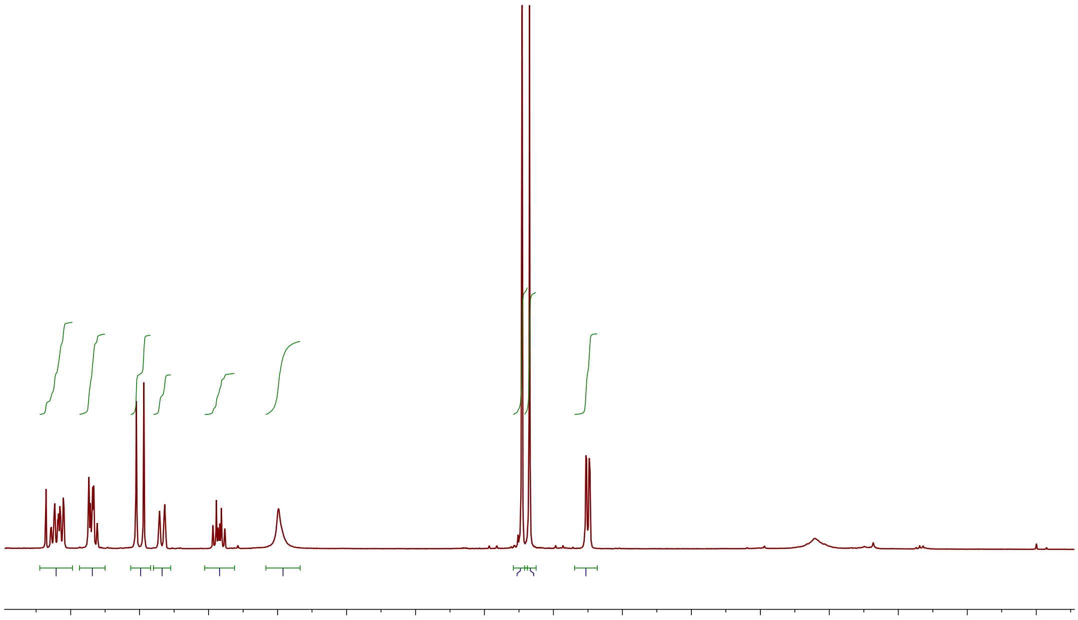
Appendix



^{1}H NMR (500 MHz, CDCl$_3$) spectrum of compound DC1



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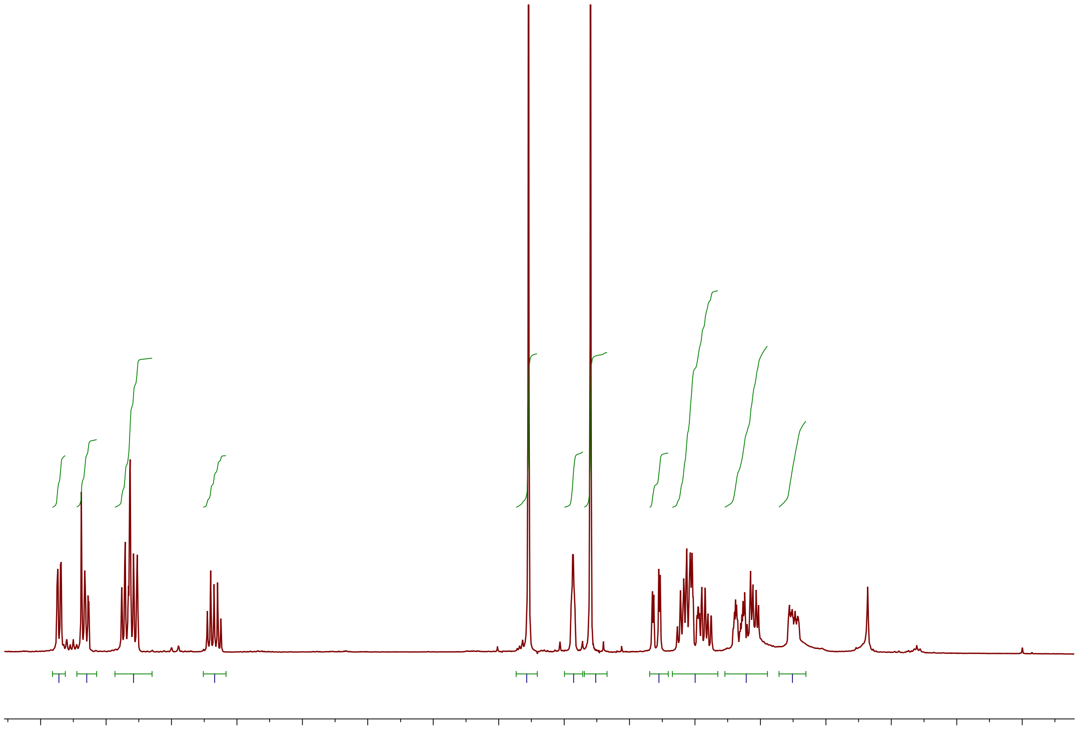

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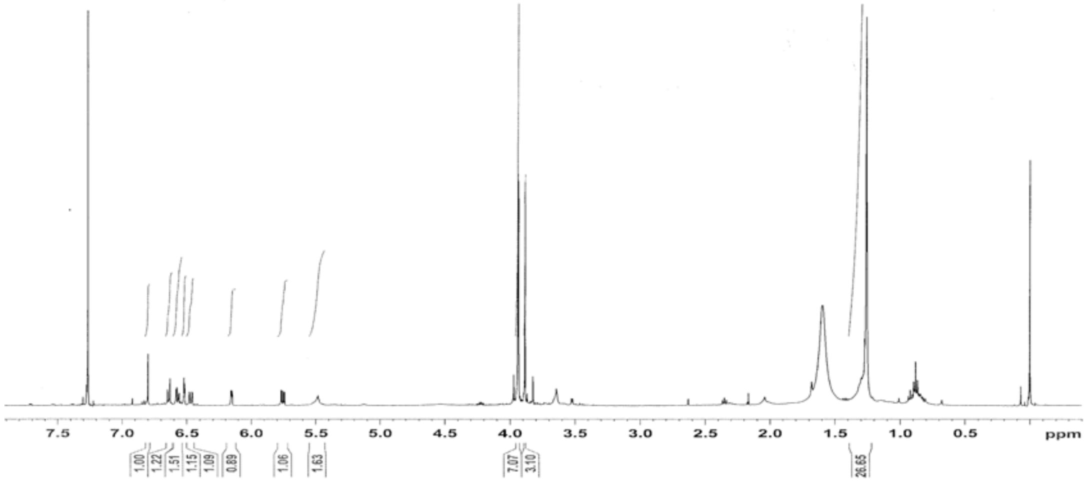

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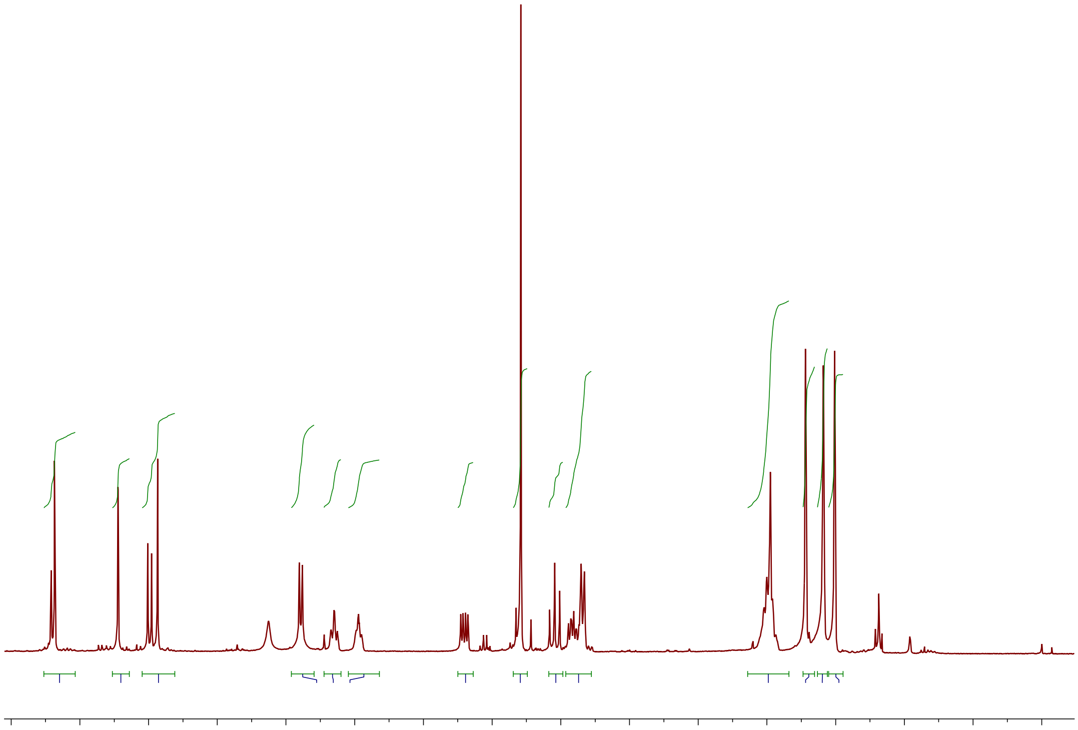

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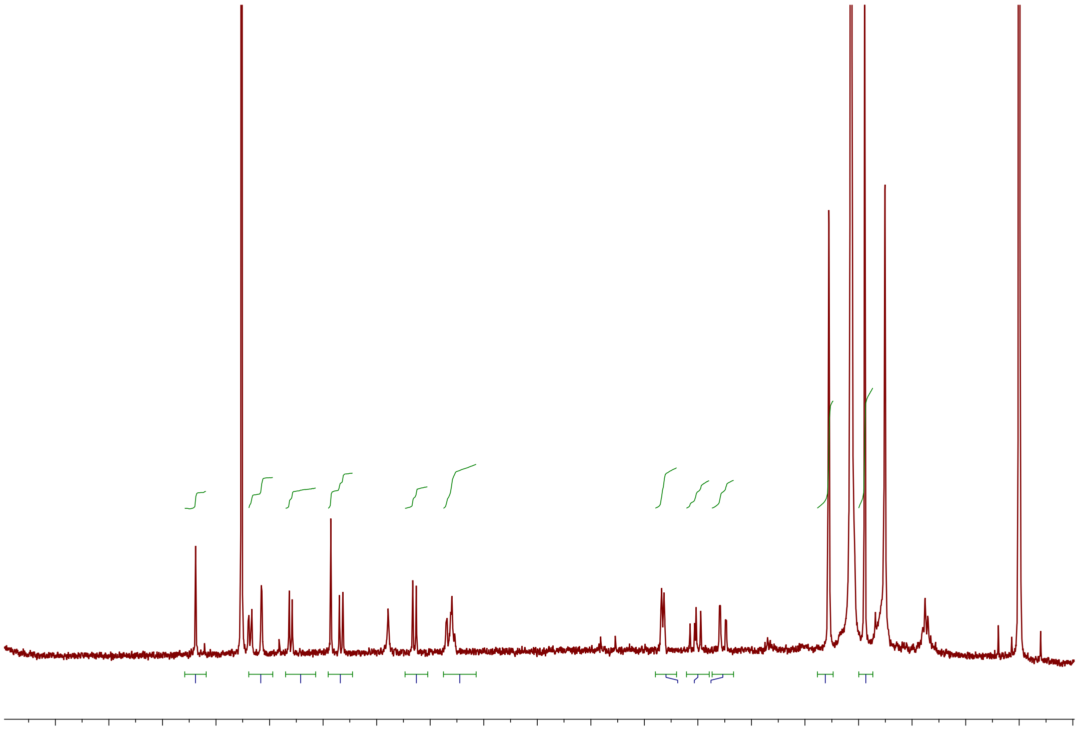

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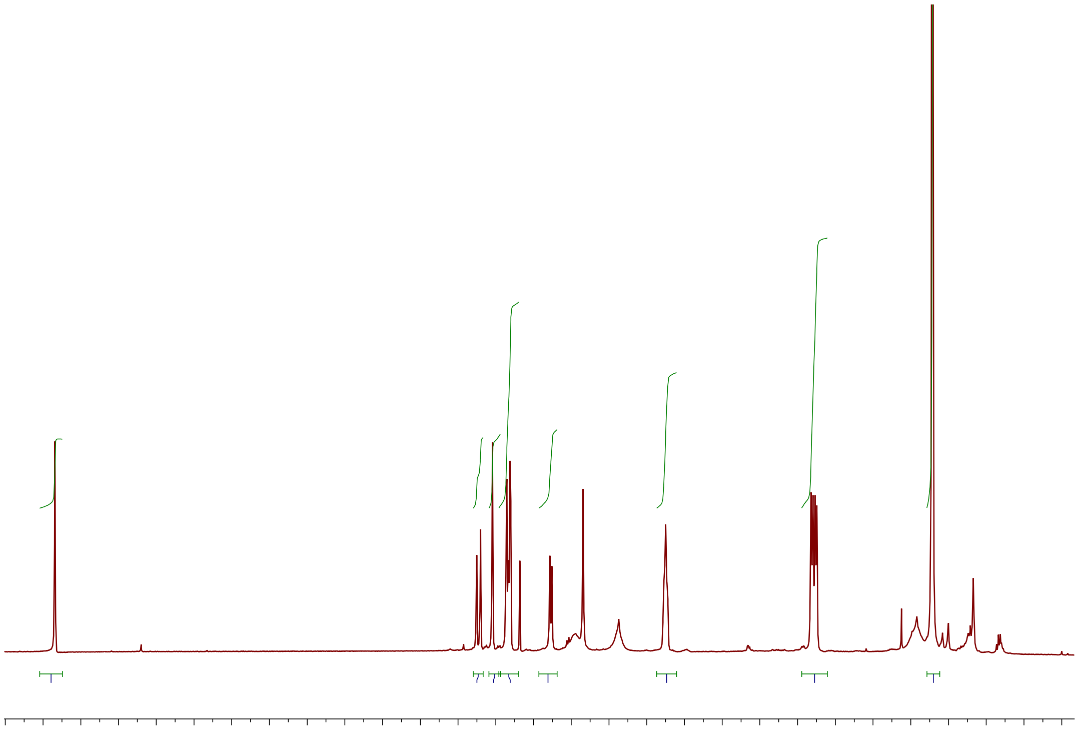

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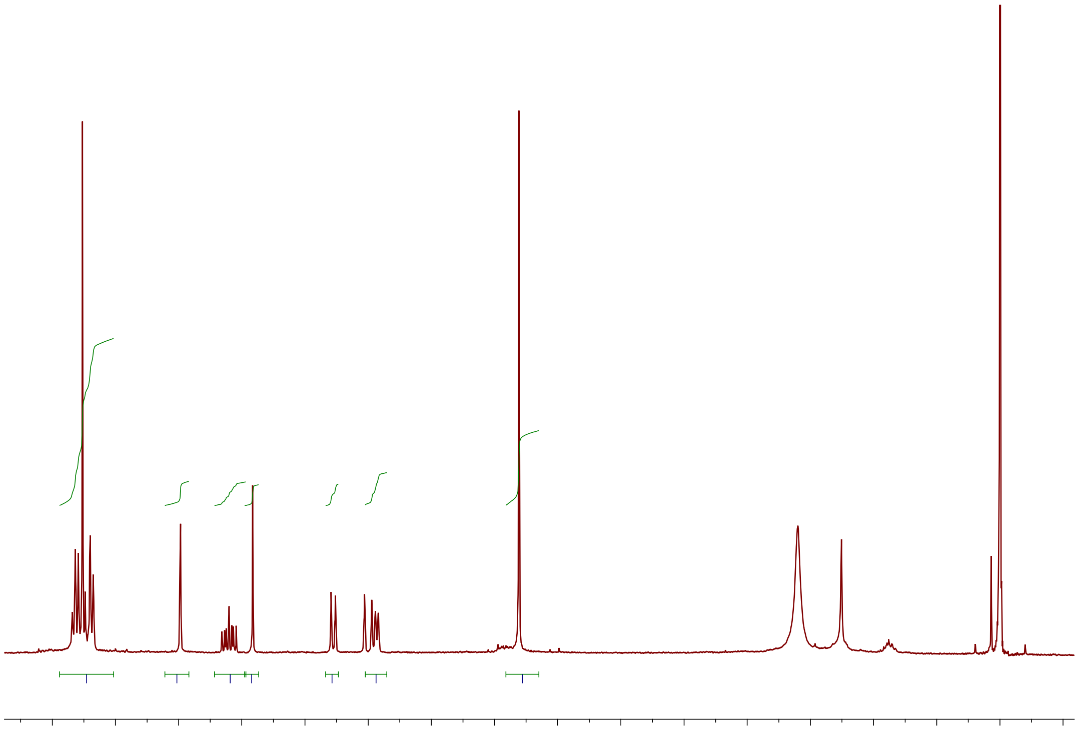

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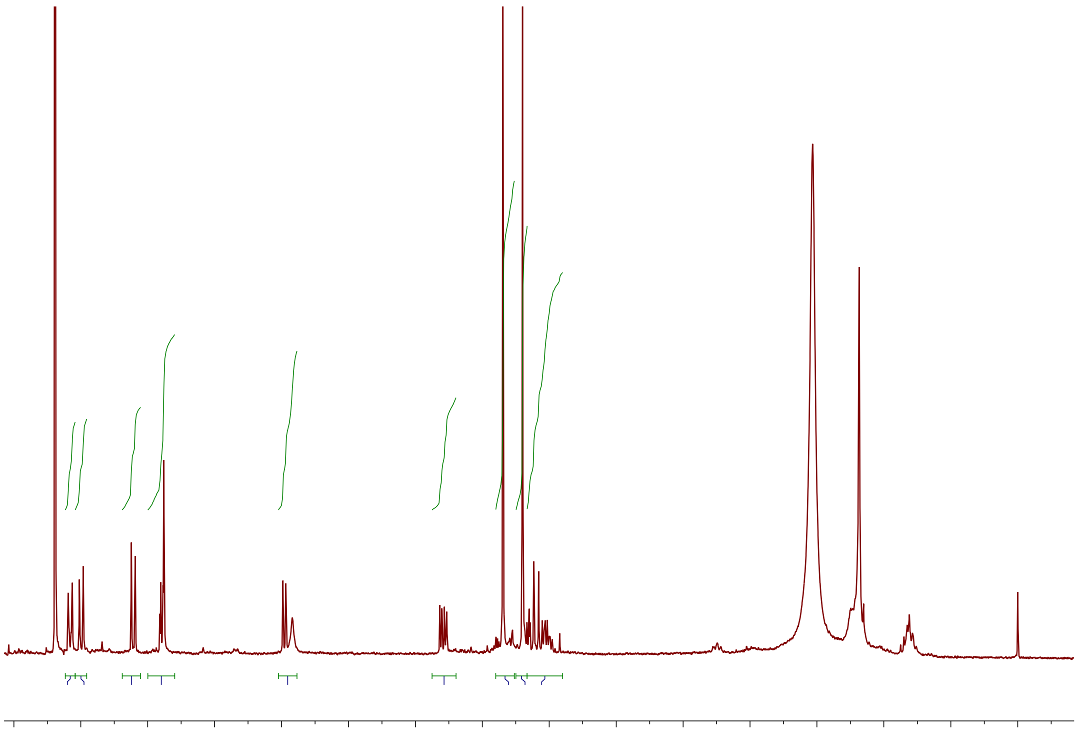

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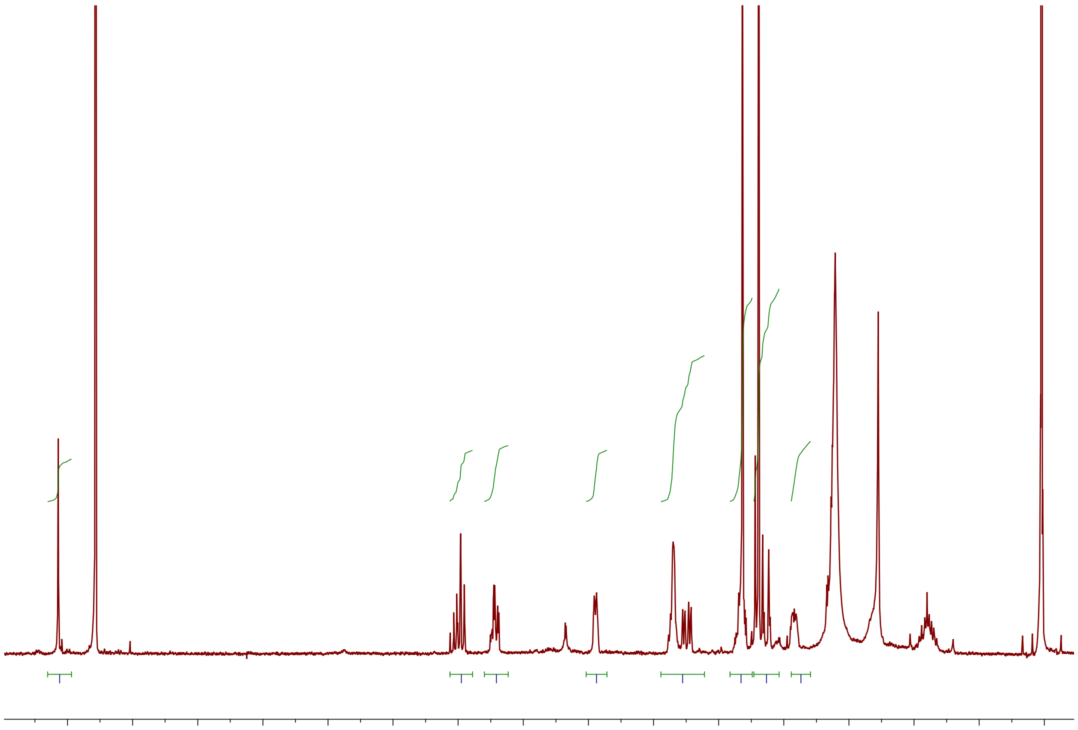

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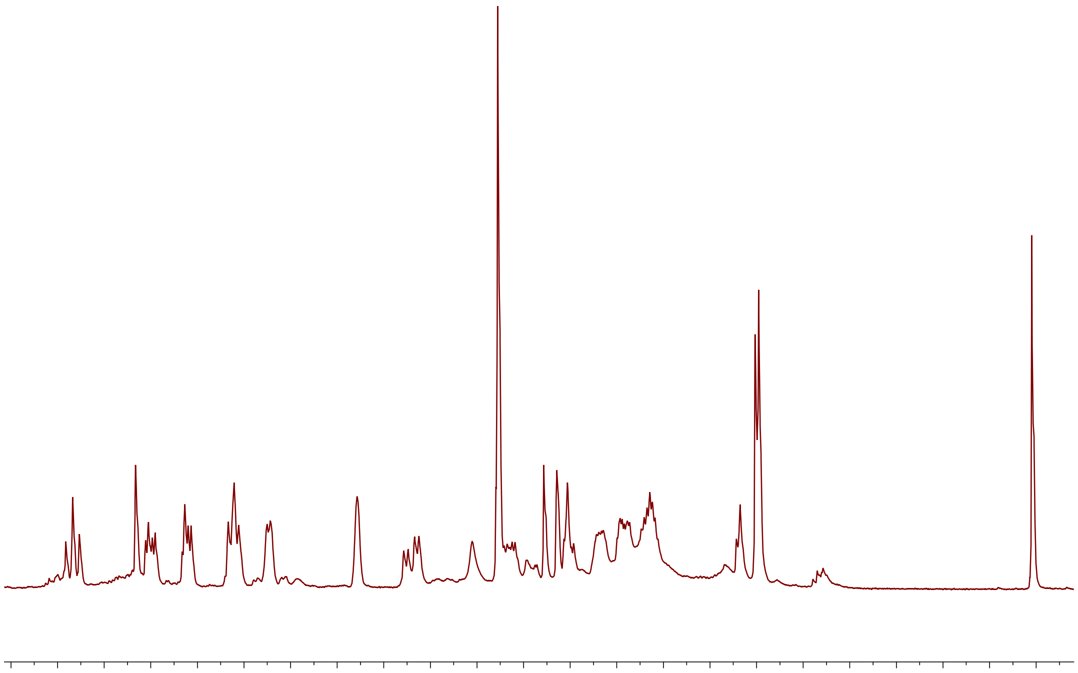

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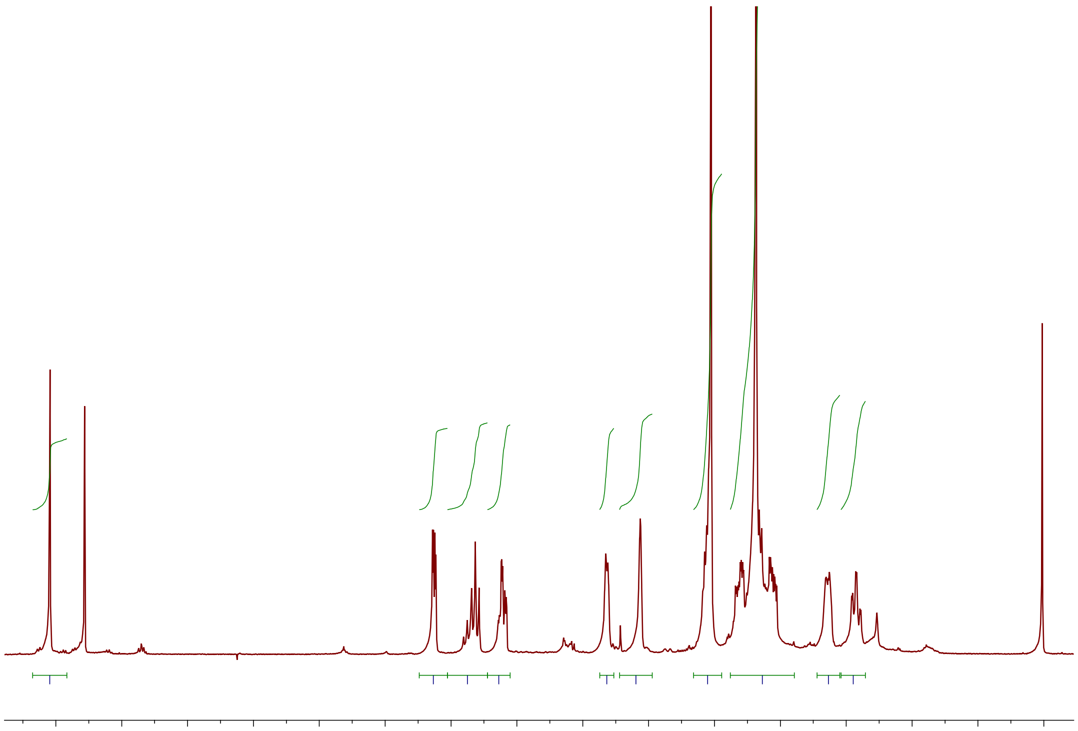

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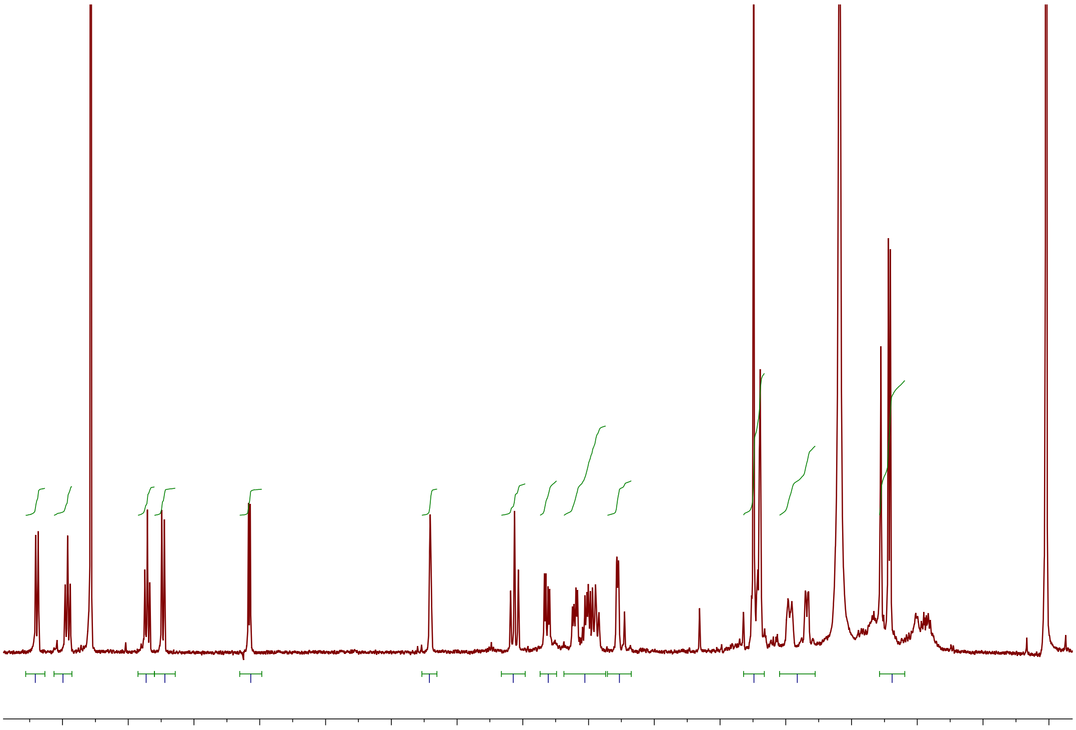

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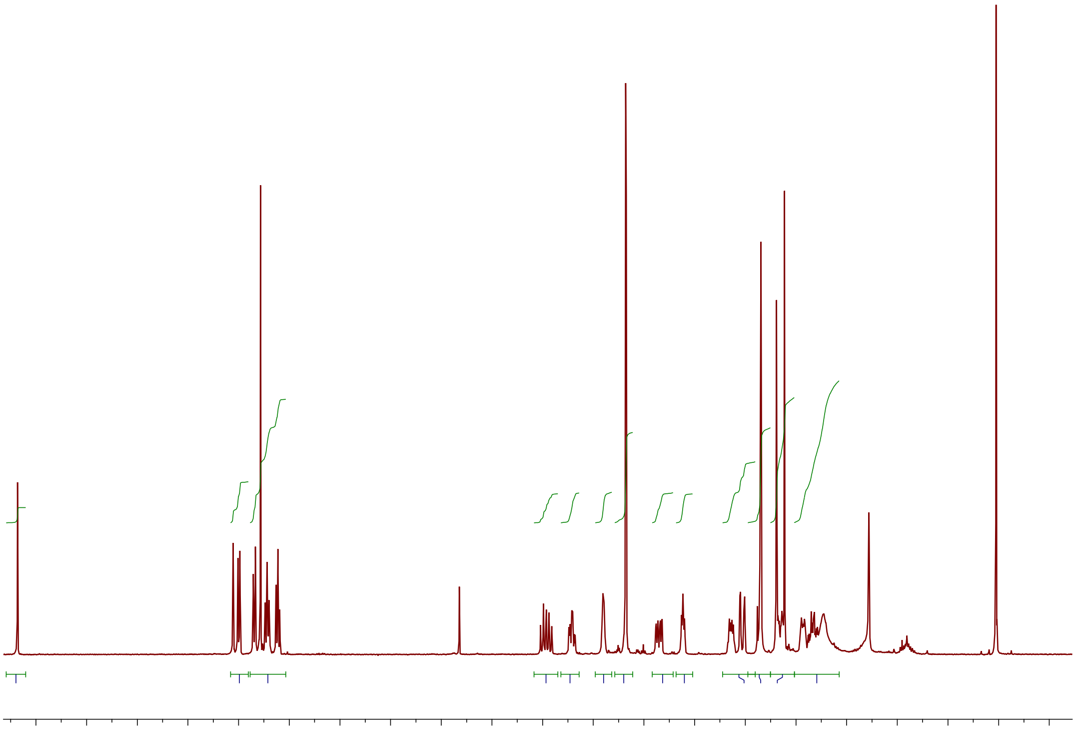

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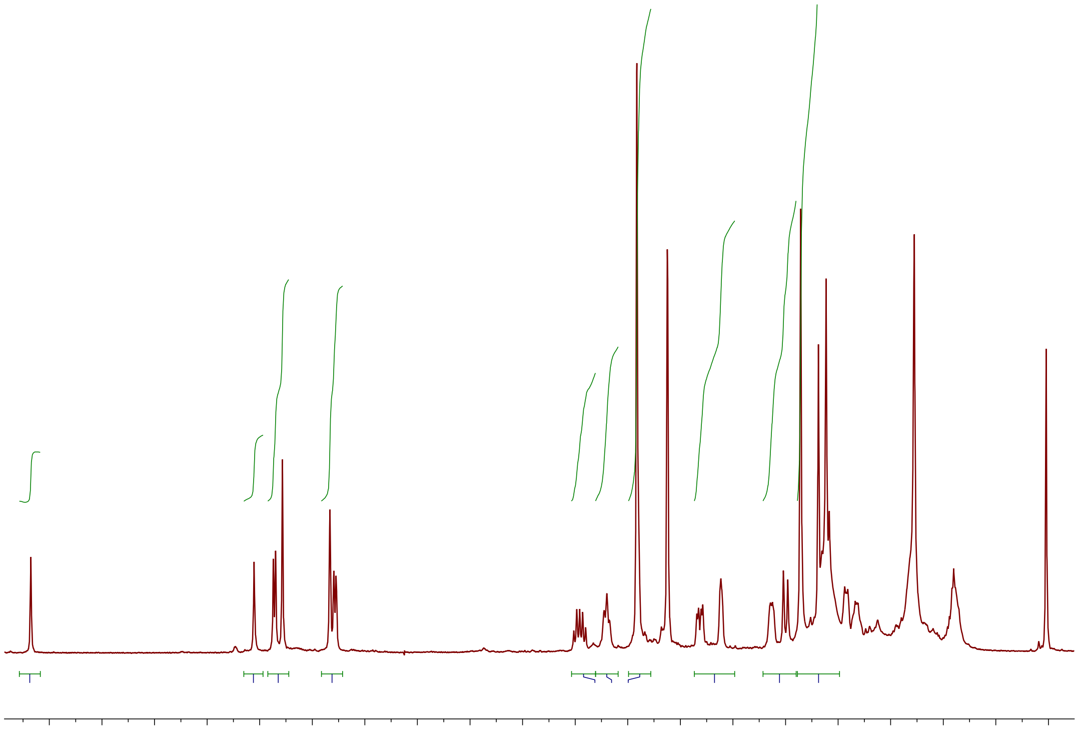

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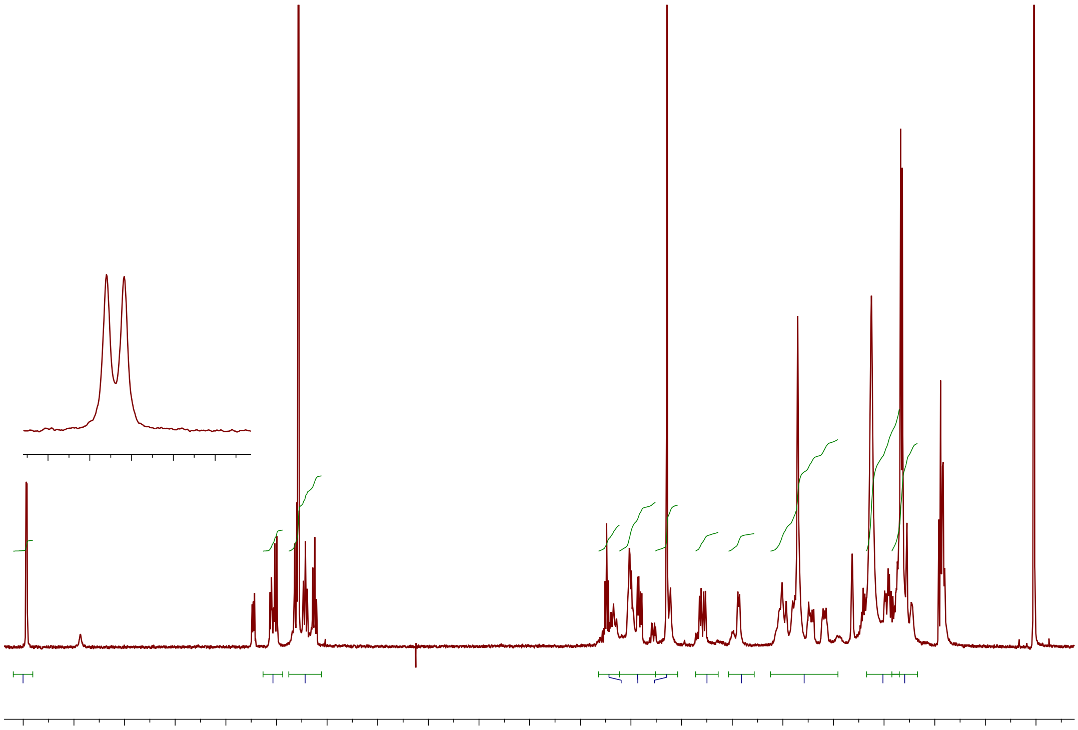

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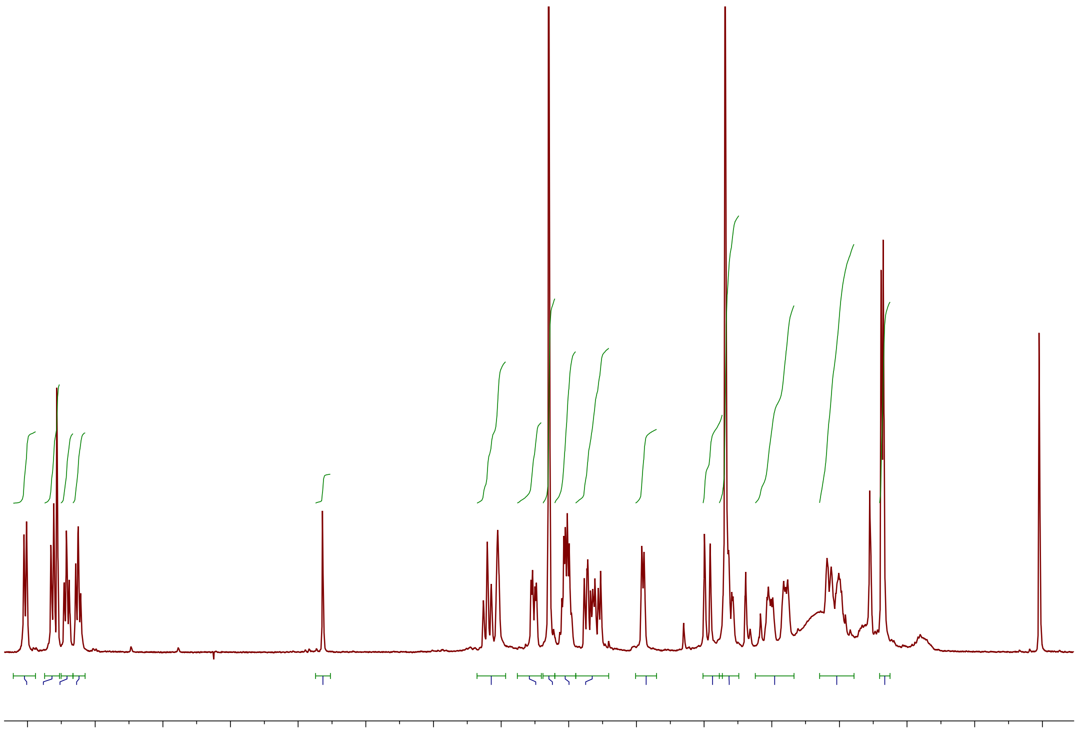

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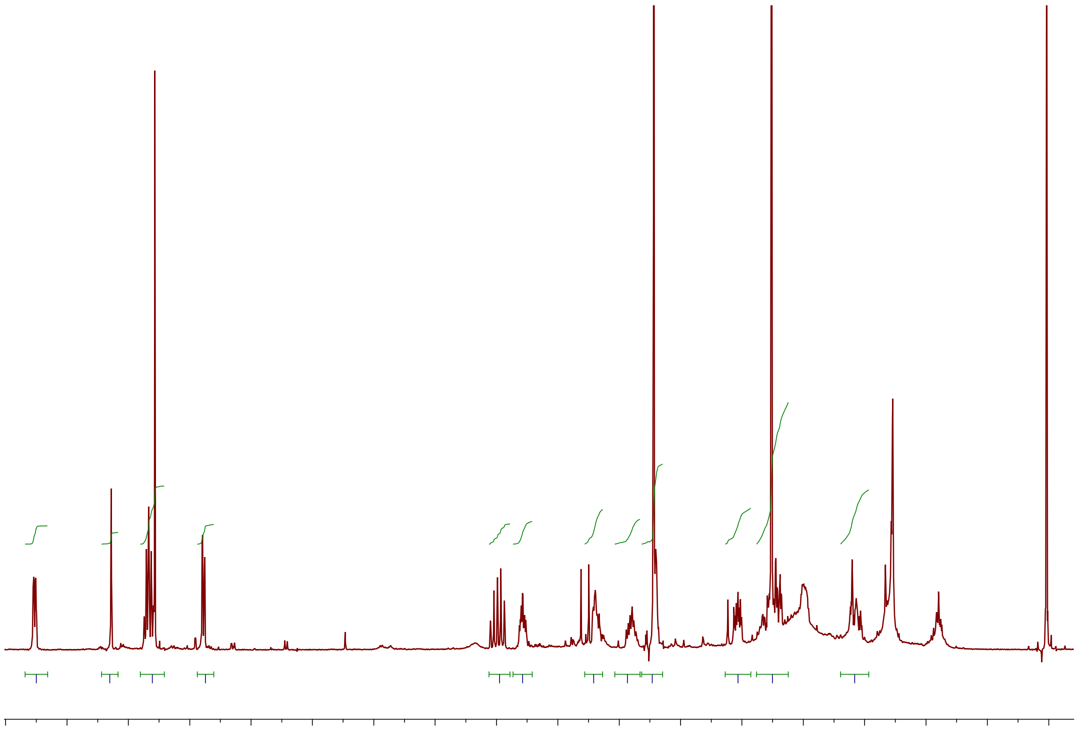

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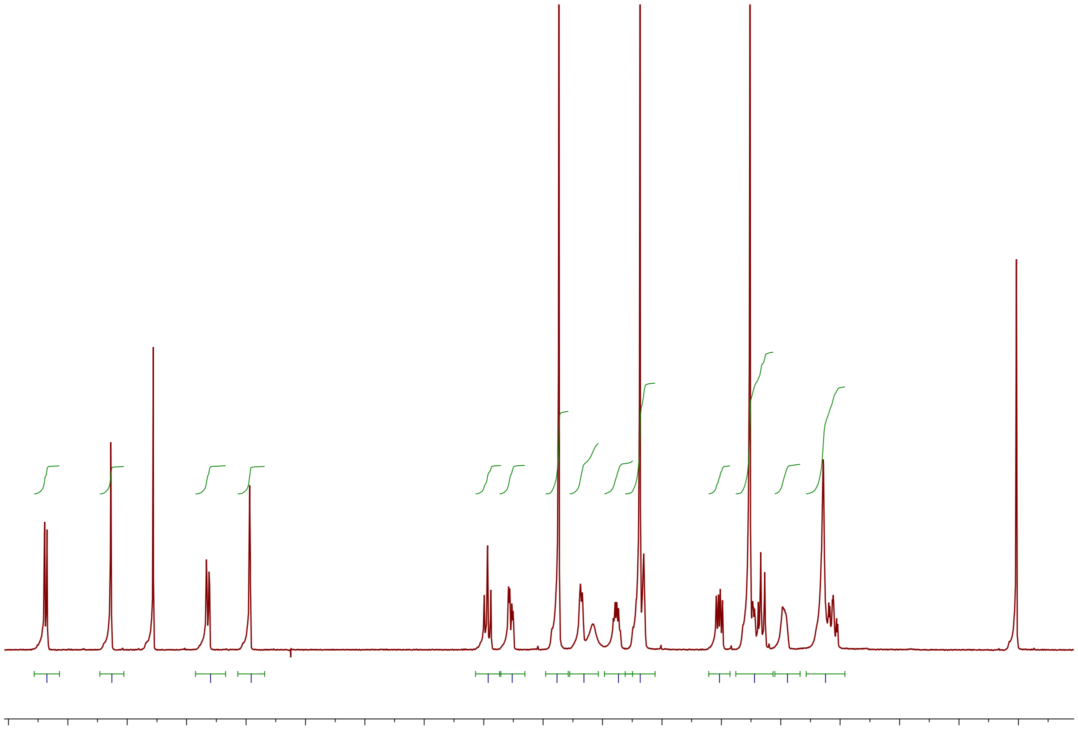

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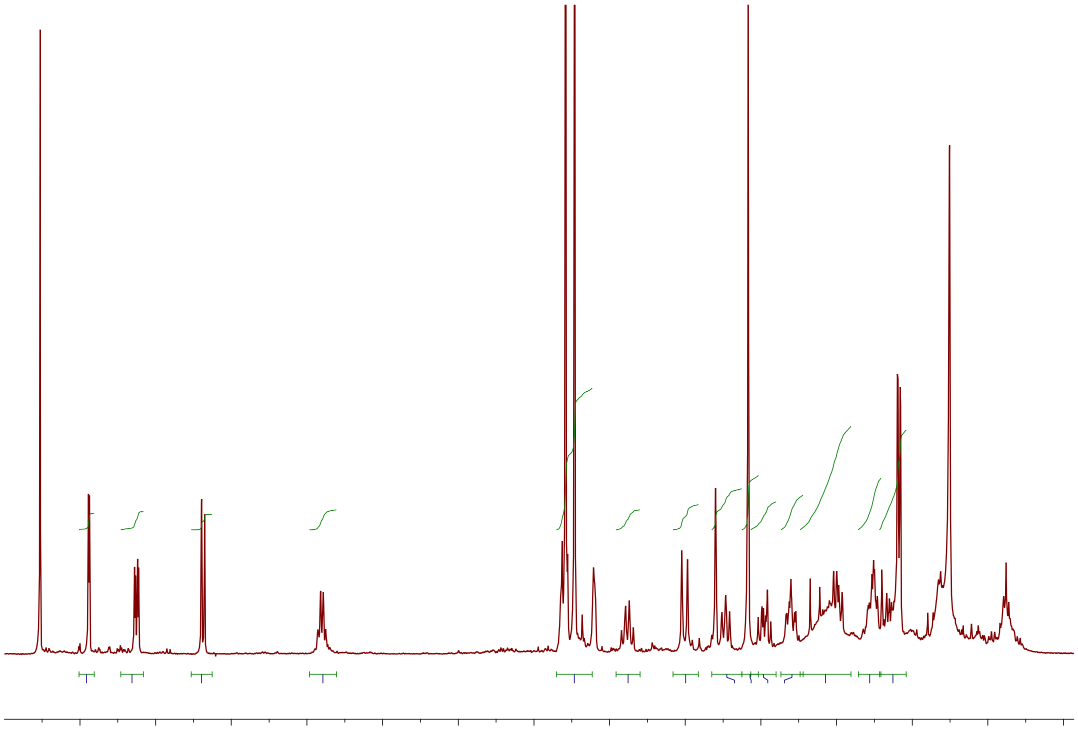

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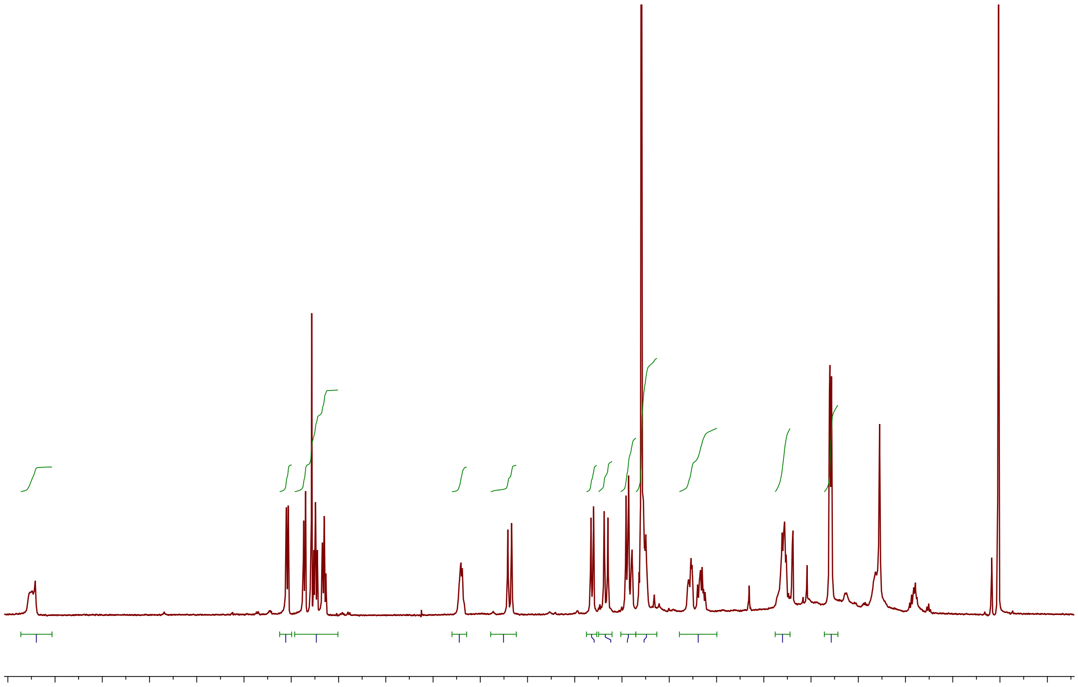

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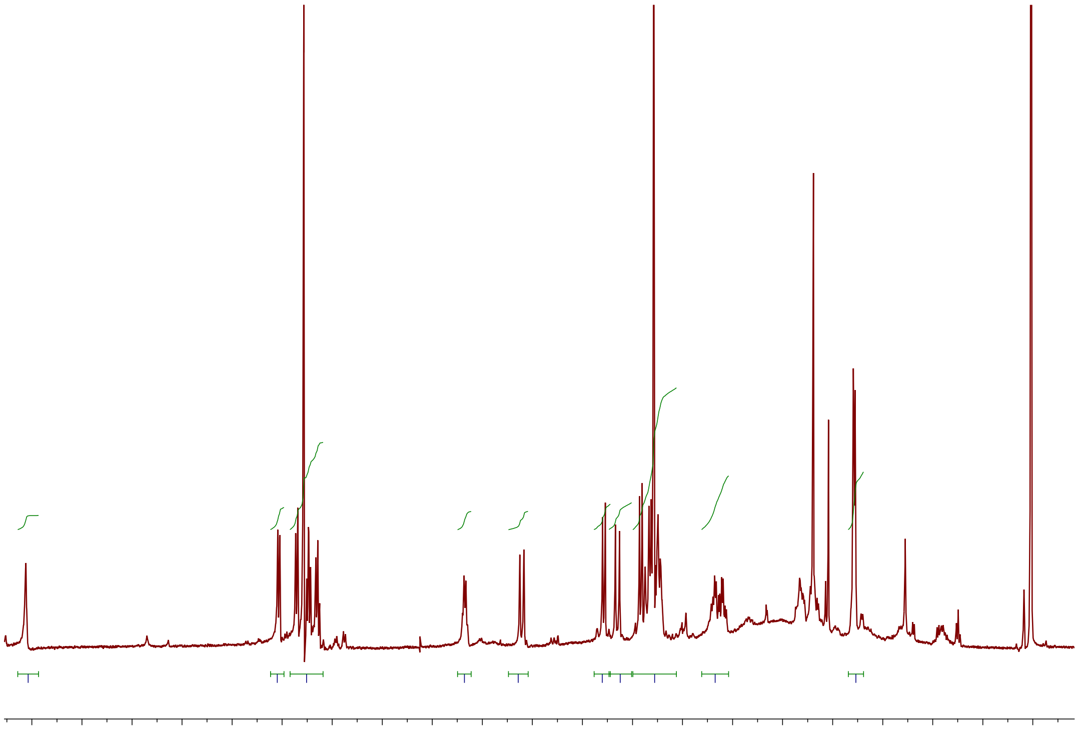

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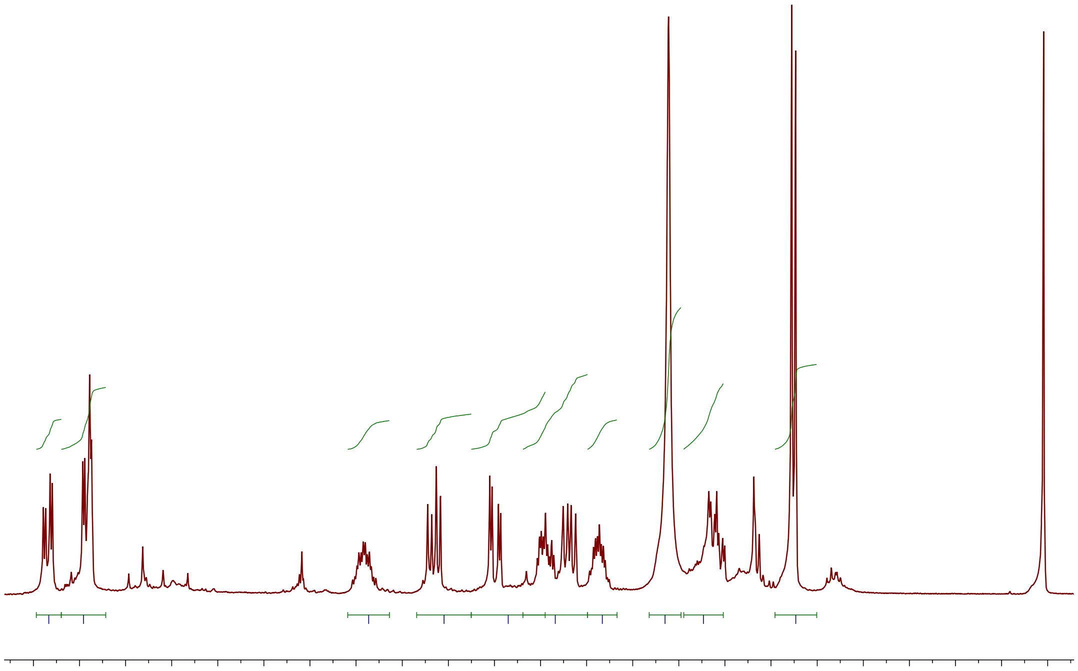

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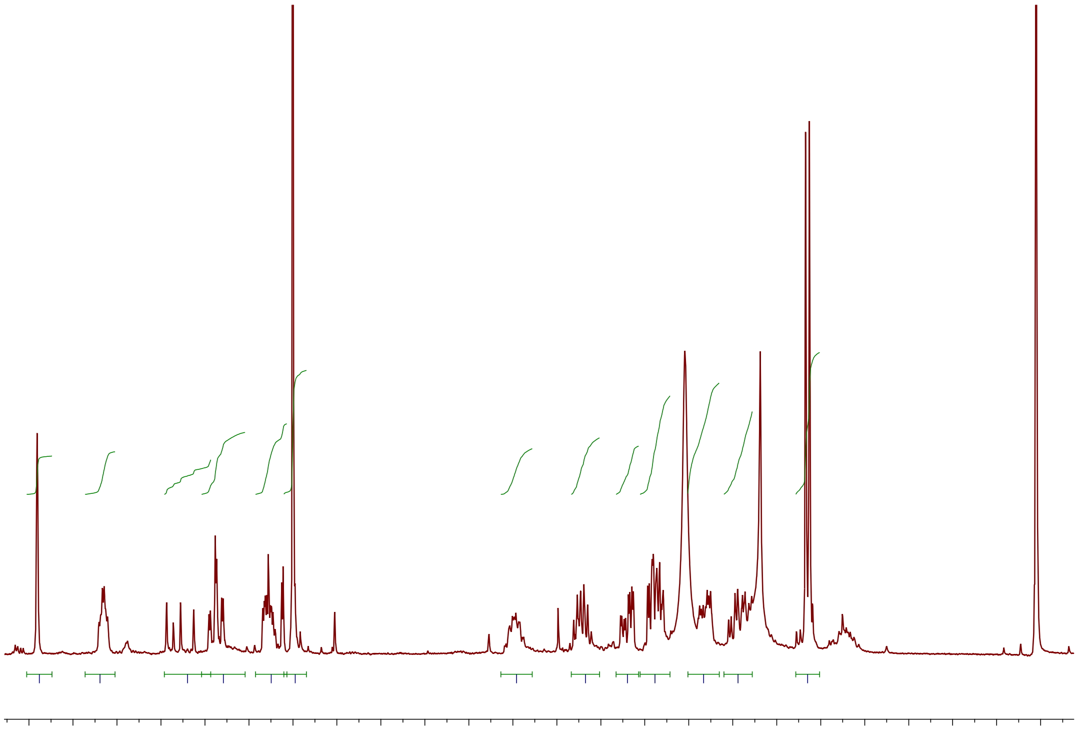

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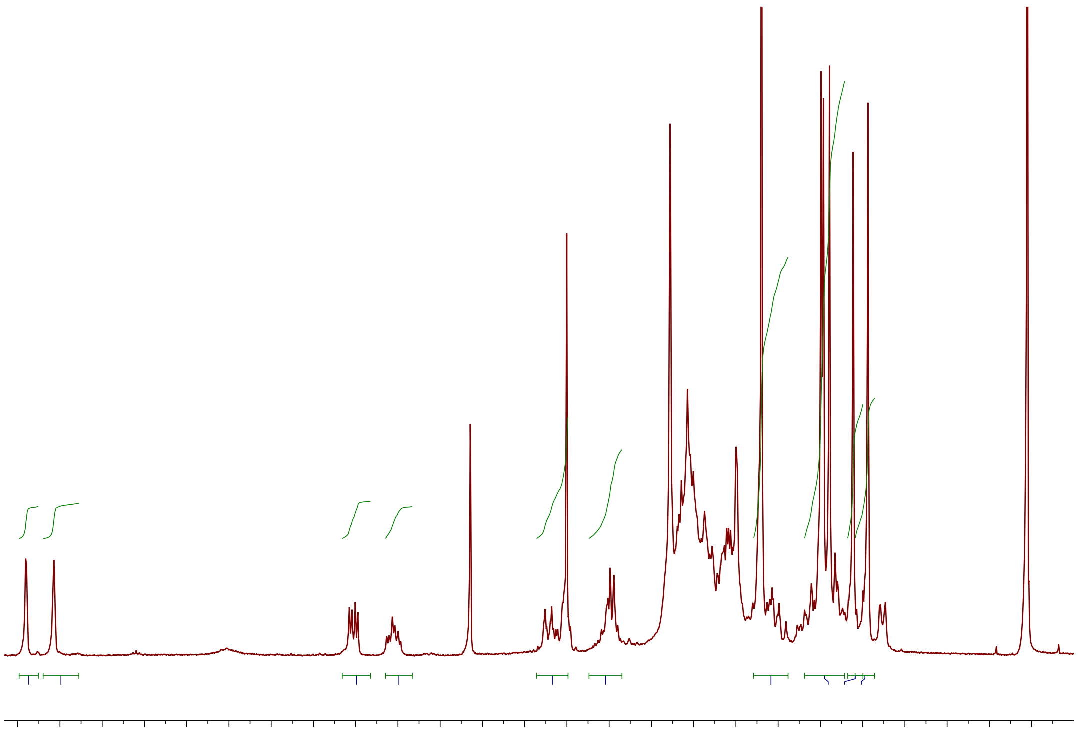

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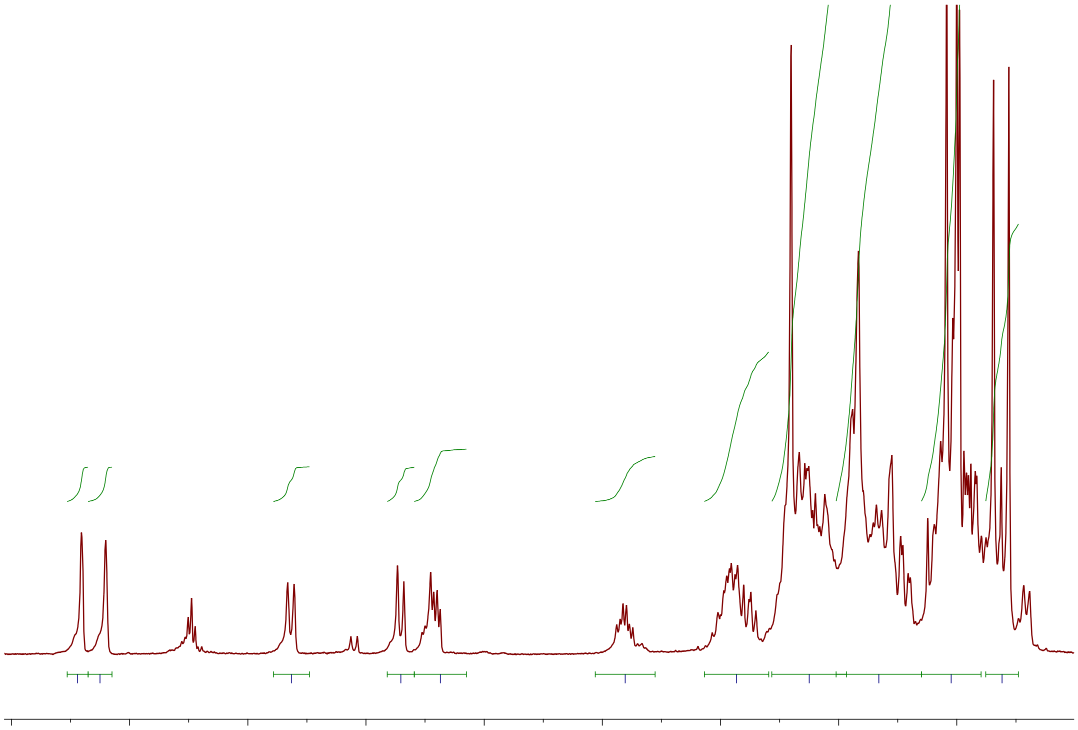


^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound ASL1

Output

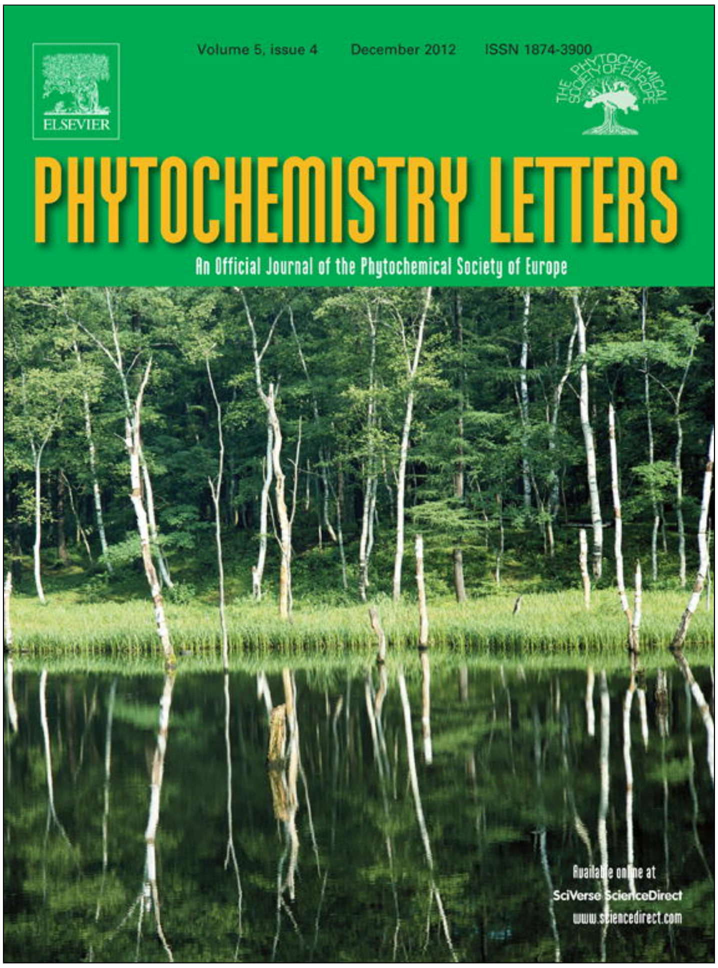
Acknowledgments

I would like to thank the Thailand Research Fund, the office of the Higher Education Commission, and University of Phayao (Grant No. MRG5480028) for financial support. I am indebted to N. Chimnoi, Chulabhorn Research Institute, Bangkok, for recording mass spectrometric data, Dr. T. Ritthiwigrom, Chaing Mai University, for recording NMR data and am also thankful to Prof. P. Sirirugsa (Prince of Songkla University) for plant identification. I am grateful to BIOTEC (Thailand) for biological assays.

I wish to express my deepest and sincere gratitude to my mentor, Associate Professor Dr. Chatchanok Karalai, for his valuable instructions, expert guidance, excellent suggestions and kindness which are more than I can describe here. Everything will always be in my mind. My sincere thanks are expressed to Assistant Professor Dr. Surat Laphookhieo, my co-worker for his kindness and valuable advices.

International Journal Publication

1. Cheenpracha, S.; Ritthiwigrom, T.; Karalai, C.; Laphookhieo, S. **2012**. Candenatenins G–K, phenolic compounds from *Dalbergia candenatensis* heartwood. *Phytochemistry Lett. 5*, 708–712.
2. Cheenpracha, S.; Ritthiwigrom, T.; Laphookhieo, S. **2013**. Alstoniaphyllines A-C, unusual nitrogenous derivatives from the bark of *Alstonia macrophylla*. *J. Nat. Prod. 76*, 723-726.


Volume 5, issue 4
December 2012
ISSN 1874-3900
ELSEVIER
THE PHYTOCHEMICAL SOCIETY OF EUROPE
PHYTOCHEMISTRY LETTERS
An Official Journal of the Phytochemical Society of Europe
Available online at
SciVerse ScienceDirect
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Phytochemistry Letters 5 (2012) 708–712

Contents lists available at SciVerse ScienceDirect

Phytochemistry Letters

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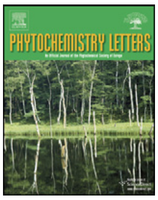

Candenatenins G–K, phenolic compounds from *Dalbergia candenatensis* heartwood

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ARTICLE INFO

Article history:
Received 1 March 2012
Received in revised form 9 July 2012
Accepted 9 July 2012
Available online 27 July 2012

Keywords:
Dalbergia candenatensis
Antioxidant activity
Cytotoxicity
Candenatenin

ABSTRACT

Five new phenolic compounds, designated candenatenins G–K (**1**–**5**), along with four known compounds, were isolated from the heartwood of *Dalbergia candenatensis*. The structures of these compounds were elucidated by HR-EI-MS, ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra. Compound **5** exhibited potent activity against DPPH radical scavenging with IC_{50} value of 25.7 μM, whereas compound **2** showed cytotoxicity against NCI-H187 cell line with IC_{50} value of 14.8 μM.

1. Introduction

Dalbergia genus (Leguminosae) is known for their deeply pigmented heartwood of varying colors. Often valued for their use in wooden crafts, the heartwood is also used in traditional medicine and some of which were proved to exhibit antibiotic and cytotoxic activities (Cheenpracha et al., 2009; Hamburger et al., 1987; Ansari et al., 2000). Phytochemical investigation on this genus resulted in the isolation of various isoflavonoids and neoflavonoids (Bekker et al., 2002; Hamburger et al., 1988). *Dalbergia candenatensis*, known locally as "Sakkhee", is a sprawling climber and distributed widely on the landward side of mangroves. The heartwood has a deep red color and is used in Thailand as a red dyestuff. Previous phytochemical investigation of the CH_2Cl_2 extract of *D. candenatensis* heartwood led to the isolation and characterization of new and known flavonoids which showed cytotoxicity against different human cancer cell lines (Cheenpracha et al., 2009). In a continuation of our study on this plant, we now report herein the isolation and structure elucidation of five new compounds, candenatenins G–K (**1**–**5**), along with four known compounds from the acetone extract of the heartwood of the same plant. The cytotoxicity against NCI-H187 cell line as well as the antioxidation of all compounds was also evaluated.

2. Results and discussion

The acetone extract of air-dried heartwood of *D. candenatensis* was separately fractionated by a combination of silica gel CC to obtain five new compounds (**1**–**5**), together with four known structures, dinklagin A (**6**) (Ngadjui et al., 2002), stipulin (**7**) (Ngameni et al., 2004), (*R*)-4-methoxydalbergione (**8**) (Chan et al., 1997) and melilotocarpan A (**9**) (Belofsky et al., 2006) (Fig. 1). All structures were established by conventional ^{1}H and ^{13}C NMR spectral methods assisted by the performance of 2D NMR techniques, COSY, HMQC, HMBC, and NOESY, thus leading to the total assignments of the ^{1}H and ^{13}C NMR spectra of the new compounds. The known compounds were identified on the basis of spectral comparison with published data.

Compound **1** was obtained as a colorless viscous oil, and its molecular formula was determined as $C_{17}H_{18}O_4$ by HR-EI-MS at $[M]^+$ m/z 286.1200 (calcd. 286.1200). The IR spectrum showed a stretching frequency of hydroxy (3385 cm^{-1}) and aromatic (1550 cm^{-1}) functionalities. The UV spectrum displayed absorption bands at λ_{max} 210 and 285 nm, suggesting the presence of a conjugated aromatic chromophore. The ^{1}H NMR spectroscopic data (Table 1) of **1** showed a presence of two methoxyl groups at δ 3.84 (6H, *s*, 2′-OMe, 2″-OMe), of a 1,2-disubstituted benzene at δ

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http://dx.doi.org/10.1016/j.phytol.2012.07.007

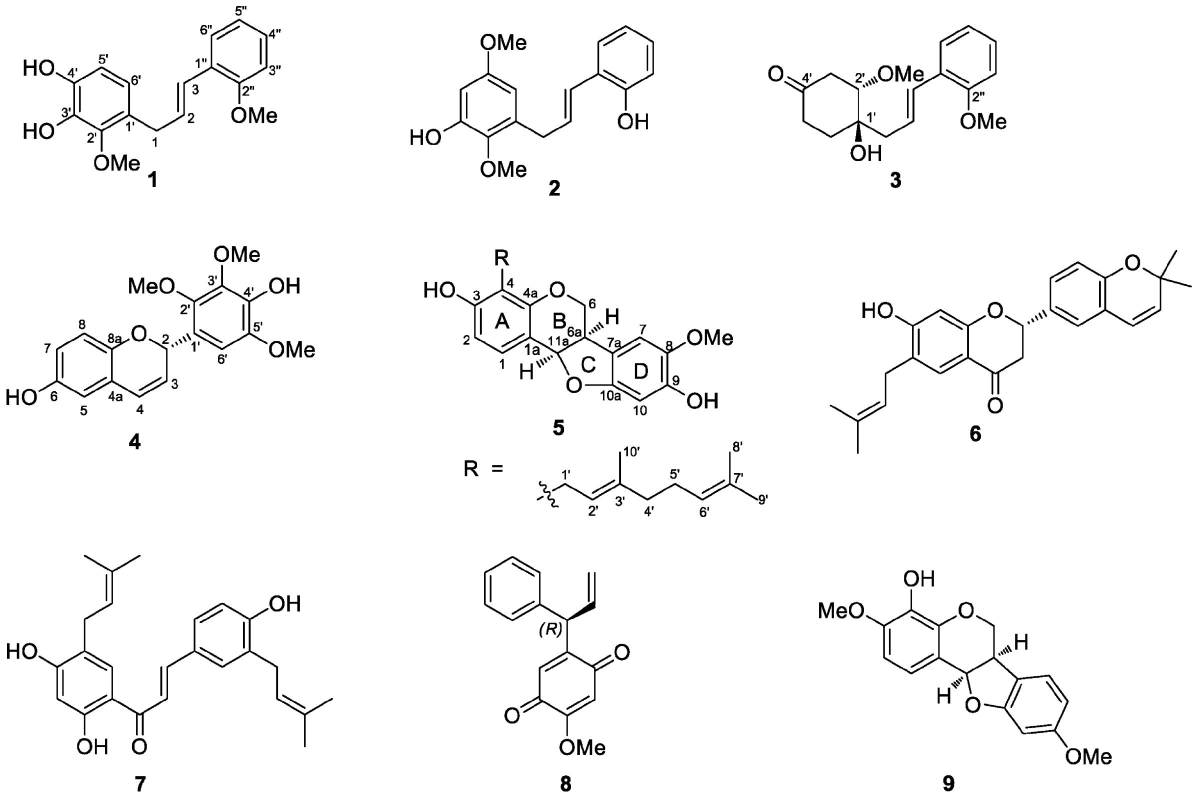

Fig. 1. Structures of compounds **1**–**9**.

7.41 (1H, *dd*, J = 7.5, 1.8 Hz, H-6″), 7.19 (1H, *td*, J = 7.5, 1.8 Hz, H-4″), 6.90 (1H, *br d*, J = 7.5 Hz, H-3″), 6.87 (1H, *br t*, J = 7.5 Hz, H-5″), of a 1,2,3,4-tetrasubstituted benzene at δ 6.69 (1H, *d*, J = 8.4 Hz, H-6′) and 6.66 (1H, *d*, J = 8.4 Hz, H-5′) and of –$CH_2CH{=}CH$– moiety at δ 6.78 (1H, *br d*, J = 15.9 Hz, H-3), 6.31 (1H, *dt*, J = 15.9, 6.6 Hz, H-2) and 3.52 (2H, *dd*, J = 6.6, 1.5 Hz, H_2-1). These data indicated that the structure of **1** was closely related to that of candenatenin A (Cheenpracha et al., 2009), except for the arrangements of OMe and OH groups in rings A and B. Only a methoxyl group was located on ring A at C-2′ and another methoxyl group was placed at C-2″ due to the ^3J HMBC correlation between methoxyl protons (δ 3.84) to C-2′ (δ 145.5) and C-2″ (δ 156.4), respectively. Accordingly, compound **1** was assigned as candenatenin G. The completed assignment of ^{13}C NMR data was shown in Table 1.

Compound **2** was isolated as a colorless viscous oil with the molecular formula $C_{17}H_{18}O_4$, determined from HR-EI-MS ($[M]^+$ m/z 286.1199, calcd. 286.1200). The ^1H NMR data (Table 1) showed signal patterns similar to those of **1**, due to two methoxyls and

Table 1
^1H and ^{13}C NMR data (300 MHz) of **1**–**3**[a] in $CDCl_3$.

No.	Candenatenin G (**1**)		Candenatenin H (**2**)		Candenatenin I (**3**)	
	δ_C	δ_H (*multi.*, J in Hz)	δ_C	δ_H (*multi.*, J in Hz)	δ_C	δ_H (*multi.*, J in Hz)
1	33.1	3.52 (*dd*, 6.6, 1.5)	29.8	3.25 (*dd*, 7.8, 1.5)	41.6	2.41 (*dd*, 14.1, 7.8) 2.59 (*m*)
2	129.6	6.31 (*dt*, 15.9, 6.6)	135.4	5.93 (*dt*, 15.4, 7.8)	124.6	6.17 (*dt*, 15.9, 7.8)
3	125.8	6.78 (*br d*, 15.9)	123.3	6.34 (*br d*, 15.4)	130.2	6.79 (*d*, 15.9)
1′	124.9		119.0		72.0	
2′	145.5		151.8		83.6	3.43 (*dd*, 4.8, 3.0)
3′	136.3		144.7		40.4	2.80 (*dd*, 14.7, 3.0) 2.50 (*m*)
4′	143.0		99.4	6.47 (*s*)	210.6	
5′	111.3	6.66 (*d*, 8.4)	140.2		37.0	2.60 (*m*) 2.17 (*m*)
6′	121.0	6.69 (*d*, 8.4)	113.2	6.52 (*s*)	32.7	2.07 (*m*) 1.74 (*m*)
1″	126.5		123.7		126.0	
2″	156.4		152.7		156.5	
3″	120.6	6.90 (*br d*, 7.5)	115.4	6.85 (*br d*, 7.5)	110.9	6.80 (*d*, 7.5)
4″	128.1	7.19 (*td*, 7.5, 1.8)	128.7	7.12 (*td*, 7.5, 1.5)	128.7	7.16 (*br t*, 7.5)
5″	110.8	6.87 (*br t*, 7.5)	120.2	6.83 (*br t*, 7.5)	120.7	6.85 (*t*, 7.5)
6″	126.6	7.41 (*dd*, 7.5, 1.8)	129.8	7.06 (*br d*, 7.5)	126.7	7.36 (*dd*, 7.5, 1.5)
2′-OMe	55.5	3.84 (*s*)	56.0	3.67 (*s*)	56.9	3.30 (*s*)
5′-OMe			56.7	3.73 (*s*)		
2″-OMe	61.5	3.84 (*s*)			55.4	3.77 (*s*)
3′-OH				5.49 (*s*)		

[a] Assignments were made using HMQC and HMBC data.

coupled ABX_2 protons. Differences included the appearance of the 1,2,3,5-tetrasubstituted aromatic protons at δ 6.52 (1H, *s*, H-6′) and 6.47 (1H, *s*, H-4′). Locations of two methoxyl and two hydroxy groups on **2** were clarified by HMBC spectrum of methylene protons H_2-1 with C-1′, C-2′ and C-6′, of 2′-OMe with C-2′, of H-6′ with C-1, C-2′, C-3′ and C-5′, and of H-3 with C-1 and C-2″, indicating the location of two methoxyl at C-2′, C-5′ and two hydroxy groups at C-3′, C-2″. The cross-peaks between H_2-1/2′-OMe and H_2-1/H-6′/5′-OMe were also observed in the NOESY experiments, confirming the assignment of the OMe at C-2′ and C-5′. In the ^{1}H NMR data, the appearance of ABX_2-type proton signals at δ 6.34 (1H, *br d*, *J* = 15.4 Hz, H-3), 5.93 (1H, *dt*, *J* = 15.4, 7.8 Hz, H-2) and 3.25 (2H, *dd*, *J* = 7.8, 1.5 Hz, H_2-1) with the coupling constant value of 15.4 Hz was signified for *trans*-configuration of $–CH_2CH{=}CH–$ unit. Thus, compound **2** was named as candenatenin H.

Compound **3**, $C_{17}H_{22}O_4$ ($[M]^+$ *m*/*z* 290.1513, calcd. 290.1499), was obtained as a colorless viscous oil. Its IR spectrum showed a carbonyl stretching frequency (1709 cm^{-1}). The ^{1}H NMR signals of **3** (Table 1) on ring B as well as the $–CH_2CH{=}CH–$ moiety were similar to those of **1**. However, the main differences are all signals in A ring. Compound **3** exhibited the presence of cyclohexanone moiety at δ 3.43 (1H, *dd*, *J* = 4.8, 3.0 Hz, H-2′), 2.80 (1H, *dd*, *J* = 14.7, 3.0 Hz, H-3′a), 2.60 (1H, *m*, H-5′a), 2.50 (1H, *m*, H-3′b), 2.17 (1H, *m*, H-5′b), 2.07 (1H, *m*, H-6′a) and 1.74 (1H, *m*, H-6′b). The remaining methoxyl proton signal at δ 3.30 was located at C-2′ because of the HMBC correlations of the oxymethine proton H-2′ (δ 3.43) with the carbon at δ 56.9 (OMe). The relative configuration of **3** was determined on the basis of the coupling constants. The small *J* values of H-2′ (*J* = 4.8 and 3.0 Hz) as well as cross-peaks between 2′-OMe with H_2-1 (δ 2.59 and 2.41) in NOESY spectrum indicated that H-2′ should be equatorially oriented and the OH and OMe groups were *trans* orientation. Therefore, compound **3** was named as candenatenin I. Since the isolation process was carried out in MeOH, candenatenin I (**3**) could be an artifact via 1,4-Michael addition of MeOH to candenatenin B (Cheenpracha et al., 2009).

Compound **4** was obtained as a colorless viscous oil and had a molecular formula $C_{18}H_{18}O_6$ as deduced from the HR-EI-MS data ($[M]^+$ *m*/*z* 330.3329, calcd. 330.3319). The IR spectrum showed absorption bands at 3425 (OH) and 1612 (aromatic ring) cm^{-1}. The UV absorption bands at λ_{max} 211 and 265 nm suggested the presence of a conjugated aromatic chromophore. The ^{1}H NMR data (Table 2) displayed the characteristic of flav-3-ene (Cheenpracha et al., 2009; Deodhar et al., 2007) at δ 6.46 (1H, *dd*, *J* = 10.2, 2.0 Hz, H-4), 6.15 (1H, *dd*, *J* = 3.5, 2.0 Hz, H-2) and 5.75 (1H, *dd*, *J* = 10.2, 3.5 Hz, H-3). The ^{1}H NMR data of **4** also showed two sets of aromatic proton signals. The first set was a 1,2,4-trisubstituted benzene ring which resonated at δ 6.63 (1H, *d*, *J* = 8.5 Hz, H-8), 6.57 (1H, *dd*, *J* = 8.5, 3.0 Hz, H-7), and 6.52 (1H, *d*, *J* = 3.0 Hz, H-5). The other set was a penta-substituted benzene ring which showed a singlet aromatic proton at δ 6.80 (1H, *s*, H-6′). In addition, three OMe signals at δ 3.94 (3H, *s*), 3.93 (3H, *s*) and 3.88 (3H, *s*) were also observed in the ^{1}H NMR spectrum which were attached to C-3′, C-2′ and C-5′, respectively, on the basis of HMBC correlations. The positions C-4′ and C-6 were attached to OH groups due to the lower field of ^{13}C NMR spectral data at δ 140.2 and 149.6, respectively. The absolute configuration at C-2 was estimated as *S* from the CD spectrum, which showed a negative Cotton effect at 280 nm (Cheenpracha et al., 2009; Garo et al., 1996). Thus, compound **4** was named as candenatenin J.

Table 2
NMR data (500 MHz, $CDCl_3$) of **4**.[a]

No.	Candenatenin J (**4**)		
	δ_C	δ_H (*multi.*, *J* in Hz)	HMBC ($^1H \rightarrow {}^{13}C$)
2	71.2	6.15 (*dd*, 3.5, 2.0)	3, 4, 1′, 2′, 6′
3	125.7	5.75 (*dd*, 10.2, 3.5)	2, 4, 1′
4	123.9	6.46 (*dd*, 10.2, 2.0)	2, 5, 8a
4a	122.1		
5	113.1	6.52 (*d*, 3.0)	4, 6, 7, 8a
6	149.6		
7	115.7	6.57 (*dd*, 8.5, 3.0)	5, 6, 8, 8a
8	116.6	6.63 (*d*, 8.5)	6, 7, 8a
8a	147.1		
1′	128.3		
2′	146.1		
3′	145.2		
4′	140.2		
5′	146.0		
6′	108.7	6.80 (*s*)	2, 1′, 2′, 4′
2′-OMe	60.8	3.93 (*s*)	2′
3′-OMe	61.2	3.94 (*s*)	3′
5′-OMe	61.9	3.88 (*s*)	5′

[a] Assignments were made using HMQC and HMBC data.

Compound **5** was isolated as a colorless viscous oil. Its molecular formula was established as $C_{26}H_{30}O_5$ by HR-EI-MS at *m*/*z* 422.2098 $[M]^+$ (calcd. 422.2088), suggesting the presence of 12 degrees of unsaturations and supported by NMR data (Table 3). The ^{1}H NMR spectrum showed the characteristic of a pterocarpan structure due to the splitting pattern of the protons at δ 5.39 (1H, *d*, *J* = 6.9 Hz, H-11a), 4.20 (1H, *dd*, *J* = 11.1, 4.5 Hz, H-6α), 3.45 (1H, *t*, *J* = 11.1 Hz, H-6β), and 3.41 (1H, *ddd*, *J* = 11.1, 6.9, 4.5 Hz, H-6a), related to the oxymethylene protons of the heterocyclic ring B, and the bridging protons of rings B and C (H-6a and H-11a) (Belofsky et al., 2006; Woo et al., 2011). The latter proton signals were assigned as *cis*-orientation by NOESY experiments. The signals of three methyl singlets [δ 1.72 (3H, *s*, H_3-10′), 1.59 (3H, *s*, H_3-8′), and 1.51 (3H, *s*, H_3-9′)], two olefinic protons [δ 5.15 (1H, *br t*, *J* = 7.2 Hz, H-2′) and 4.97 (1H, *m*, H-6′)], and three methylenes [δ 3.35 (2H, *m*, H_2-1′), 2.00 (2H, *m*, H_2-5′), and 1.96 (2H, *m*, H_2-4′)] were recognized to geranyl moiety (Woo et al., 2011). In addition, the remaining of two singlet signals of aromatic protons at δ 6.72 (1H, *s*, H-7), and 6.43 (1H, *s*, H-10), and two *ortho*-coupled doublets centered at δ 7.19 (1H, *d*, *J* = 8.4 Hz, H-1), and 6.49 (1H, *d*, *J* = 8.4 Hz, H-2) gave clear evidence of a 3,4,8,9-tetrasubstitution pattern for the pterocarpan moiety (Belofsky et al., 2006). The HMBC correlations

Table 3
NMR data (500 MHz, $CDCl_3$) of **5**.

No.	Candenatenin K (**5**)		
	δ_C	δ_H (*multi.*, *J* in Hz)	HMBC ($^1H \rightarrow {}^{13}C$)
1	129.3	7.19 (*d*, 8.4)	11a, 3, 4a
1a	112.6		
2	110.0	6.49 (*d*, 8.4)	1a, 3, 4
3	155.9		
4	114.9		
4a	153.8		
6	66.8	4.20 (*dd*, 11.1, 4.5) 3.45 (*t*, 11.1)	 6a, 11a, 4a, 7a
6a	40.3	3.41 (*ddd*, 11.1, 6.9, 4.5)	6, 4a, 7a
7	107.9	6.72 (*s*)	6a, 7a, 8, 9, 10a
7a	117.1		
8	141.1		
9	146.7		
10	98.1	6.43 (*s*)	7a, 8,9, 10a
10a	154.0		
11a	78.8	5.39 (*d*, 6.9)	6, 6a, 1, 1a, 4a
1′	22.3	3.35 (*m*)	3, 4, 4a, 2′
2′	121.5	5.15 (*br t*, 7.2)	1′, 4′, 10′
3′	138.4		
4′	39.7	1.96 (*m*)	2′, 3′, 5′, 6′, 10′
5′	26.4	2.00 (*m*)	3′, 4′, 6′, 7′
6′	123.9	4.97 (*m*)	
7′	131.9		
8′	25.6	1.59 (*s*)	6′, 7′, 9′
9′	17.7	1.51 (*s*)	6′, 7′, 8′
10′	16.2	1.72 (*s*)	2′, 3′, 4′
8-OMe	57.0	3.79 (*s*)	8

(Table 3) of the OMe protons at δ 3.79 with C-8 and of an aromatic proton at δ 6.72 (H-7) with C-6a, C-8, C-9 and C-10a established the attachment of the OMe and OH groups at C-8 and C-9, respectively. Furthermore, the methylene protons at δ 3.35 (H_2-1′) showed HMBC connectivity to C-3, C-4, and C-4a, confirming the location of substituents on the A ring. In the NOESY spectrum, this spectral evidence finalized the complete structure of **5** which was named as candenatenin K.

Compounds **1**–**9** were investigated for DPPH scavenging activity and cytotoxicity against small cell lung cancer (NCI-H187). Compound **1** possessed the moderate activity with IC_{50} value of 76.7 μM, whereas compounds **2** and **5** exhibited potent activity against DPPH radical with IC_{50} values of 41.0 and 25.7 μM, respectively, which were higher than the standard compound, ascorbic acid with an IC_{50} of 44.4 μM. The rest of the compounds (**3**, **4**, **6**–**9**) were found to be inactive against DPPH radical. Unfortunately, only compound **2** exhibited cytotoxicity against NCI-H187 cell line with IC_{50} value of 14.8 μM.

3. Experimental

3.1. General

The specific rotation ($[\alpha]_D$) values were determined with a JASCO P-1020 polarimeter. The CD spectra were measured using a JASCO J-810 spectropolarimeter. The UV spectra were recorded with PerkinElmer UV–vis spectrophotometer. The IR spectra were recorded with PerkinElmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 300 and 500 MHz Bruker FTNMR Ultra Shield spectrometers. Chemical shifts are reported in parts per million (δ) in $CDCl_3$ with TMS as an internal reference. The EIMS data was obtained from a Micromass LCT and MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F_{254} was used for analytical purposes.

3.2. Plant material

The heartwood of *D. candenatensis* was collected in April 2010 in Sikhao district, Trang Province, Southern Thailand. Botanical identification was achieved through comparison with a voucher specimen number SC10 in the Herbarium of Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand by Prof. Puangpen Sirirugsa.

3.3. Extraction and isolation

The dried and chopped heartwood of *D. candenatensis* (2.5 kg) was extracted successively with CH_2Cl_2 and acetone (3× 5 L, 5 days) at room temperature. Filtration and subsequent evaporation of the combined acetone extract to dryness *in vacuo* afforded a dark brown gum (7.31 g) which was subjected to silica gel QCC with a gradient of hexanes–EtOAc (100:0 to 0:100, v/v) to give 9 fractions (A–I). Fraction C (51.8 mg) was purified by CC with CH_2Cl_2–hexanes (3:7, v/v) to give **6** (3.0 mg) and **8** (5.4 mg). Fraction E (1.53 g) was separated by QCC eluting with CH_2Cl_2–hexanes (1:1, v/v) to give seven subfractions (E1–E7). Subfraction E2 (40.5 mg) was subjected to CC with acetone–hexanes (1:4, v/v) to afford **2** (20.2 mg), whereas **5** (15.0 mg) was derived from subfraction E3 (36.3 mg) with the same procedure. Subfraction E6 (92.5 mg) was purified by CC with EtOAc–CH_2Cl_2 (1:49, v/v) to give **7** (50.0 mg). Fraction G (3.11 g) was separated by QCC with acetone–hexanes (3:17, v/v) to give five subfractions (G1–G5). Subfraction G3 (135.0 mg) was purified by CC with EtOAc–CH_2Cl_2 (3:97, v/v) to give **3** (7.3 mg). Subfraction G5 (100.3 mg) was purified by CC with $CHCl_3$–hexanes (9:1, v/v) to give **9** (2.0 mg). Fraction H (110.0 mg) was separated by CC with EtOAc–hexanes (3:97, v/v) to give **1** (3.3 mg) and **4** (2.3 mg).

3.4. Candenatenin G (*1*)

Viscous oil; UV (MeOH) (log ε): 210 (4.09), 285 (3.31) nm; IR (neat): 3385, 2924, 1659, 1550,1463, 1376, 1245, 1166 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-EI-MS: $[M]^+$ m/z 286.1200 (calcd. for $C_{17}H_{18}O_4$, 286.1200).

3.5. Candenatenin H (*2*)

Viscous oil; UV (MeOH) (log ε): 210 (4.07), 240 (3.49), 291 (3.31) nm; IR (neat): 3418, 3004, 2926, 1602, 1223 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-EI-MS: $[M]^+$ m/z 286.1199 (calcd. for $C_{17}H_{18}O_4$, 286.1200).

3.6. Candenatenin I (*3*)

Viscous oil; $[\alpha]_D^{26}$ −62.5 (*c* 0.30, $CHCl_3$); UV (MeOH) (log ε): 210 (4.30), 253 (4.09), 300 (3.65) nm; IR (neat): 3426, 2928, 1709, 1598, 1243 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HR-EI-MS: $[M]^+$ m/z 290.1513 (calcd. for $C_{17}H_{22}O_4$, 290.1499).

3.7. Candenatenin J (*4*)

Viscous oil; $[\alpha]_D^{27}$ −24.4 (c 0.09, MeOH); UV (MeOH) (log ε): 211 (3.80), 265 (3.26) nm; CD (CH_2Cl_2, *c* 0.20): $\Delta\varepsilon_{246}$ +0.4, $\Delta\varepsilon_{280}$ −1.4; IR (neat): 3425, 1612, 1273 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 2; HR-EI-MS $[M]^+$ m/z 330.3329 (calcd. for $C_{18}H_{18}O_6$: 330.3319).

3.8. Candenatenin K (*5*)

Viscous oil; $[\alpha]_D^{26}$ −99.7 (*c* 0.30, $CHCl_3$); UV (MeOH) (log ε): 210 (4.18), 298 (3.30) nm; IR (neat): 3418, 2925, 1613, 1217 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 3; HR-EI-MS: $[M]^+$ m/z 422.2098 (calcd. for $C_{26}H_{30}O_5$, 422.2088).

3.9. Radical scavenging activity

The DPPH radical-scavenging activity was determined using the method proposed by Wojdylo et al. (2007). Briefly, 1 ml of 15 μM methanolic solution of DPPH was mixed with 1 ml of sample solution. The mixture was then vortexed vigorously and left for 45 min at room temperature in the dark. The absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. All determinations were performed in triplicate.

3.10. Cytotoxic assay

The procedures for cytotoxic assay of small cell lung cancer (NCI-H187) were performed by resazurin microplate assay (REMA) which was a modified method of fluorescent dye for the mammalian cell cytotoxicity (Brien et al., 2000). The standard compounds were ellipticine which had IC_{50} value of 7.0 μM.

Acknowledgements

We would like to thank the Thailand Research Fund, the office of the Higher Education Commission (Grant No. MRG5480028) and University of Phayao (Grant No. R020054116022), for financial support. We appreciate Prof. Puangpen Sirirugsa for plant identification and are also thankful to Department of Chemistry, Faculty of Science, Prince of Songkhla University, for recording the

NMR spectrum. We are grateful to BIOTEC (Thailand) for cytotoxic assay.

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Note

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Alstoniaphyllines A–C, Unusual Nitrogenous Derivatives from the Bark of *Alstonia macrophylla*

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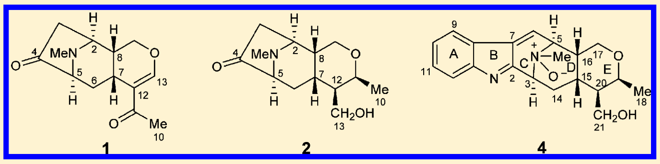
Supporting Information

ABSTRACT: Chemical investigation of an alkaloidal extract of *Alstonia macrophylla* bark led to the isolation and identification of two new nitrogenous derivatives, alstoniaphyllines A (**1**) and B (**2**), a new indole alkaloid, alstoniaphylline C (**4**), and eight known alkaloids (**3**, **5**–**11**). Alstonisine (**9**) exhibited antiplasmodial activity against *Plasmodium falciparum*, with an IC_{50} of 7.6 μM.



Alstonia macrophylla, known as "Tung fa" in Thailand, usually grows as a shrub or tree distributed within the tropical regions of America, Africa, Australia, and Asia.[1] *A. macrophylla* stem bark is used as a tonic and aphrodisiac.[2] The plant is also a rich source of unique monoterpene indole skeletons. Some of the indole alkaloids isolated from *A. macrophylla*, especially villalstonine and macrocarpamine, have attracted significant attention, as they possess a range of biological activities, such as anticancer and antimalarial properties.[3−7] As part of our research program focusing on the isolation of bioactive compounds from Thai medicinal plants,[8−10] the alkaloidal extract of the bark of *A. macrophylla* was examined for antimalarial activity (IC_{50} 3.07 μg/mL). Herein, we describe the isolation and structure elucidation of two new unusual nitrogenous derivatives (**1** and **2**) and a new indole alkaloid (**4**) from the MeOH extract of the bark of *A. macrophylla*, in addition to eight known alkaloids: angustimaline (**3**),[11] alstonerinal (**5**),[12] alstophyllal (**6**),[13] talcarpine (**7**),[14] macrocarpine B (**8**),[15] alstonisine (**9**),[14] N_b-demethylalstophylline oxindole (**10**),[14] and 10-methoxycathafoline (**11**)[12] (Figures 1 and S1, Supporting Information). The known compounds were identified by comparison of their physical and spectroscopic data with published values.[11−15]

The bark of *A. macrophylla* was extracted with MeOH and partitioned between EtOAc and 1% HCl. The aqueous layer was adjusted to pH 8−9 with saturated Na_2CO_3, and the water-soluble materials were extracted with EtOAc. The EtOAc-soluble material was purified by silica gel column chromatography to afford 11 alkaloidal compounds, compounds **1**, **2**, and **4** being new entries.

Alstoniaphylline A (**1**) was isolated as a light yellow gum. Its molecular formula, $C_{13}H_{17}NO_3$, was determined by the pseudomolecular ion peak at m/z 236.1287 $[M + H]^+$ (calcd for 236.1281) in the APCITOFMS. The IR absorptions for a ketocarbonyl group at 1743 cm^{-1} and conjugated carbonyl at 1651 cm^{-1}, and the UV absorptions at λ_{max} 210 and 257 nm, supported the presence of an α,β-unsaturated carbonyl in the structure.[11] The ^{13}C NMR and DEPT spectroscopic data showed the presence of 13 carbons attributable to two methyl, [δ 42.4 (*N*-Me) and 25.1 (C-10)], three methylene, one of which was an oxymethylene carbon [δ 67.0 (C-9), 37.2 (C-3), and 32.3 (C-6)], five methine, one of which was an olefinic carbon [δ 157.4 (C-13), 69.1 (C-5), 60.3 (C-2), 36.9 (C-8), and 23.4 (C-7)], and three quaternary carbons, two of which were ketocarbonyls [δ 219.0 (C-4),[15] 195.5 (C-11), and 120.8 (C-12)]. The ^1H NMR data (Table 1) displayed the presence of a lower field signal at δ 7.57 (1H, s, H-13) attributable to a vinylic proton associated with a vinyl ether function, an *N*-methyl singlet at δ 2.31 (3H, s, *N*-Me), and an acetyl group at δ 2.19 (3H, s, H-10). These data were in good agreement with the E-ring of a typical type-B macroline such as angustimaline (**3**), which was previously isolated from *A. angustifolia*.[4,11] However, the C-4 oxymethine in **3** was replaced by a carbonyl group (δ 219.0) in **1**. Assignments of the protonated carbons of **1** were achieved by the combined analysis of COSY (Figure 2) and HMQC spectroscopic data. The connectivity of H-2/H_2-3, H-2/H-8, H-8/H_2-9, H-7/H_2-6, and H_2-6/H-5 in the COSY spectrum led to the characteristic assignment of macroline compounds, i.e., C(3)−C(2)−C(8)−C(9) and C(5)−C(6)−C(7) fragments, respectively.[12,16] The molecule was therefore assembled accordingly and required only insertion of a carbonyl functionality to complete the structure of alstoniaphylline A (**1**), which was in perfect agreement with the HMBC data (Figure 2). The HMBC cross-peaks of the methine proton [δ 3.44 (H-2)] with the carbons at δ 219.0 (C-4), 67.0 (C-9), 36.9 (C-8), and 23.4 (C-7), of the methylene protons [δ 2.30, 1.63 (H_2-6)] with the carbons at δ 219.0 (C-4), 69.1 (C-5), and 23.4 (C-7), and of *N*-Me (δ 2.31) with the carbons at δ 69.1(C-5) and 60.3 (C-2) confirmed a C-4 ketocarbonyl group. The

Received: October 5, 2012
Published: April 8, 2013

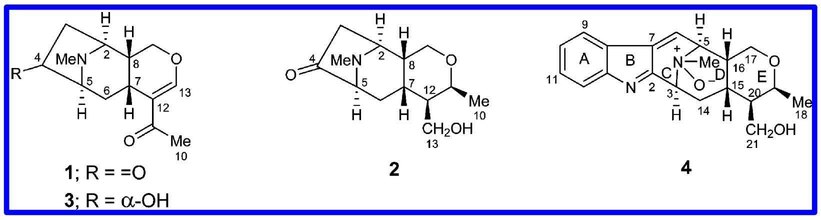


Figure 1. Structures of compounds **1**−**4** from *A. macrophylla* bark.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data of **1**, **2**, and **4** in $CDCl_3$[a]

	alstoniaphylline A (**1**)		alstoniaphylline B (**2**)		alstoniaphylline C (**4**)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
2	60.3, CH	3.44, d (7.8)	60.8, CH	3.44, m	182.2, C[b]	
3	37.2, CH_2	2.77, dd (17.5, 7.8) 2.13, d (17.5)	37.0, CH_2	2.68, dd (18.7, 11.5) 2.00, dd (18.7, 3.7)	68.4, CH	4.70, br s
4	219.0, C[b]		220.0, C[b]			
5	69.1, CH	2.85, m	69.2, CH	2.92, br s	56.3, CH	3.27, br d (5.8)
6	32.3, CH_2	2.30, m 1.63, m	26.7, CH_2	2.01, m 1.77, m	107.8, CH	6.08, d (5.8)
7	23.4, CH	2.85, m	27.4, CH	2.26, m	137.2, C	
8	36.9, CH	1.92, m	40.6, CH	1.87, m	120.8, C	
9	67.0, CH_2	4.48, t (11.6) 4.22, ddd (11.6, 2.4, 2.0)	67.1, CH_2	4.13, t (11.8) 3.80, dd (11.8, 5.4)	125.3, CH	7.69, d (8.1)
10	25.1, CH_3	2.19, s	20.0, CH_3	1.20, d (6.1)	118.8, CH	6.85, t (8.1)
11	195.5, C		70.1, CH	3.44, m	136.6, CH	7.45, t (8.1)
12	120.8, C		46.6, CH	1.68, m	107.4, CH	6.75, d (8.1)
13	157.4, CH	7.57, s	60.9, CH_2	3.65, dd (11.8, 5.0) 3.30, m	152.0, C	
14					27.1, CH_2	2.21, dd (7.6, 2.8) 1.84, dd (7.6, 1.8)
15					25.9, CH	2.18, m
16					39.7, CH	1.95, br d (11.8)
17					67.0, CH_2	4.05, t (11.8) 3.82, dd (11.8, 4.8)
18					20.0, CH_3	1.21, d (6.3)
19					70.0, CH	3.45, m
20					46.9, CH	1.62, m
21					61.4, CH_2	3.60, dd (10.8, 4.8) 3.51, dd (10.8, 6.3)
N-Me	42.4, CH_3	2.31, s	42.2, CH_3	2.31, s	41.7, CH_3	2.24, s

[a] Chemical shifts (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses. [b] ^{13}C NMR chemical shifts obtained from HMQC and HMBC spectra.

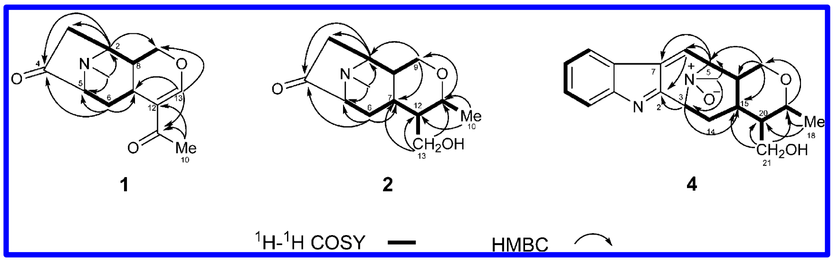


Figure 2. Selected 2D NMR correlations of **1**, **2**, and **4**.

HMBC correlations of the olefinic proton [δ 7.57 (H-13)] with the carbons at δ 195.5 (C-11), 120.8 (C-12), 67.0 (C-9), and 23.4 (C-7), the methyl group [δ 2.19 (H_3-10)] with the carbons at δ 195.5 (C-11) and 120.8 (C-12), and the methylene protons [δ 4.48, 4.22 (H_2-9)] with the carbons at δ 157.4 (C-13), 36.9 (C-8), and 23.4 (C-7) indicated that alstoniaphylline A possessed the nonindole portion of a type-B macroline alkaloid.[17] The ring junction stereochemistry between rings C, D, and E in **1** was assumed to follow the same pattern as for the known macrolines angustimaline (**3**)[11] and alstonerinal (**5**)[12] by comparison of the chemical shifts and NOE data. The interactions between H-2/*N*-Me/*N*-5 and H-7/H-8 were observed for **1** in the NOE experiments (Figure 3), confirming the C/D and D/E *cis* ring junctions. This

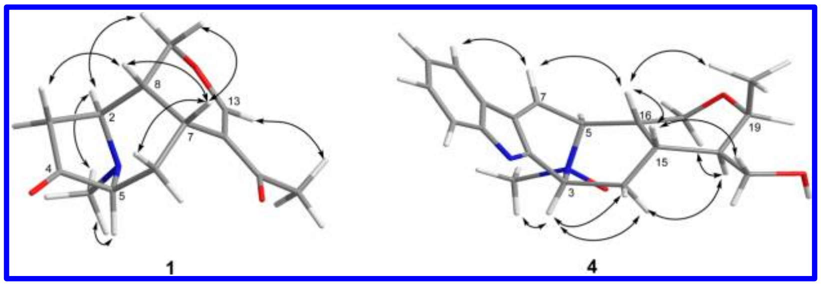


Figure 3. Key NOE correlations of **1** and **4**.

compound is probably derived from fragmentation of a macroline-type precursor.[12,17]

Alstoniaphylline B (**2**) was obtained as a light yellow gum. The molecular formula was established as $C_{13}H_{21}NO_3$ by ESITOFMS data, according to the $[M + H]^+$ ion peak at *m/z* 240.1591 (calcd for 240.1600). The IR spectrum showed the absorption bands of hydroxy (3405 cm^{-1}) and ketocarbonyl (1743 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectroscopic data (Table 1) revealed a nonindole portion resembling **1**, except for the absence of the vinylic proton at δ 7.57 (1H, s, H-13) and the presence of a methine proton at δ 3.44 (1H, m, H-11) and a doublet at δ 1.20 (3H, d, *J* = 6.1 Hz, H_3-10). In addition, the acetyl group at δ 2.19 (3H, s, H-10) in **1** was replaced by the oxymethylene protons at δ 3.65 (1H, dd, *J* = 11.8, 5.0 Hz, H-13a) and 3.30 (1H, m, H-13b) in **2**. The COSY correlations of H_3-10/H-11/H-12/H_2-13 and H-12/H-7/H_2-6/H-5, together with HMBC correlation (Figure 2) of H-11 with C-9, confirmed that **2** should be a type-A macroline compound.[4] The orientation of the substituents at C-11 and C-12 was confirmed by the NOE interaction between H_2-13/H-8, H-11/H-9a, and H-11/H-6α. Alstoniaphylline B (**2**) retains all the features of the nonindole portion of a type-A macroline compound.[4]

Alstoniaphylline C (**4**) was isolated as a colorless oil with the molecular formula $C_{20}H_{24}N_2O_3$, as determined by ESITOFMS ($[M + H]^+$, *m/z* 341.1853, calcd 341.1865). IR absorptions at 3397 cm^{-1} implied the presence of a hydroxy functionality. The UV spectrum showed absorption maxima at λ_{max} 210, 250, 289, and 320 nm, indicating the presence of a typical modified indolenine chromophore.[16] This was confirmed by the characteristic chemical shift of C-2 at δ 182.2 in the ^{13}C NMR spectrum due to the conjugated imino carbon.[18,19] The ^1H NMR spectroscopic data (Table 1) revealed the presence of a substituted indole moiety due to the signals of four aromatic protons [δ 7.69 (1H, d, *J* = 8.1 Hz, H-9), 7.45 (1H, t, *J* = 8.1 Hz, H-11), 6.85 (1H, t, *J* = 8.1 Hz, H-10), and 6.75 (1H, d, *J* = 8.1 Hz, H-12)] and an E-ring from a typical type-A macroline (e.g., macrocarpine A[15] and **2**) [δ 4.05 (1H, t, *J* = 11.8 Hz, H-17α), 3.82 (1H, dd, *J* = 11.8, 4.8 Hz, H-17β), 3.60 (1H, dd, *J* = 10.8, 4.8 Hz, H-21a), 3.51 (1H, dd, *J* = 10.8, 6.3 Hz, H-21b), 3.45 (1H, m, H-19), 2.18 (1H, m, H-15), 1.95 (1H, br d, *J* = 11.8 Hz, H-16), 1.62 (1H, m, H-20), and 1.21 (3H, d, *J* = 6.3 Hz, H_3-18)]. The full ^1H and ^{13}C NMR spectroscopic assignments for **4** based on 2D NMR experiments (Table 1) indicated that **4** had the same skeleton as macrocarpine A,[15] except for the absence of methylene proton signals [δ 3.25 (1H, dd, *J* = 17, 7 Hz, H-6a), 2.47 (1H, m, H-6b)] and *N*(1)-Me [δ 3.62 (3H, s)]. A ^1H NMR resonance at δ 6.08 (1H, d, *J* = 5.8 Hz, H-6), which correlated with the methine proton at δ 3.27 (1H, br d, *J* = 5.8 Hz) in the ^1H−^1H COSY spectrum, was attributed to the C-6 vinylic proton. This finding was confirmed by a HMBC experiment, demonstrating correlations of H-6 with C-2, C-5, and C-7 (Figure 2). In addition, alstoniaphylline C (**4**) possessed an N_4-oxido group, deduced from the downfield chemical shifts at δ 68.4 (C-3), 56.3 (C-5), and 41.7 (N_4-Me) compared to δ 54.8 (C-3), 53.6 (C-5), and 23.2 (N_4-Me) in **5**.[12] The relative configuration of **4** was readily confirmed from the observed NOE interactions (Figure 3) and comparison with the known, published compound macrocarpine A.[15]

The isolated compounds were tested for their antimalarial activity and cytotoxicity. Only alstonisine (**9**) showed moderate in vitro antiplasmodial activity against *Plasmodium falciparum*, with an IC_{50} value of 7.6 μM; none of the compounds were cytotoxic to the oral cavity cancer cell line KB (IC_{50} = >100 μM). Notably alkaloid **10**, with a C-11 methoxy group, had a reduced antimalarial activity, compared to compound **9**.

■ EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotation values were determined with a Bellingham & Stanley ADP400 polarimeter. The UV spectra were recorded with a PerkinElmer UV−vis spectrophotometer. The IR spectra were recorded with a PerkinElmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker FTNMR Ultra Shield spectrometers. Chemical shifts are reported in parts per million (δ) in $CDCl_3$ with TMS as an internal reference. The TOFMS data were obtained from a Micromass LCT and MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

Plant Material. The bark of *A. macrophylla* was collected in April 2011 in Wangwisat District, Trang Province, Southern Thailand. Botanical identification was achieved through comparison with a voucher specimen, number QSBG-2009-983, in the Herbarium Collection of Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand, by Prof. Puangpen Sirirugsa.

Extraction and Isolation. Dried and chopped bark of *A. macrophylla* (2.0 kg) was extracted with MeOH (2 × 5 L, 5 days) at room temperature, and the solvent was evaporated under vacuum. The residue was dissolved in 1% HCl (2 L) and partitioned with EtOAc (2 × 3 L). The aqueous layer was treated with saturated aqueous Na_2CO_3 to adjust the pH to 8−9 and extracted with EtOAc (2 × 3 L) to give an alkaloidal fraction (6.0 g). The alkaloidal fraction was purified by QCC with a gradient of hexanes−EtOAc−MeOH (100:0:0−0:0:100, v/v) to give nine fractions (A−I). Fraction C (1.5 g) was further subjected to silica gel QCC to provide nine subfractions (C1−C9). Subfraction C6 (395.7 mg) was separated by CC and eluted with acetone−CH_2Cl_2 (1:25, v/v) to give eight subfractions (C6a−C6f). Compounds **1** (5.1 mg), **5** (6.2 mg), and **8** (28.9 mg) were isolated from subfraction C6d (72.3 mg) by CC and elution with MeOH−CH_2Cl_2 (1:99, v/v). Recrystallization of subfraction C6 (298.4 mg) with acetone−hexanes (1:4, v/v) led to the isolation of compound **9** (20.3 mg). Subfraction C8 (93.5 mg) was subjected to CC and eluted with acetone−CH_2Cl_2 (1:9, v/v) to afford compounds

4 (2.0 mg) and **11** (3.4 mg). Fraction E (441.3 mg) was purified by CC with acetone−CH_2Cl_2 (1:5, v/v) followed by CC with acetone−hexanes (1:4, v/v) to give compounds **2** (4.8 mg) and **10** (5.1 mg). Compounds **6** (2.6 mg) and **7** (5.9 mg) were derived from fraction F (119.8 mg), eluting with MeOH−CH_2Cl_2 (1:49, v/v), while compound **3** (11.5 mg) was isolated from fraction G (2.06 g) by repeated CC with MeOH−CH_2Cl_2 (1:19, v/v).

Alstoniaphylline A (**1**): light yellow gum; $[\alpha]^{26}_{D}$ +50 (c 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.09), 257 (3.31) nm; IR ($CHCl_3$) ν_{max} 2926, 1743, 1651, 1618, 1195 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; APCITOFMS m/z 236.1287 $[M + H]^+$ (calcd for $C_{13}H_{18}NO_3$, 236.1281).

Alstoniaphylline B (**2**): light yellow gum; $[\alpha]^{26}_{D}$ +20 (c 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 221(3.64) nm; IR ($CHCl_3$) ν_{max} 3405, 2931, 1743, 1616, 1467, 1122 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESITOFMS m/z 240.1591 $[M + H]^+$ (calcd for $C_{13}H_{22}NO_3$, 240.1600).

Alstoniaphylline C (**4**): colorless oil; $[\alpha]^{26}_{D}$ +133 (c 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (4.10), 250 (3.96), 289 (3.88), 320 (3.78) nm; IR ($CHCl_3$) ν_{max} 3397, 2926, 1684, 1637, 1602, 1466, 1127 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESITOFMS m/z 341.1853 $[M + H]^+$ (calcd for $C_{20}H_{25}N_2O_3$, 341.1865).

Antimalarial Assay. Antimalarial activity against the parasite *P. falciparum* (K1, multidrug resistant) was evaluated using the method of Trager and Jensen.[20] Quantitative assessment of antimalarial activity in vitro was performed using the microculture radioisotope technique, based on the method described by Desjardins et al.[21] The concentrations of the compounds leading to a 50% reduction in parasite growth (IC_{50}) were determined by measuring the uptake of $[^3H]$-hypoxanthine by *P. falciparum* in vitro. The standard antimalarial compound was dihydroartemisinin (IC_{50} = 1.41 nM).

Cytotoxicity Assay. The cytotoxicity of the compounds to the oral cavity cancer (KB) cell line was assessed using the resazurin microplate assay, which is a modified method of the fluorescent dye-uptake assay for measuring mammalian cell cytotoxicity.[22] Ellipticine (IC_{50} = 4.87 μM) and doxorubicin (IC_{50} = 0.55 μM) were used as standard, reference cytotoxic substances.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra for compounds **1−2** and **4**. These material are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank the Thailand Research Fund, the office of the Higher Education Commission, and University of Phayao (Grant No. MRG5480028) for financial support. We are indebted to N. Chimnoi, Chulabhorn Research Institute, Bangkok, for recording mass spectrometric data and are also thankful to Prof. P. Sirirugsa (Prince of Songkla University) for plant identification. We are grateful to BIOTEC (Thailand) for biological assays.

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