Study of Chemical Constituents in Dichloromethane Extract from Root of *Polyalthia debilis* (Piere) Finet & Gagnep.

การศึกษาองค์ประกอบทางเคมีของรากก้นครกในส่วนสกัดหยาบไดคลอโรมีเทน

Daungrudee Yodbuddee (ดวงฤดี ยอดบุดดี) Dr. Kwanjai Kanokmedhakul (ดร. ขวัญใจ กนกเมธากุล)***
*Dr. Somdej Kanokmedhakul (ดร. สมเดช กนกเมธากุล)***

ABSTRACT

Isolation and chromatographic separation of crude dichloromethane extract from root of *Polyalthia debilis* (Piere) Finet & Gagnep. afforded 4 principle compounds (compound **1-4**). Structure of these compounds were elucidated by spectroscopic methods such as IR, ^{1}H NMR, ^{13}C NMR, MS and Elemental analysis. Compound **1** was identified as a sterol, **β-sitosterol**. While, compound **2** and **3** were new compounds, which were isolated from nature. Their structure belong to a symmetric of aporphine alkaloids, which we named as **Bisdebiline A** and **Bisdebiline B**, respectively. Their structures were elucidated by 1D and 2D NMR technique such as DEPT, COSY, HMBC and HMQC. Compound **4** was lactone which its DEPT, COSY, HMBC and HMQC spectrum indicated a mixture of isomers. Therefore, the acetylation derivative, compound **4a**, was prepared and it could confirmed the presence of hydroxyl group in lactone ring. From present evidence, compound **4** and **4a** were identified only as partial structure of 3 parts of 3,5-disubstituent γ-lactone part, diene which one was terminal alkene part, and a long chain alkane part. However, the connection to the complete structure need more spectrum data.

Bioactivity testing found that the two new compounds, compound **2** and **3** exhibited a good potential antimalarial activity against *Plasmodium falciparum* with EC_{50} = 4.11 and 5.4 μg/ml, respectively.

บทคัดย่อ

จากการสกัดแยกองค์ประกอบทางเคมีของรากก้นครก *Polyalthia debilis* (Piere) Finet & Gagnep. ในส่วนสกัดหยาบไดคลอโรมีเทนโดยวิธีโครมาโทรกราฟีได้สารที่น่าสนใจ 4 สาร จากการพิสูจน์โครงสร้างของสารเหล่านี้โดยวิธีทางสเปกโทรสโกปี ได้แก่ IR, ^{1}H NMR, ^{13}C NMR, MS และ Elemental analysis พบว่า สาร **1** เป็น **β-sitosterol** สาร **2** และสาร **3** เป็นสารใหม่ที่แยกได้จากธรรมชาติ มีโครงสร้างเป็น aporphine alkaloid ที่เป็น dimer แบบสมมาตรซึ่งได้ให้ชื่อว่า **Bisdebiline A** และ **Bisdebiline B** ตามลำดับ โครงสร้างของสารใหม่นี้พิสูจน์โดยใช้เทคนิค 1D และ 2D NMR เช่น DEPT, COSY, HMBC และ HMQC สำหรับสาร **4** เป็น lactone ซึ่งจากข้อมูล เช่น DEPT, COSY, HMBC และ HMQC พบว่ามีไอโซเมอร์ผสมอยู่ จึงเตรียมอนุพันธ์จากปฏิกิริยา Acetylation ได้สาร **4a** ซึ่งสามารถยืนยันได้ว่ามีหมู่ hydroxy ในวง Lactone จากข้อมูลที่มีอยู่ขณะนี้สามารถพิสูจน์โครงสร้างได้เป็น 3 ส่วนคือ ส่วนที่เป็น 3,5-disubstituent γ-lactone ส่วนที่เป็น diene ที่มีพันธะคู่ 1 คู่อยู่ปลาย และส่วนที่เป็น alkane สายโซ่ยาว อย่างไรก็ตามยังต้องการข้อมูลเพิ่มเติมในการประกอบเป็นโครงสร้างทั้งหมด

สำหรับการทดสอบฤทธิ์ในทางชีวภาพพบว่าสารใหม่คือสาร 2 และสาร 3 ให้ผลดี โดยแสดงการยับยั้งเชื้อ *Plasmodium faltiparum* ซึ่งเป็นสาเหตุของโรคมาลาเรีย ด้วยค่า EC_{50} = 4.11 และ 5.4 μg/ml ตามลำดับ

Keywords: *Polyalthia debilis*, Bisdebiline A, Bisdebiline B
คำสำคัญ: ก้นครก บิสดีบิลิน เอ บิสดิบลีน บี

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* A master's degree graduate. Master of Science Program in Chemistry, Faculty of Sciences, Khon Kaen University.
** Assistant Professor, Department of Chemistry, Faculty of Sciences, Khon Kaen University.

Introduction

Malaria is one of the 10 most prevalent and deadly disease in the world, and kills more people than any other communicable disease except tuberculosis. In many developing countries and especially in Africa, malaria exacts an enormous tolls in lives, in medical costs and in days of labour lost. (WHO, 1999) Between 300 to 500 million clinical cases occur every year with over 1.2 to 2.7 million deaths. More than 90% of these occur in Sub-Sahara Africa. About 800-1,000 cases of malaria are reported in Australia each year (Disclaimer, 2000). Malaria is endemic in a total of 101 countries and territories: 45 countries in WHO's African Region, 21 in WHO's Americas Region, 4 in WHO's European Region, 14 in WHO's Eastern Mediterranean Region, 8 in WHO's South-East Asia Region, and 9 in WHO's Western Pacific Region (WHO, 1999).

The recent malaria situation in Thailand during the first half of the year 2000 reported a total of 70,968 cases of patients were found to be associate with this disease (ACT Malaria, 2001). Malaria remains a significant public health challenge in Thailand although in rather restricted.

Malaria is a serious disease caused by a parasite, *Plasmodium protozoa.* There are four kinds of *Plasmodium* that can infect humans : *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae.* (WHO, 1999) of these, *P. falciparum* accounts for the majority of infections and is the most lethal. The high-risk groups are young children, women during pregnancy, and non-immune travellers, refugees, displaced persons and labourer entering endemic areas. Malaria is transmitted by Anopheles mosquitoes.

S. Kanokmedhakul *et al* (1997). investigated root of *Polyalthia debilis* (Piere) Finet & Gagnep and found that crude extract of n-hexane, dichloromethane and methanol shown significantly antimalarial activity against *P. falciparum* with EC_{50} = 3.90 x 10^{-5}, 1.35 x 10^{-6} and 3.9 x 10^{-5} g/ml, respectively. Four compounds from dichloromethane extract were isolated. However, their structures have not completely identified. Therefore, this study was a continue project to further isolate and identify the chemical constituents from dichloromethane extract and to search for the active principles.

Experimental

Apparatus

All melting points (uncorrected) were recorded in °C and were measured on Electrothermal IA9000 SERIES Digital Melting Point Apparatus. IR spectra were measured with Perkin-Elmer 683 infrared Spectrometer and FTIR-8601PC SHIMADZU spectrometer. Nuclear magnetic resonance spectra were recorded on Jeol JNM-EX 90A or Brucker DMX 400 spectrometer. Mass spectra were taken with a Finnigan Mat INCOS 50 spectrometer. Elemental analysis were measured on Perkin Elmer Series II CHNS/O Analyser 2400.Silica gel 60 (Merck) 70-230 mesh, silica gel 60 (Merck) 230-400 mesh and aluminium oxide 90 active, neutral 70-230 mesh (Merck) were used as adsorbents.

Plant Material

The root of *P. debilis* (Pierre) Finet & Gagnep was collected at Khon Kaen University, Khon Kaen, Thailand in August 1998, and was identifield by Associate Prof. Dr Pranom Chantaranothai, Department of Biology, Khon Kaen University. A plant specimen (voucher number SK 002) was deposited in the Herbarium of the Department of Biology, Khon Kaen University.

Extraction and Isolation

The root of *P. debilis* (2.6 kg) was dried and extracted with hexane 4 L, at room temperature for 3 days, then filtered, the process was repeated three times. Filtrates were combined and the solvent was removed *in vacuo* to yield a brown crude hexane extract 30 g (1.15%). The marc was then extracted with dichloromethane (4 L x 3) to yield a dark brown crude dichloromethane extract 32 g (1.23%). Finally, the marc was further extracted with methanol (4 L x 3) to yield black brown crude methanol extract 59 g (2.26%). Crude dichloromethane (32 g) was separated on silica gel flash column chromatography using 20-90% EtOAc: Hexane and 10-50% MeOH: EtOAc mixtures as eluent which gradually increasing polarity to yield 98 fractions of 150 ml each. Then each fraction was monitoring by TLC to give 10 combined fractions

(F_1-F_{10}). Fraction F_2, F_3 and F_4 were selected for further separation due to their TLC profiles and their adequate amount for investigation.

Fraction F_2 chromatographed on a silica gel column which gradually eluted with Hexane and EtOAc by increasing polarity of the solvents and afforded 9.7 mg of white needles of **compound 1** (9.7 mg).

Fraction F_3 (1.463 g) was purified by column chromatography eluted with 80% CH_2Cl_2: Hexane. A total of 13 fractions were collected and combined to 5 fractions (F_3A, F3B, F_3C, F_3D and F_3E) by TLC guiding. The fraction F_3D was further purified by PLC, using 80% CH_2Cl_2: Hexane as an eluent. Among two separating bands obtained, the second band (R_f = 0.52) gave compound 2 (8.2 mg).

Fraction F_4 (7.968 g) was redissolved in hexane to obtain white solid (234.6 mg), which further recrystallized from CH_2Cl_2: Hexane to give white plates of compound 4 (192.1 mg). The filtrate was evaporated to dryness (7.626 g) and was subjected over silica gel column chromatography which was gradually eluted with 10-90% EtOAc: Hexane, EtOAc and MeOH and a 100 ml aliquot was collected for each fraction. A total of 50 fractions were collected and combined to 5 fractions (F_4A, F_4B, F_4C, F_4D and F_4E) by TLC guiding.

Fraction F_4B was chromatographed on a silica gel colunm which was gradually eluted with Hexane and EtOAc by increasing polarity of the solvents and a 100 ml aliquot was collected for each fraction. A total of 53 fractions were collected and combined to 5 fractions (F_4B_1, F_4B_2, F_4B_3, F_4B_4 and F_4B_5). The fraction F_4B_2 was dissolved with hexane and yellow solid was filtered off. The yellow solid was further purified by PLC using 80% CH_2Cl_2: Hexane as an eluent. Among three bands obtained, the first band (R_f = 0.52) gave yellow amorphous of **compound 2** (12.9 mg). The second band (R_f = 0.45) gave yellow amorphous of **compound 3** (25.2 mg).

Fraction F_4B_3 was chromatographed on a silica gel column which was gradually eluted with 80% CH_2Cl_2: Hexane as an eluent and a 100 ml aliquot was collected for each fraction. A total of 10 fractions were collected and combined to 3 fractions ($F_4B_3.1$, $F_4B_{3.2}$, and $F_4B_{3.3}$) by TLC guiding The fraction $F_4b_{3.2}$ afforded 41.2 mg of yellow amorphous of **compound 2.**

Fraction F_4C and F_4D were redissolved in hexane and white solid of **compound 4** 38.73 and 448.95 mg were filtered off respectively

Compound 1 (β-sitosterol) was obtained as white needles, 9.7 mg (0.30%), R_f = 0.50 (80% EtOAc: Hexane); mp 137-139 °C.

IR (KBr) ν_{max} cm^{-1} : 3420 cm^{-1} (O-H, *br*), 2960-2820 cm^{-1} (C-H, *m*), 1460 cm^{-1} (C-H, *m*), 1380 cm^{-1} (C-H, *s*), 1050 cm^{-1} (C-O, *m*).

^{1}H NMR (90 MHz,$CDCl_3$) : δ 5.35 (1H, *d*, *J* = 4.28, C = CH-), 5.10 (1H, *m*), 3.50 (1H, *br*, *s*, OH), 2.30-1.1 (3OH, *m*, -CH, CH_2), 1.02 (3H, *s*, 19-CH_3), 0.99-0.78 (12H, *m*, 21-CH_3, 26-CH_3, 27-CH_3, 29-CH), 0.70 (3H, *s*,18-CH_3).

^{13}C NMR (90 MHz, $CDCl_3$,TMS) : δ 140.8, 121.7, 78.5, 77.0, 75.6, 71.9, 56.8, 56.1, 50.2, 45.9, 42.4, 39.9, 37.3, 36.6, 36.2, 34.0, 31.9, 31.7, 29.2, 28.3, 26.2, 24.3, 23.2, 21.1, 19.8, 19.4, 19.1, 18.8, 12.0, 11.9.

Compound 2 was obtained as a yellow amorphous, 71.2 mg (0.22%), R_f = 0.52 (80% CH_2Cl_2: Hexane), mp 191.2 °C (decompose).

IR (KBr) ν_{max} cm^{-1} : : 3370 cm^{-1}(N-H, w), 2926 cm^{-1} (C-H, vw), 2828 cm^{-1} (C-H, vw), 1624 cm^{-1} (C=C, *m*), 1597 cm^{-1} (C=C, *s*), 1458 cm^{-1} (N-H, *m*), 1208 cm^{-1} (C-O, *s*), 1044 cm^{-1} (C-N, *s*).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ 8.62 (1H, *d*, *J* = 2.60 Hz), 7.08 (1H, *d*, *J* = 9.01 Hz), 7.06 (1H, *s*), 6.95 (1H, *dd*, *J* = 9.01 and 2.60 Hz), 6.30 (2H, *s*), 4.22 (1H, *br*, N-H), 3.94 (3H, *s*), 3.28-3.40 (3H, *s*), 3.30-3.19 (2H, *m*).

^{13}C NMR (100 MHz, $CDCl_3$,TMS) : δ 155.9, 145.6, 142.4, 139.2, 128.6, 127.9, 125.2, 117.8, 117.2, 110.1, 108.6, 108.5, 106.7, 101.4, 55.9, 41.7, 31.3.

EIMS (positive ion mode): *m/z* 584 $[M]^+$(100), 552 (11), 538 (19), 508 (23), 292 (45), 276 (33), 260 (55), 232 (14), 195 (25), 32 (13).

Elemental analysis: C 72.41%, H 6.56% , N 4.41% calcd. for $C_{36}H_{28}N_2O_6$, C 71.76%, H 4.98%, N 4.65%.

Compound 3 was obtained as yellow amorphous, 25.2 mg (0.079%), R_f = 0.45 (80% CH_2Cl_2 : Hexane), mp 143.8 °C (decompose).

IR (KBr) ν_{max} cm^{-1} : 3383 cm^{-1}(N-H, w), 2929 cm^{-1} (C-H, vw), 2833 cm^{-1} (C-H, w), 1624 cm^{-1} (C=C, *m*), 1597 cm^{-1} (C=C, *s*), 1582 cm^{-1} (C=C, *s*), 1533 (N-H, *m*), 1211 cm^{-1} (C-O, *s*), 1051 cm^{-1} (C-N, *s*).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ 8.78 (1H, *dd*, *J* = 8.12 and 1.39 Hz), 7.25 (1H, *t*, *J* = 8.12 Hz), 7.00 (1H, *s*), 6.83 (1H, *dd*, *J* = 8.12 and 1.39 Hz), 6.24 (2H, *d*, *J* = 1.38, -OCH_2O-), 3.40-3.15 (4H, *m*), 3.05 (3H, *s*, -OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$, TMS) : δ 156.3, 144.8, 141.8, 138.5, 127.8, 125.8, 125.5, 121.8, 120.5, 117.3, 110.5, 109.0, 107.9, 100.7, 56.8, 41.5, 31.2.

EIMS (positive ion mode): *m/z* 584 [M]+(20), 199 (4), 185 (8), 171 (12), 145 (28), 131 (42), 117 (44), 105 (33), 91 (50), 79 (29), 67 (35), 55(41), 43 (100).

Elemental analysis: C 72.99%, H 6.90% , N 4.56% calcd. for $C_{36}H_{28}N_2O_6$, C 71.76%, H 4.98%, N 4.65%.

Compound 4 was obtained a white plates, 679.78 mg (2.12%), R_f = 0.62% (50% EtOAc : Hexane), mp 57.7-59.5 °C.

IR (KBr) ν_{max} cm^{-1} : 3464 cm^{-1} (O-H, *br*), 3080 cm^{-1} (C = CH), 2920, 2849 cm^{-1} (C-H), 1769 cm^{-1} (C = O), 1640 cm^{-1} (C=C) 1472 cm^{-1} (C-H), 1173 cm^{-1} (C-O), 1078 cm^{-1} (C-O), 1047 cm^{-1} (C-O), 941 cm^{-1} (C-H).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ 6.24 (1H, *dt*, *J* = 15.78 and 2.94 Hz), 5.75 (1H, *m*), 5.46 (1H, *d*, *J* = 15.78 Hz), 4.97 (2H, *dd*, *J* = 15.80 and 8.70 Hz), 4.56 (1H, *sex*, *J* = 3.06 Hz), 3.84 (1H, *ddd*, *J* = 12.30, 4.80 and 3.06 Hz), 3.62 (1H, *ddd*, *J* = 12.30, 4.80 and 3.06 Hz), 2.68 (1H, *m*), 2.29 (1H, *ddt*, *J* = 9.00 and 4.68 Hz), 2.27 (1H, *ddt*, *J* = 9.00 and 4.68 Hz), 2.22 (2H, *t*, *J* = 7.02 Hz), 1.99 (1H, *m*), 1.40 (1H, *m*), 1.42 (2H, *m*), 1.35 (2H, *m*), 1.22-1.30 (10H, *m*).

^{13}C NMR (100 MHz, $CDCl_3$,TMS) : δ 179.5 , 115, 146.8, 137.2, 115.3, 109.1, 78.2, 64.5, 39.5, 32.4-32.5, 31.2, 30, 29.5, 29.2, 27.2-29.5, 19.1.

Compound 4a was obtained from acetylation of **compound 4** as following method: **Compound 4** (19.21 mg) was dissolved in pyridine (14 ml) and was treated with acetic anhydride (14 ml), allowed to stir at room temperature for 9 hours. Then the reaction mixture was poured into cold water and extracted with ethyl acetate. The organic layer after washing with water, was dried over anhydrous Na_2SO_4. The acetylation product was recrystallized from Hexane: CH_2Cl_2 to give white pallets of **compound 4a** 11.11 mg (58.9%), R_f = 0.80% (50% EtOAc: Hexane), mp 47.9-48.5 °C.

IR (KBr) ν_{max} cm^{-1} : 2920, 2848.7 cm^{-1} (C-H), 1773 cm^{-1} (C = O), 1732 cm^{-1} (C = O), 1471.6 cm^{-1} (C-H), 1244 cm^{-1} (C-O), 1173 cm^{-1} (C-O), 950 cm^{-1} (C-H).

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ 6.29 (1H, *td*, *J* = 15.88 and 6.81 Hz), 5.8 (1H, *m*), 5.53 (1H, *d*, *J* = 15.88 Hz), 5.05 (1H, *d*, *J* = 15.24 Hz) 5.00 (1H, *d*, *J* = 8.70 Hz), 4.73 (1H, *sex*, *J* = 3.66 Hz), 4.28 (1H, *dd*, *J* = 12.15 and 3.39 Hz), 4.15 (1H, *dd*, *J* = 12.15 and 5.45 Hz), 2.68 (1H, *m*), 2.33 (2H, *t*, *J* = 7.0 Hz), 2.25 (2H, *m*), 2.11 (3H, *s*), 1.55 (2H, *sex*, *J* = 7.18 Hz), 1.48, 1.87 (2H, *m*), 1.20 (*br*).

^{13}C NMR (400 MHz, $CDCl_3$,TMS): δ 179.2, 170.9, 147.4, 137.7, 115.8, 109.5, 75.5, 65.9, 39.5, 33.1, 32.9, 31.5, 31.1, 29.8, 28.6-29.7, 28.7, 27.6, 21.1, 19.6.

Results and Discussion

Structure Identification

Extraction and isolation of root of *Polyalthia debilis* (Pierre) Finet & Gagnep obtained compound 1-4. Structures of these compounds were established on the basic of spectroscopic means as the following.

Compound 1 was obtained as white solid. Crystallization from hexane gave white needles. The IR spectrum showed broad O-H stretching band at 3420 cm^{-1}. Aliphatic C-H antisymmetric and symmetric

appeared as strong absorption at 2960-2820 cm^{-1}. An absorption at 1460 and 1380 cm^{-1} were characterized for methylene bending and methyl bending, respectively. The C-O stretching showed at 1050 cm^{-1}, which suggested a saturated primary or secondary alcohol, alicyclic, five or six membered ring alcohol. The 90 MHz ^1H NMR spectrum exhibited the doublet resonance signal at δ 5.35 which was assigned to one olefinic proton. A multiplet signal at δ 5.10 was assigned to methin proton which beared to hydroxyl group. Weak broad singlet signal at δ 3.50 was assigned to hydroxy proton. Several methine, methylene and methyl protons appeared as multiplets between δ 2.30-0.70. The ^{13}C NMR showed resonance signals of olefinic carbons at (140.8 and 121.7. The aliphatic carbon with hydroxyl substituent showed its signal at δ 71.9 whereas methine, methylene and methyl carbons appeared between δ 56.8-11.9. Comparison of the NMR spectral data, mix-mp and mix-TLC with authentic sample indicated that **compound 1** was a known sterol, β-sitosterol.

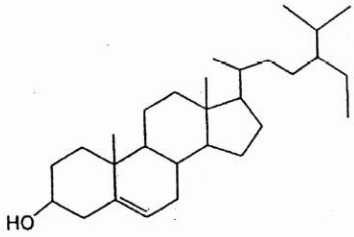


Figure 2.1 Compound 1 (β-sitosterol).

Compound 2 was obtained as yellow amorphous and its EI mass spectrum *m/z* 584 and DEPT spectrum established a molecular formula of $C_{36}H_{28}N_2O_6$. Spectroscopic evidences indicated that the structure was a dimer of aporphine alkaloid type. The IR spectrum showed a characteristic of N-H stretching absorption as a weak band at 3370 cm^{-1}. The absorption bands at 1624 and 1597 cm^{-1} were characterized as aromatic ring, at 1208 cm^{-1} was characterized as C-O stretching, at 1458 cm_{-1} was assigned to N-H bending and at 1044 cm^{-1} was assigned to C-N stretching. ^1H NMR spectrum (Table 2.1) showed a characteristic of aromatic protons as a singlet signal at δ 7.06 of H-3, doublet at δ 8.62 (J = 2.60 Hz) of H-11, doublet of doublet at δ 6.95 (J = 9.01 and 2.60 Hz) of H-9 and a doublet signal at 7.08 (J = 9.01 Hz) of H-8. The singlet signal at δ 6.30 was assigned to protons of methylene dioxy group (-O-CH_2-O-). The protons of methoxy group appeared as a singlet signal at δ 3.95 and multiplet signal at δ 3.40-3.28 and δ 3.30-3.19 were assigned to methylenes H-4 and H-5. The N-H resonance appeared as a broad signal at δ 4.22. The ^{13}C NMR spectrum (Table 2.1) exhibited signals of 13 aromatic carbons including C-7, whereas the methylene dioxy carbons appeared at δ 101.4. Two methylene carbons showed signals at 41.7 and 31.3, while methoxy carbons showed at δ 55.9. The DEPT spectrum indicated ten quarternary carbons, four methine carbons, three methylene carbons and one methyl carbon. The COSY spectrum showed correlation between (H-8) $\leftrightarrow$ (H-9), (H-9)$\leftrightarrow$ (H-11) and (H-4)$\leftrightarrow$ (H-5) whereas the correlation between protons and carbons indicated by HMQC spectrum. The HMBC spectrum showed 2-3 positions correlation between protons and carbons (Figure 2.2) to confirm a connection of carbon atoms in the structure.

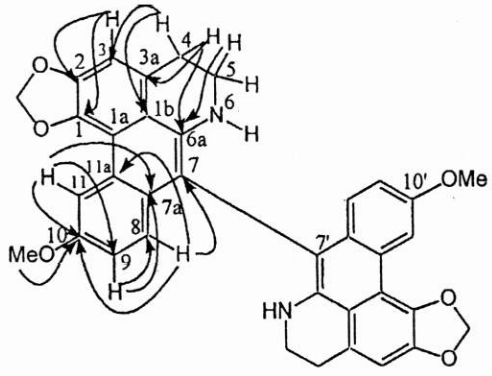


Figure 2.2 HMBC correlations of **compound 2**

The EI mass spectrum of **compound 2** which its *m/z* 584 and *m/z* 292 ions suggested a dimer structure. From literature, the known dimeric aporphenoid type at 7,7′positions has been reported as **Bipowinone** (Figure 2.3) which was first isolated from Popawia pisocarpa (Annonaceae)[5]. For the most closely related symmetry bis aporphine dimer, **Bisdehydronorglaucine** (Figure 2.4) has been reported as a dimerized product from treatment of **dehydronorglaucine** with sodium. (Kanokmedhakul S. et al. 1997).

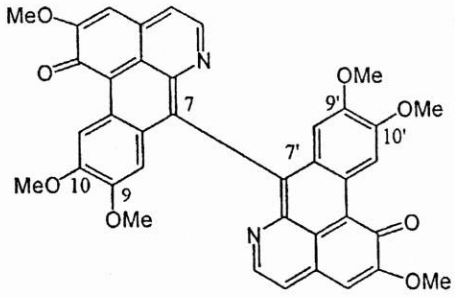
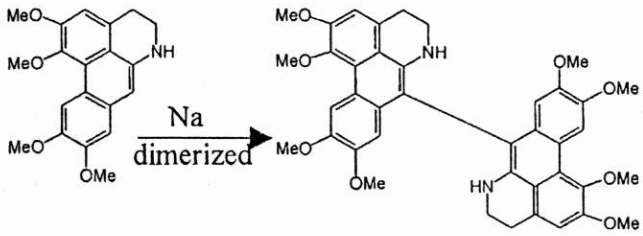


Figure 2.3 Bipowinone



Bisdehydronorglaucine **Dehydronorglaucine**

Figure 2.4 Dimerization of Dehydronorglaucine

For our knowledge, compound 2 was a new type of symmetry dimeric aporphine alkaloid. Therefore, we named this new compound as Bisdebiline A and its structure was shown in Figure 2.5.

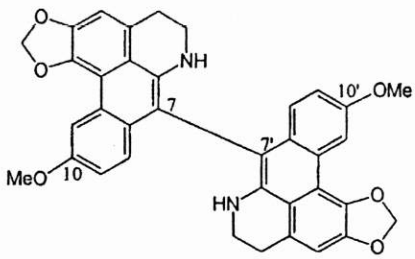


Figure 2.5 Compound 2 (Bisdebiline A).

Compound 3 was obtained as yellow amorphous and its EI mass spectrum (*m/z* 584) and DEPT spectrum were indicated a molecular formula of $C_{36}H_{28}N_2O_6$. From spectroscopic evidences which were similar to those of **compound 2,** a dimer of aporphine alkaloid type was focus. The IR spectrum showed a characteristic of N-H stretching absorption as a weak band at 3383 cm^{-1}. The absorption band at 1624 and 1597 cm^{-1} were characterized as aromatic ring, at 1211 cm^{-1} was characterized as C-O stretching and at 1533 cm^{-1} was assigned to N-H bending and at 1051 cm^{-1} was assigned to C-N stretching. The ^1H NMR spectrum (Table 2.1) showed resonance signals of aromatic protons at δ 7.00 which was assigned to H-3, doublet of doublet at δ 6.83 (J = 8.12 and 1.39 Hz), triplet at δ 7.25 (J = 8.12 Hz) and doublet of doublet at δ 8.78 (J = 8.12 and 1.39 Hz) were assigned to H-9, H-10 and H-11, respectively. The methylene dioxy protons (-O-CH_2-O-) appeared as doublet at δ 6.24 (J = 1.38 Hz). The multiplet signal at ? 3.40-3.15 represented to H-4 and H-5. The singlet signal at δ 3.05 was characterized as methoxy protons at C-8 which comparable to bisaporphine alkaloid [61]. The ^{13}C NMR and DEPT spectrum (Figure 15 and 16 in Appendix and Table 2.1) indicated ten quarternary carbons, four CH aromatic carbons, three methylene carbons and one methyl carbon. The comparison of spectroscopic data with those of compound 2 and a known compound Beccapoline[15] (Figure 2.6) suggested that compound 3 had a difference positions of methoxy groups (-OCH_3) in molecule as shown in Figure 2.7. For our knowledge, **compound 3** was a new symmetric dimerized of bis-7,7′-dihydroaporphine and we named as **Bisdebiline B** (Figure 2.7).

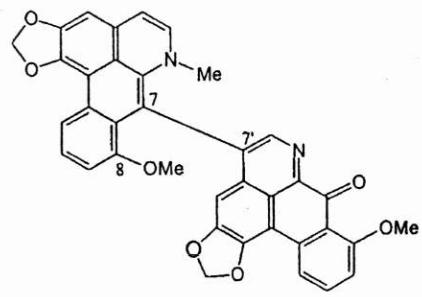


Figure 2.6 Beccapoline.

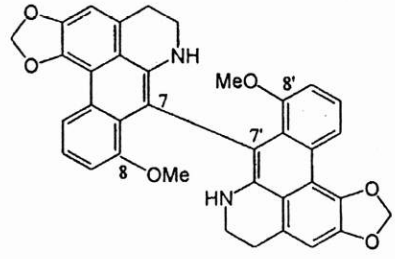


Figure 2.7 Compound 3 (Bisbediline B).

Table 2.1 ^{1}H and ^{13}C NMR spectral data of **compound 2** and **3** in $CDCl_3$.

No	δH		δC	
	2[a]	3[b]	2[a]	3[b]
1	-	-	142.4	141.8
1a	-	-	117.8	117.3
2	-	-	145.6	144.8
3	7.06, *s*	7.0, *s*	108.5	107.9
3a	-	-	128.6	125.8
4	3.40-3.28, *m*	3.40-3.15, *m*	31.3	31.2
5	3.30-3.19, *m*	3.40-3.15, *m*	41.7	41.5
6	4.22, *br*	4.40, *br*	-	-
6a	-	-	139.2	138.5
7	-	-	106.7	109.0
7a	-	-	127.9	127.8
8	7.08, *d* (*J* = 9.01 Hz)	-	125.2	156.3
9	6.95, *dd* (*J* = 9.01,2.60 Hz)	6.83, *dd* (*J* = 8.12, 1.39 Hz)	117.2	121.8
10	-	7.25, *t* (*J* = 8.12 Hz)	155.9	120.5
11	8.62, *d* (*J* = 2.60 Hz)	8.78, *dd* (*J* = 8.12, 1.39 Hz)	110.1	110.5
11a	-	-	108.6	125.5
12	6.30, *s*	6.24, *d* (*J* = 1.38 Hz)	101.4	100.7
-OCH_3	3.94, *s*	3.05, *s*	55.9	56.8

[a] recorded on 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR

[b] recorded on 90 MHz for ^{1}H NMR and 22.5 MHz for ^{13}C NMR.

Compound 4 was obtained as colourless plates. The IR spectrum showed a broad absorption of hydroxy group at 3464 cm^{-1}. The weak absorption at 3080 and 1640 cm^{-1} indicated the olefinic group. The strong and medium absorption at 2920 and 1472 cm^{-1} indicated a saturated C-H in molecule. The strong absorption at 1769 and 1173 cm^{-1} consistent with a five membered ring lactone, while at 1078 and 1047 cm^{-1} were assigned to C-O stretching of ether or alcohol. The absorption at 941 cm^{-1} was assigned to out of plan bending of C=C-H. The ^{1}H NMR spectrum showed two set of resonance signals of two alkene protons at δ 6.24 (*dt*, *J* = 15.78 and 2.94 Hz), 5.75 (*m*), 5.46 (*d*, *J* = 15.78 Hz) and 4.97 (*dd*, 15.80 and 8.70 Hz). The signal at δ 4.97 was assigned to terminal protons of alkene which was confirmed by DEPT spectrum. A part of protons of lactone ring were assigned to H-3, two double doublet of triplet signals at 2.29 (*J* = 9.00 and 4.68 Hz) and 2.27 (*J* = 9.00 and 4.68 Hz) were assigned to H-4. The sextet signals at δ 4.56 (*J* = 3.06 Hz) were assigned to H-5. The signals of double doublet of doublet at δ 3.84 (*J* = 12.30 and 3.06 Hz), 3.62 (*J* = 12.30, and 4.80 Hz) were assigned to germinal protons H-1″(-CH_2-OH), whereas the signals of multiplet at δ 1.99 and 1.40 were assigned to methylene protons, H-1′. The broad

singlet signal at δ 1.20 was characterized as long chain methylene protons, whereas a triplet signal at δ 0.87 was assigned as an impurity. The ^{13}C NMR spectrum showed signal at δ 179.5 of lactone carbonyl (C-2). The signals at δ 78.2 and 64.5 were corresponding to carbons bearing to oxygen atom, C-5 and C-1″, respectively. The resonance signals of long chain methylene carbons showed between δ 32.4-19.1. The four alkene carbons showed resonance signals at δ 109.1, 115.3, 137.2 and 146.8. The DEPT spectrum indicated one carbonyl group, five methine groups, thirteen methylene groups including a terminal alkene. The COSY spectrum confirmed a correlation between protons which could suggest a connection of the partial structure as 3 parts; I, II and III (Figure 2.8). The COSY correlation of protons of these 3 pasts was shown in Figure 2.9.

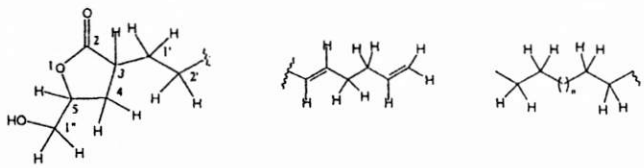


Figure 2.8 partial structures of **compound 4**

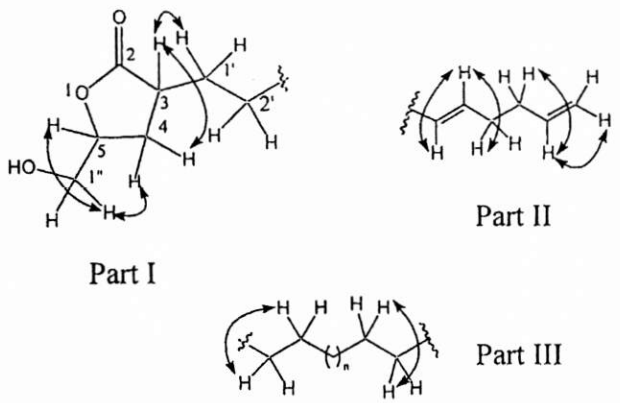


Figure 2.9 COSY correlation of protons of partial structures of **compound 4**

The HMBC and HMQC spectrum of compound 4 were not clear due to overlapping of proton signals of methylene groups and some side signal of carbon resonances which suggested a mixture of isomers. Attempted purification by chromatographic method was unsuccessful. We therefore, prepared an acetylated derivative, compound 4a, for structure determination.

Compound 4a was obtained from acetylation reaction of compound 4 as a white plates. The IR spectrum showed absorption of lactone carbonyl at 1773 cm^{-1} and acetoxy carbonyl at 1732 cm^{-1}. The C-O stretching of acetoxy group appeared as strong absorption at 1244 cm^{-1}. The absorption of C-O of lactone showed at 1173cm^{-1}. The out of plan bending of C=C-H showed at 950 cm^{-1}. The ^{1}H NMR spectrum showed the same pattern of resonance signals as those of compound 4. A methyl group of acetoxy appeared as singlet at δ 2.11. The ^{13}C NMR spectrum showed two carbonyl signals at δ 179.2 and 170.9 of lactone and acetoxy group, respectively, whereas a methyl signal of acetoxy appeared at δ 21.1. The remaining signals were corresponding to those of compound 4. Two double bond carbons showed signals at δ 147.4, 137.7, 115.8 and 109.5. The resonance signals at δ 75.5 and 65.9 were assigned to C-5 and C-1″, respectively. The DEPT spectrum (Figure 27 in Appendix) indicated three quaternary carbons including two carbonyl groups, five methine groups which three of them were alkenes, fifteen methylene groups including a terminal alkene and one methyl group. The COSY spectrum (Figure 28 in Appendix) showed the same protons correlation pattern as those in compound 4. H-H COSY spectrum of partial structure I showed a correlation between protons of lactone ring, H-3↔ H-4↔ H-5 and between protons of lactone ring and side chain protons H-3 ↔ H-1′and H-5 ↔ H-1″(Figure 2.10).

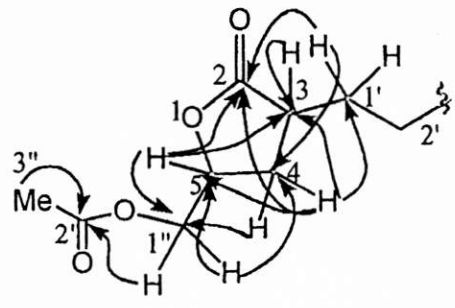


Figure 2.10 COSY correlation of protons of partial structure I of **compound 4a.**

The HMBC spectrum of partial structure I **of compound 4a** (Figure 29 in Appendix) exhibited 1-2 and/or 1-3 correlation between protons and carbons such as H-1″, H-5, H-1′, H-3 to C-4; H-4, H-1″ to C-5; H-3, H-4, H-5, H-1′ to C-2; H-1′, H-2′, H-4, H-5 to C-3; H-5, H-4 to C-1″; H-4, H-3, H-2′ to C-1″ Figure 2.11). The 2D NMR spectral data suggested that partial structure of compound 4a contained 3,5-disubstituented δ-butyrolactone.

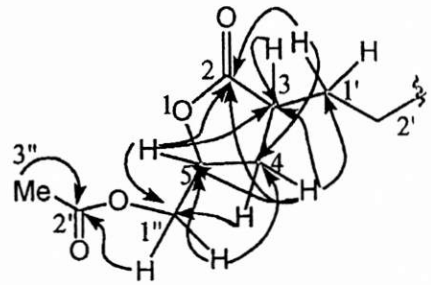
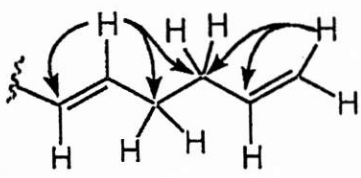


Figure 2.11 HMBC correlation of partial structure I of **compound 4a.**

The ^{1}H NMR spectrum of partial structure II showed resonance signals of terminal alkene as two doublets at δ 5.05 (J = 15.24 Hz) and 5.00 (J = 8.70 Hz) which coupling to signal at δ 5.8 (m). The other trans alkene protons appeared at δ 6.29 (*dt*, J = 15.88 and 6.81 Hz) and δ 5.53 (*d*, J = 15.88 Hz). The HMBC spectrum of partial structure II showed correlation between protons of alkene and carbons (Figure 2.12). Whereas, the partial structure III was proposed as a long chain. The two triple signals of methylene groups together with no signal of methyl group suggested a cyclic structure. Unfortunately, the correlation of quaternary carbons with protons was not clear. Thus, only partial structures could be assigned under these evidence.



Part II

Figure 2.12 HMBC correlation of partial structure II.

Therefore, the complete structure of **compound 4** and **4a** need more data such as mass spectrometry and elemental analysis.

Bioactivities Testing

Compound 2, 3 and **4** were tested for antimalarial activity against *Plasmodium fulciparum*. The result found that **compound 4** showed no activity while **compound 2** and **3** exhibited a good potential activity with EC_{50} of 4.11 and 5.4 μg/ml, respectively. This will provide a challenge research for chemist and related fields to study and develop the utilization of **compound 2** and **3** as an antimalarial drug in the future.

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