

Natural Resources in the Tropics

Sustaining Tropical
Natural Resources
through
Innovations,
Technologies &
Practices

Editors: Mohd Effendi Wasli |
Hamsawi Sani | Fasihuddin Badruddin
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**Faculty of Resource Science & Technology,
Universiti Malaysia Sarawak**



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PREFACE

Under the Malaysia Development Plan, one major goal of the nation's plan was to promote the adoption of an integrated and holistic approach in addressing environmental and natural resources issues to attain sustainable development while striving for economic excellence. As part of our nation's goal in achieving a balanced development progress, critical agendas in the tropics which encompass broad issues in sustainable utilization and management of existing natural resources to support and enhance the national and regional economic developments.

Important issues in the tropics such as conservation of tropical biodiversity, environmental management, legislative issues, socio-economics and marketing strategies of natural resources should also be addressed properly in materialising this goal. In addition, issues of alternative resources such as new potential commercial crops, suitable tree species for forest plantation, alternative sources of energy such as biofuel, agriculture technologies, sustainable fisheries and aquaculture and commercialization of timber and non-timber forest products are also essential as part of realising the nation's plan towards a sustainable development process.

This publication is a compilation of scientific papers from various fields of study as presented at the *4th Regional Conference on Natural Resources in the Tropics, 2012 (NTrop4)* on 19 – 20 September 2012 at DeTAR PUTRA, Universiti Malaysia Sarawak. This scientific conference which focused on various issues regarding the latest R&D findings, innovations and ideas on issues of tropical natural resources. Besides as a platform for establishment of research networking among researchers, practitioners and stakeholders of tropical natural resources, this conference is an important forum for discussing the future of our tropical natural resources in Malaysia.

Editors

November, 2012

KEYNOTE PAPER

INVESTMENT IN MALAYSIAN BIODIVERSITY: IT'S IMPORTANCE IN SUSTAINING TROPICAL NATURAL RESOURCES

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It is now about 14 years after the launching of the National Biodiversity Policy which had taken a centre stage in Malaysian environmental management and policy agenda. The government's agencies together with non-government organizations have been demanding that biodiversity be conserved and utilized for future socio- economic development. The country's tropical forest and the marine ecosystems are endowed with one of the richest biodiversity assets in the region and efforts to conserve and utilize the rich flora and fauna have been carried out in the country since the last decade through the establishment of National Biotechnology Policy and other commercial government and private initiatives. To-day about 7.6% of the forest of all types had been set aside for the conservation of biodiversity but their inventories are yet to be carried out fully. The country has claimed that these protected areas have captured most of the diverse ecosystems and species of plants and animals found. Plants and animals including microorganisms biodiversity is represented by numerous species with flowering plants constituting about 80% whilst the diversity of fauna species is represented by more than 5,000 species excluding invertebrates. The investment in biodiversity objective should be to achieve a long term capital growth for subsequent investment of biodiversity asset in biotechnology especially medical and agricultural biotechnology, health care and ecotourism. To this effect Malaysia has just formulated the National Biotechnology Policy that envisaged the sustainable use of biodiversity. The genetic resources especially the seeds, DNA manipulation and microorganism cultures may enhance both the medical and agricultural products and by-products and some salient features in potential products of biodiversity for commercial use and management would be discussed.

NEW LIMONOIDS FROM *Chisocheton ceramicus*

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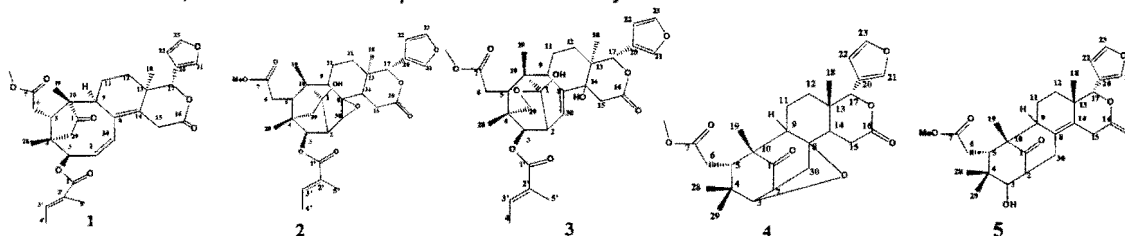
Abstract

An investigation of the bark of *Chisocheton ceramicus* has yielded five limonoids, three new limonoids, chisomicine A 1, chisomicine B 2, and chisomicine C 3, with two known mexicanolides, limonoid 4 and limonoid 5. The absolute structures were determined by 2D-NMR, CD, and computational methods. Chisomicine A 1 exhibited no production inhibitory activity in J774.1 stimulated by LPS dose-dependent at high cell viability.

Keywords: Limonoids, *chisocheton ceramicus*, chisomicine A-C

1. INTRODUCTION

Limonoids from Meliaceae have been the subject of various studies due to their significant biological activities such as antifeedants, insecticides, antitumor, and antimalarial activities [1, 2]. In addition their diverse structures with the oxidized backbone and the side chain moiety bonded to ring D in the intact tetranortriterpenoid nucleus have attracted great interest. [3] In continuation of our research on Meliaceae family, [4] we have found the alcoholic bark extracts of *Chisocheton ceramicus* are rich sources in interesting limonoids [5]. We have isolated five limonoids, ceramicine E 1 with A 2, B-seco limonoid ring, ceramicine F 2 with phragmalin-type limonoid, and the other one was ceramicine G3 with oxidized phragmalin-type limonoid, and limonoids 4 [6] and 5 [7] were known mexicanolide type compounds. Limonoid 4 (14-Deoxyxylocensin K) was first reported as a synthetic compound from natural xylocensin K, and we reported for the first time on its occurrence as a natural product [8]. We now wish to report the isolation and structure elucidation of three new limonoids, chisomicine A 1, chisomicine B 2, and chisomicine C 3, as well as the NO production inhibitory of the chisomicine A 1.



2. Materials and Methods

2.1 General experimental procedures

2.1.1 Plant material

The barks of *C. ceramicus* were collected in 2000 from Hutan Simpan Bukit Enggang, Malaysia. The plant species was identified by Mr. Teo Leong Eng with a Voucher specimen (No. KL 4973) and herbarium specimen was deposited in the herbarium of the Chemistry Department, University of Malaya.

2.1.2 Extraction and isolation

The dried and powdered bark of *C. ceramicine* (900g) was extracted successively with methanol and the methanol extract (200g) was partitioned with 10% aq MeOH and EtOAc. The EtOAc-soluble materials (10g.) were subjected to a silica gel column (hexane/ EtOAc. 1:0→0:1), in which a fraction eluted with hexane / EtOAc 30%: 70% was further purified on a silica gel column with CH₂Cl₂ - hexane - EtOAc (5:3:2) to give chisomicine A **1** (250 mg; 2.5% yield). The second fraction with hexane / EtOAc 20%: 80% was further purified on a silica gel column with EtOAc 65%: Acetone 10% : Hexane 25%, the first sub-fraction has been subjected to semi-preparative HPLC, developed with H₂O-0.1%FA/MeOH-0.1%FA iso- (25-75) Flow rate 2.5 ml/min. at RT 20.60 to give pure chisomicine B **2** (25mg; 0.25% yield). While Second sub-fraction from the same column was subjected to the plate TLC with the solvent system EtOAc 65%: Acetone 10%: Hexane 25%, to get pure chisomicine C **3** (16mg; 0.16% yield).

Chisomicine A (1): white, amorphous powder; $[\alpha]_D^{27}$ -125 (c 0.7, MeOH); IR (KBr) ν_{\max} 2938, 1734, and 1266 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ) 202 (4.15), and 214 (sh, 4.02); CD (MeOH) λ_{\max} 201 ($\Delta\epsilon$ -36.2), 213 (0), 227 (7.73), 290 (1.24) nm; ¹H NMR data (Table 1) and ¹³C NMR data (Table 2); ESIMS m/z 573 (M + Na)⁺; HRESIMS m/z 573.2464 (M + Na)⁺; calcd for C₃₂H₃₈O₈Na, 573.2464.

Chisomicine B (2): colorless needles; mp 176-178 °C; $[\alpha]_D^{27}$ -66 (c 1.0, MeOH); IR (KBr) ν_{\max} 3391, 2972, 1735, 1703, and 1268 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ) 216 (3.92); CD (MeOH) λ_{\max} 205 ($\Delta\epsilon$ 0), 209 (0.71), 213 (0), 223 (-3.42), 236 (0), 245 (0.92) nm; ¹H NMR data (Table 1) and ¹³C NMR data (Table 2); ESIMS m/z 591 (M + Na)⁺; HRESIMS m/z 569.2706 (M + H)⁺; calcd for C₃₂H₄₁O₉, 569.2751.

Chisomicine C (3): white, amorphous powder; $[\alpha]_D^{27}$ -86 (c 1.0, MeOH); IR (KBr) ν_{\max} 3441, 2980, 1732, 1718, 1706, and 1269 cm⁻¹; UV (MeOH) λ_{\max} (log ϵ) 206 (4.16); CD (MeOH) λ_{\max} 201 ($\Delta\epsilon$ -4.86), 208 (0), 211 (0.44), 217 (0), 221 (-0.3), 227 (0), 235 (0.48), 263 (0.93) nm; ¹H NMR data (Table 1) and ¹³C NMR data (Table 2); ESIMS m/z 607 (M + Na)⁺; HRESIMS m/z 607.2512 (M + Na)⁺; calcd for C₃₂H₄₀O₁₀Na, 607.2519

3. Results and Discussion

Chisomicine A **1** was afforded as white amorphous solid. The HRESIMS of chisomicine A **1** displayed a pseudomolecular ion peak at 573.2464 (M+Na)⁺, compatible to the molecular formula of C₃₂H₃₈O₈Na. IR absorptions indicated the presence of carbonyl group at 1734 cm⁻¹. The ¹³C/DEPT NMR spectra revealed thirty two carbon resonances due to four carbonyls, four sp² quaternary carbons, three sp³ quaternary carbons, six sp² methines, four sp³ methines, five sp³ methylenes, and six methyls. Among them, two sp³ methines (δ_C 76.8 and 80.2), one sp³ methyl (δ_C 52.0), and two sp² methines (δ_C 141.7 and 142.8) were ascribed to those bearing an oxygen atom.

Five partial structures **a** (C-2, C-3, and C-30), **b** (from C-5 to C-6), **c** (from C-9 to C-12), **d** (from C-22 to C-23), and **e** (from C-3' to C-4') were deduced from ¹H-¹H COSY analysis of **1** in CDCl₃ (Figure 1). The presence of a bicyclo[5.2.1]dec-3-en-8-one unit containing the partial structure **a** was supported by HMBC correlations as shown in Fig. 1. HMBC correlations for H-3, H-5, H₃-28, H₂-29 of C-4 (δ_C 43.3) gave rise to the connectivity of the partial structures **a** and **b** through C-4 atom. The presence of a cyclopentanone ring connected with the partial structure **b** was assigned by the HMBC correlations for H₂-29 of C-1 (δ_C 220.6), C-5 (δ_C 40.5), and C-10 (δ_C 54.2), and for H-5 of C-1 and C-10. Connection among partial structures **a**, **b**, and **c** could be assigned HMBC correlations for H₃-19 of C-5, C-9 (δ_C 44.4), and C-10, and for H-9 of C-8 (δ_C 131.0) and C-10. The presence of a methoxy carbonyl group connected to the partial structure **b** was supported by the HMBC correlations for H₂-6 and H₃-OMe of C-7 (δ_C 174.1). Partial structure **e** constructing (*E*)-2-methylbut-2-enoic acid was attached at C-3 by the HMBC correlations for H-3 and H-3' of C-1' (δ_C 167.1). The presence of a β -furyl ring at C-17 was also assigned by the HMBC correlations as shown in Figure 1. In addition, the HMBC correlations for H₃-18 of C-12 (δ_C 28.5), C-13 (δ_C 37.8), C-14 (δ_C 131.6), and C-17 (δ_C 80.2), and for H-15 of C-8, C-13, C-14, and C-16 (δ_C 169.2) indicated the presence of an isochromenone containing the partial structure **c** and a tetrahydropyran-2-one ring. Thus, chisomicine A **1** was concluded to be a unique limonoid possessing a bicyclo[5.2.1]dec-3-en-8-one ring system, an isochromenone, and a β -furyl ring at C-17.

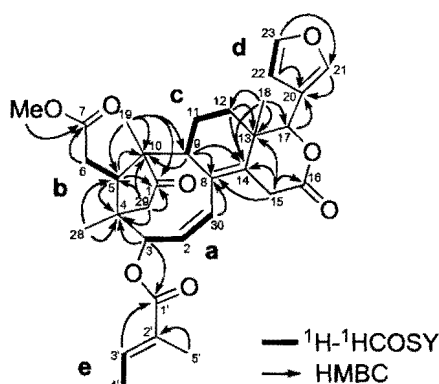


Figure 1 Selected 2D NMR correlations of Chisomicine A 1

NOESY correlations among H-2, H-3, and H-29b indicated that the ester at C-3 and Me-28 at C-4 assumed a α -configuration. Furthermore, the relative configurations at C-5, C-13, and C-17 were deduced from NOESY correlations among H-5, H-12a, and H-17 as shown in the computer-generated 3D drawing as depicted in Figure 2. The relative configurations at C-9 and C-10 could be assigned by NOESY correlations of H-9/H-30 and H₃-19, and of H-30/H₂-15.

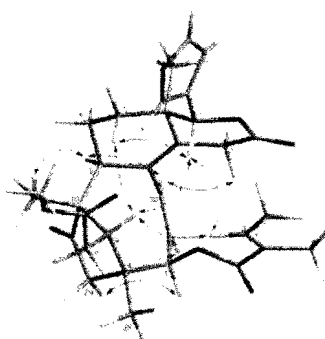


Figure 2 Selected NOESY correlations for Chisomicine A 1

Chisomicine B 2, ($[\alpha]_D^{27}$ -66 (c 1.0, MeOH)) was isolated as a colourless crystal. The HRESIMS showed a $[M+H]^+$ peak at m/z 569.2076 corresponding to the molecular formula of $C_{32}H_{40}O_9$. IR absorption implied the presence of esteric ketone group at 1731 cm^{-1} . The ^1H NMR spectrum showed the presence of β -mono-substituted furan moiety and its position was confirmed by characteristic chemical shifts of H-17 singlet at δ_H 5.4. Furthermore, H-21 singlet at δ_H 7.7, H-22 at δ_H 6.40 (d, $J = 1.2$ Hz), and H-23 at δ_H 7.4 (t-like). Additionally, two more proton signals have been appeared, one of them at δ_H 4.8 (d, $J=10.9$) which therefore indicated the presence of oxygen belong to H-3, while the second proton signal appeared at δ_H 3.25 (d, $J=3.2$) which is more up fielded in comparison with H-3 belongs to H-30, therefore indicates to the presence of oxygen in the form of epoxy, it was in the agreement of 1D NMR data⁹. Four methyl singlets detected at δ_H 1.0 (Me-18), 1.0 (Me-19), 0.86 (Me-28), and 1.80 (Me-5'), meanwhile, one methyl doublet (Me-4') detected at (δ_H 1.3, $J=9$) and a methoxy singlet appeared at δ_H 3.69. One double bond proton signals, H-3', was detected at δ_H 6.97 (qd $J=7, 1.5$ Hz). $^1\text{H}-^1\text{H}$ COSY cross signals observed (H-3'/H₃-4', H-3/H-2, H-5/H₂-6b, H₂-12a/H₂-12b, H₂-15a/H₂-15b, H₂-6a/H₂-6b, and H₂-29a/H₂-29b. The $^{13}\text{C}/\text{DEPT}$ NMR spectra revealed thirty two carbon resonances due to three carbonyls, two for ester at δ_C 168.4 (C-1') and δ_C 173.8 (C-7), and one for lactone (C-16) at δ_C (169.4), two sp^2 quaternary carbons, five sp^3 quaternary carbons, four sp^2 methines, seven sp^3 methines, five sp^3 methylenes, and six methyls. Among them, two sp^3 quaternary carbons (δ_C 80, 61.3), three sp^3 methines (δ_C 78.6, 77.6, 59.6), one sp^3 methyl (δ_C 52.0), and two sp^2 methines (δ_C 141.4 and 143.1) were ascribed to those bearing an oxygen atom. Figure 3

shows selected 2D NMR correlations for **2**. HMBC correlations of H-17 to C-20, C-21, and C-22 indicated the presence of a β -furyl ring at C-17. The presence of a α -methyl crotonate at C-3 was confirmed based on the HMBC correlation of H-3 (δ_H 4.8) to C-1' (δ_C 168.4). Additionally, methyl propionate substituent at C-5 found in typical A, B, D-secos limonoids, was observed from the chemical shifts (δ_H 3.69 and 2.30 for MeO and H₂-6 respectively and δ_C 52.0 Me, δ_C 34.2 C-6, and 173.75 C-7), the HMBC correlations of the methoxy peak, H-5, and H₂-6 to C-7 suggested that the methoxy group was attached to C-7 and methyl propionate substituent attached to C-5. The position of Δ 8-30 should be oxygenated in the form of epoxy, it was confirmed by HMBC correlations of H-2 to C-29 (δ_C 43.3), C-8 (δ_C 61.3), and C-30 (δ_C 59.6); H-30 to C-2 (δ_C 43.1) and C-1 (δ_C 80); and H-3 to C-30 (δ_C 59.6). In the δ -lactone ring (ring-D), the geminal proton of H₂-15 showed the HMBC correlations to carbons of C-8 (δ_C 61.3), C-14 (δ_C 44.8), and C-16 (δ_C 169.4). three methyls of C-18, C-19 and C-28 were attached to C-13, C-10, and C-4 respectively, by HMBC correlations of H₃-18 to C-12, C-13, C-14 and C-17, and of H₃-19 to C-1, C-5, C-9, and C-10, while H₃-28 correlated C-3, C-4, and C-5. Thus the gross structure of **2** was suggested to possess phragmalin-type skeleton with β -furan ring, δ -lactone ring, and α -methyl crotonate as shown in Figure 3.

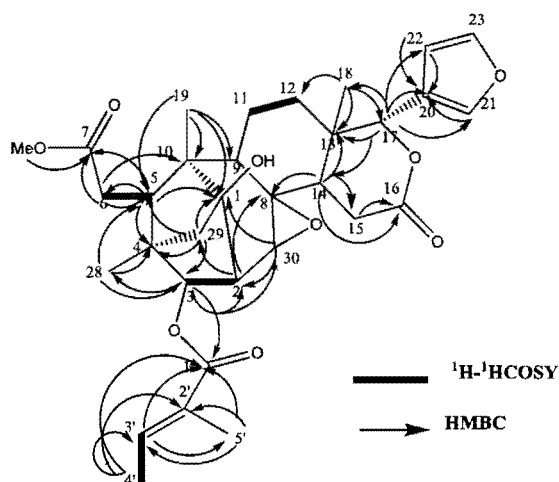


Figure 3 Selected 2D NMR correlations of chisomicine B 2

Chisomicine B **3**, ($[\alpha]_D^{27}$ -86 (c 1.0, MeOH) was isolated as white amorphous powder. The HRESIMS showed a $[M+Na]^+$ peak at m/z 607.2512, corresponding to the molecular formula of $C_{32}H_{40}O_{10}Na$. IR absorption implied the presence of esteric ketone (1732cm^{-1}) groups. The ^1H NMR spectrum showed the presence of β -mono-substituted furan moiety and its position by characteristic chemical shifts of H-17 singlet at δ_H 5.38. Furthermore, H-21 singlet at δ_H 7.78, H-22 a broad singlet at δ_H 6.46, and H-23 at δ_H 7.39 (t-like). Additionally, three down field sp^3 proton signals have been appeared, one of them at δ_H 4.8 (d, $J=10.0$) which therefore indicated the presence of oxygen belong to H-3, the other two were geminal protons; one appeared at 3.93 (d, $J=9.6$), while the second geminal proton appeared at δ_H 3.48 (d, $J=9.6$), it was in the agreement of 1D NMR data⁹. Additionally, four methyl singlets signals were detected at δ_H 1.01 (Me-18), 0.63 (Me-19), 1.01 (Me-28), and 1.77 (Me-5'), meanwhile, one methyl doublet signals (Me-4') detected at (δ_H 1.6, $J=7$) and a methoxy singlet at (δ_H 3.69). Two sp^2 proton signals, were detected at δ_H 6.92 as multiplet and at 5.48 (d, $J=6.2$) belong to H-3' and H-30 respectively. $^1\text{H}-^1\text{H}$ COSY cross signals observed (H-3/H-2 and H-2/H-30, but no correlation found between H-3 and H-30 which is approve of the position of carbon - carbon double bond between C-8 and C-30, meanwhile $^1\text{H}-^1\text{H}$ COSY cross signals observed (H-5/H₂-6, H₂-12a/H₂-12b, H₂-15a/H₂-15b, H₂-6a/H₂-6b, and H₂-29a/H₂-29b, H-3'/H₃-4'). The $^{13}\text{C}/\text{DEPT}$ NMR spectra revealed thirty two carbon resonances due to three carbonyls, three sp^2 quaternary carbons, five sp^3 quaternary carbons, five sp^2 methines, six sp^3 methines, five sp^3 methylenes, and six methyls. Among them, two sp^3 quaternary carbons (δ_C 97.3 and 72.9), two sp^3 methines (δ_C 75.3, 76.9), one sp^3 methyl (δ_C 52.2), one methylene (δ_C 67.9), and two sp^2 methines (δ_C 142.1 and 143.0) were ascribed to those bearing an oxygen atom. In addition, the ^{13}C spectrum indicated the presence of three carbonyls, two for ester at δ_C 167.7 (C-1') and δ_C 173.96 (C-7), and one for lactone

(C-16) at δ_C (169.3). According to the ^{13}C NMR spectral data, C-1 (δ_C 97.3) could be an acetal or hemiacetal carbon and C-3, C-14, C-17, C-29, (δ_C 75.3, 67.9, 72.94, and 67.89, respectively) should be oxygenated. Figure 4 shows selected 2D NMR correlations for **3**.

HMBC correlations of H-17 to C-20, C-21, and C-22 indicated the presence of a β -furyl ring at C-17. The presence of a α -methyl crotonate at C-3 was confirmed based on the HMBC correlation of H-3 (δ_H 4.8) to C-1' (δ_C 167.7). Additionally, methyl propionate substituent at C-5 found in typical A, B, D-seco limonoids, was observed from the chemical shifts (δ_H 3.69 and 2.30 for MeO and H₂-6 respectively and δ_C 52.2-OMe, δ_C 31.8 C-6, and 173.9 C-7), the HMBC correlations of the methoxy peak, H-5, and H₂-6 to C-7 suggested that the methoxy group was attached to C-7 and methyl propionate substituent attached to C-5. There was a double bond between C-8 and C-30, that was confirmed by COSY and HMBC correlations of H-2 to C-30 (δ_C 121.8), C-8 (δ_C 140.9), C-1 (δ_C 97.3), and C-3 (δ_C 75.3), in addition, H-30 to C-1 (δ_C 97.3), C-9 (δ_C 43.78), and C-14 (δ_C 72.94). It was in the agreement of 1D NMR data⁷. In the δ -lactone ring (ring-D), the geminal proton of H₂-15 showed the HMBC correlations to carbons of C-8 (δ_C 140.9), C-14 (δ_C 72.9), and C-16 (δ_C 169.3). Three methyls of C-18, C-19 and C-28 were attached to C-13, C-10, and C-4 respectively, by HMBC correlations of (H₃-18 to C-12, C-13, C-14 and C-17), (H₃-19 to C-1, C-5, C-9, and C-10), and (H₃-28 to C-3, C-4, C-5 and C-29).

Thus the gross structure of **3** was suggested to possess oxydized phragmalin-type skeleton with β -furan ring, δ -lactone ring, and α -methyl crotonate as shown in Figure 4.

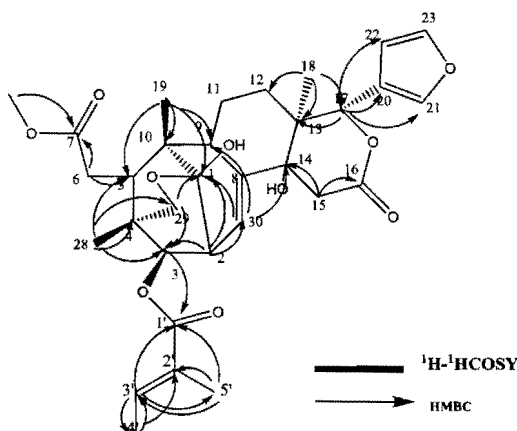


Figure 4 Selected 2D NMR correlations of chismicine C 3

The absolute configuration of all three compounds could be assigned by comparing their experimental CD spectra with the calculated CD spectra (CD calculations were performed by Turbomole 6.110 using RI-TD-DFT-BP86/aug-cc-pVDZ11 level of theory on RI-DFT-BP86/SVP11 optimized geometries.). The calculated CD spectra showed similar CD patterns to those of 1, 2, and 3 as shown in Figure 5 (should be changed). Therefore, their absolute stereochemistries were proposed as shown in the structures.

Table 1 1H NMR spectral data of compounds (1-3)

H	1	2	3
2	5.85 (1H, dd, 11.6, 6.4)	2.94 (1H, dd, 10.9, 3.4)	2.96 (1H, m)
3	4.79 (1H, dd, 6.4, 1.6)	4.8 (1H, d, 10.9)	4.8 (1H, d, 10.0)
5	3.82 (1H, brd, 12.0)	3.03 (1H, dd, 11, 2.3)	2.9 (1H, dd, 12.0, 10.0)
6a	2.52 (1H, dd, 16.0, 12.8)	2.33 (1H, dd, 17.2, 2.3)	2.37 (1H, d, 11.3)
6b	2.35 (1H, m)	2.27 (1H, m)	2.30 (1H, d, 11.3)
9	2.66 (1H, brd, 6.0)	1.8 (1H, m)	2.44 (1H, dd, 5.5, 4.6)
11a	1.87 (1H, brd, 14.4)	1.9 (1H, brd, 11.1)	1.66 (1H, bd, 14.0)
11b	1.63 (1H, m)	1.81 (1H, m)	1.51 (1H, m)

H	1	2	3
12a	1.30 (1H, m)	1.62 (1H, m)	2.0 (1H, m)
12b	1.04 (1H, m)	1.34 (1H, d, 11.1)	1.17 (1H, m)
14		2.04 (1H, dd, 6, 1.2)	
15a	3.07 (2H, brs)	2.5 (1H, dd, 18.4, 7.3)	2.94 (1H, d, 18.8)
15b		2.3 (1H, dd, 18.4, 1.8)	2.79 (1H, d, 18.8)
17	5.44 (1H, s)	5.4 (1H, s)	5.38 (1H, s)
18	1.09 (3H, s)	1.0 (3H, s)	1.01 (3H, s)
19	0.97 (3H, s)	1.0 (3H, s)	0.63 (3H, s)
21	7.54 (1H, s)	7.7 (1H, s)	7.78 (1H, s)
22	6.46 (1H, d, 1.2)	6.4 (1H, d, 1.2)	6.46 (1H, s)
23	7.39 (1H, t like,)	7.4 (1H, t like,)	7.39 (1H, s)
28	1.13 (3H, s)	0.86 (3H, s)	1.01 (3H, s)
29a	2.40 (1H, d, 17.6)	2.0 (1H, d, 11)	3.93 (1H, d, 9.6)
29b	2.05 (1H, d, 17.6)	1.33 (1H, dd, 11, 1.5)	3.48 (1H, d, 9.6)
30	5.83 (1H, brd, 11.6)	3.25 (1H, d, 3.2)	5.48 (1H, d, 6.2)
OMe	3.72 (3H, s)	3.69 (3H, s)	3.69 (3H, s)
3'	7.29 (1H, qd, 7.0, 1.6)	6.97 (1H, qd, 9.0, 1.6)	6.92 (1H, d, 7.0)
4'	1.70 (3H, d, 7.0)	1.3 (3H, d, 9.0)	1.6 (3H, d, 7.0)

Table 1 ^{13}C NMR Data of compounds (1- 3) in CDCl_3 .

C	1	2	3
1	220.6	80	97
2	135.5	43.1	45.2
3	76.8	78.6	75.3
4	43.3	44.8	43.3
5	40.5	39.4	34.8
6	33.8	34.2	31.8
7	174.1	173.8	173.9
8	131.0	61.3	140.9
9	44.4	41.6	43.8
10	54.2	45.1	41.4
11	19.1	21.5	19.1
12	28.5	33.7	28.6
13	37.8	36.0	41.2
14	131.6	44.8	72.9
15	33.0	27.3	39.3
16	169.2	169.4	169.3
17	80.2	77.6)	76.9
18	16.4	22.0	14.9
19	22.9	18.8	14.7
20	120.7	120.8	120.0
21	141.7	141.4	142.1
22	109.9	109.7	109.9
23	142.8	143.1	143.1
28	22.6	15.5	15.5
29	46.4	43.3	67.9
30	129.1	59.6	121.8
OMe	52.0	52.0	52.2

C	1	2	3
1'	167.1	168.4	167.7
2'	127.9	128.0	127.4
3'	139.5	139.3	140.0
4'	12.0	14.2	14.7
5'	14.3	12.1	11.8

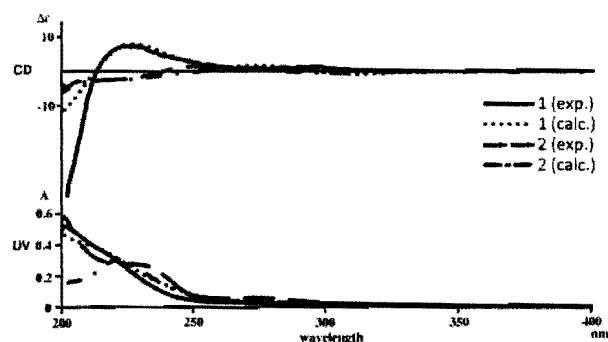


Figure 5 Actual and simulated CD and UV spectra of chisomicine A ¹

Chisomicine A 1 inhibited NO production in J774.1 cells dose-dependently stimulated by LPS and also showed little effect on cell viability (Figure 6: IC₅₀ 20.2 μM).¹² However chisomicine B 2, chisomicine C 3, 14-deoxyxylocensin K 4 and proceranolide 5 did not show NO production inhibitory activity.

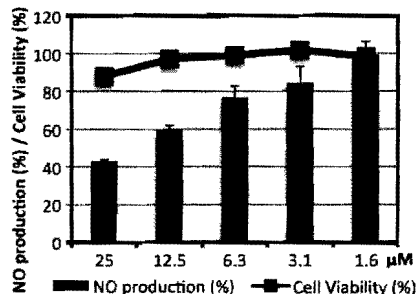


Figure 6 NO production ratio in J774.1 stimulated by LPS of chisomicine A 1.

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HMBC SPECTRA OF ALKALOIDS FROM LAURACEAE SPECIES: NINE STARS HALO-N THEORIES

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Abstract

Four theories related to the field of natural products were discovered by Dr. Halo-N and was published in a book entitled *Al Fathun Nawa*, Volume 1, 2011. These theories are:

1. Nine Stars Halo-N Theory,
2. Nawiah 9 x 45 (1) Theory,
3. Nawiah 9 x 45 (2) Theory,
4. Halo-N 9.2 Homolength Theory

These theories have similarities with depiction of correlation spots in HMBC (Heteronuclear Multiple Bond Coherence) spectrum, which is obtained through the NMR (Nuclear Magnetic Resonance) machine. The HMBC spectra of various alkaloids isolated from Lauraceae species were studied and these spectra were used to prove these theories. The theories were experimentally proven based on the HMBC spectra.

Keywords: Lauraceae, Nine Stars Halo-N Theory, HMBC spectra, alkaloids.

1. INTRODUCTION

1.1 NINE STARS HALO-N THEORY

“Each specialty of Mass of Bio-Nature will occur under the arrangement conducted by nine stars (Called Code Nine Stars L System: 2.4.1.2.) in righteous equilibrium coordinate” [1].

Figure 1 shows the findings of The Route of Mass $[(7+2) = (9)]$ which is described in the *Code Nine Stars L System: 2.4.1.2.* Thus, from the compass directions formed in nature, it is able to determine the existence of a special mass, in which it could be a mass that had already been discovered or a new mass which is yet to be discovered by bio-chemistry researchers.

Referring to Figure 1, the red horizontal line is the connecting line between the correlation points, consists of 7 correlation points (in yellow), which is in the clusters of 2, 4 and 1. Meanwhile, the vertical 90° angle straight line is the connecting line from the horizontal line to the middle point of 2 correlation points, the 8th and the 9th spots, to form an L-shape. Any correlation point which is the nearest to the 8th and the 9th correlation points, and touches the vertical 90° angle line, is the 10th correlation spot and the sought 'special mass'. Thus, whichever HMBC spectrum for a natural product that matches the *Nine Stars Halo-N Theory* will be a sign, that the natural product can be a vaccine for the humans.

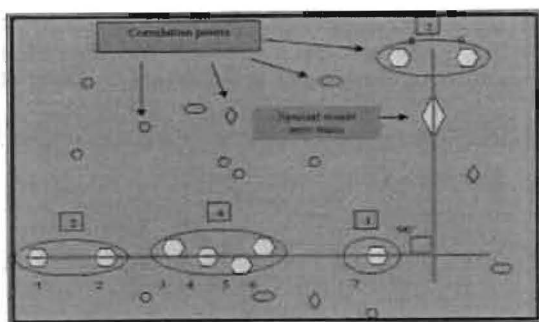


Figure 1 Nine Stars L System: 2.4.1.2.

1.1 Nawiah 9 x 45 (1) Theory

“Positive or negative property of a carbon compound which is found through the Nine Stars Halo-N Theory, can be determined by the position of any correlation points, in which it touches or otherwise, the intersection point between a straight line drawn 45° from the 90° angle and a straight line formed from the first correlation point connected to the middle point of the eighth and ninth correlation points” [2].

Figure 2 describes the optical rotation property of a compound, in which it can be positive or negative, from the Nawiah 9 x 45 (1) Theory's point of view. The blue straight line (Nawiah Line 1) is the connecting line between the first (1) correlation point and the middle point of the eighth (8) and ninth (9) correlation points. Meanwhile, P is the intersection point between Nawiah Line 1 and a straight line with the angle 45°. If there is any correlation points located on the intersection point P, thus it describes that the compound has a positive (+ve) property. On the contrary, if there is no correlation point on the intersection point P, it implies that the compound has a negative (-ve) property. The positive or negative characteristic of a compound is important to determine the effects of the compound as a vaccine. Vaccine with the positive property will treat the external illness, while vaccine with the negative property will treat the internal illness.

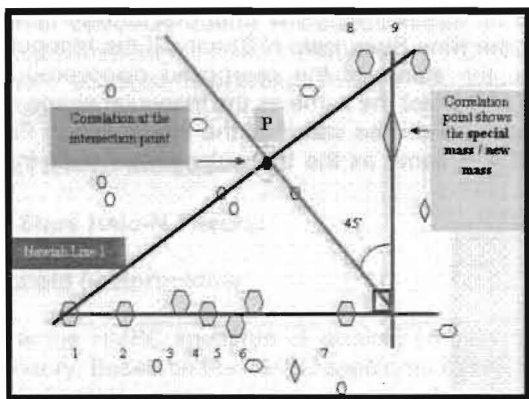


Figure 2 Nawiah 9x45 (1) Theory

1.3 Nawiah 9 x 45 (2) Theory

“Positive or negative property of a carbon compound found through the Nine Stars Halo-N Theory can be determined by the existence of the correlation point located on the straight line formed between the first correlation point and special mass / new mass.” [3].

Figure 3 describes the optical rotation direction of a compound, whether it is positive or negative, from Nawiah 9 x 45 (2) Theory's point. The green straight line (Nawiah Line 2) is the straight line formed between the first correlation point and special mass / new mass. If there is a correlation point located on

the Nawiah Line 2, then it means the compound has a negative (-ve) property. On the contrary, if there is no correlation point located on the Nawiah Line 2, then it means the compound has a positive (+ve) property. Once again the positive or negative property of a compound is important to determine the usefulness of the compound as a vaccine. If a vaccine compound has a negative property, thus it can be used to treat internal illness, where as a vaccine compound with a positive property can be used to treat external illness.

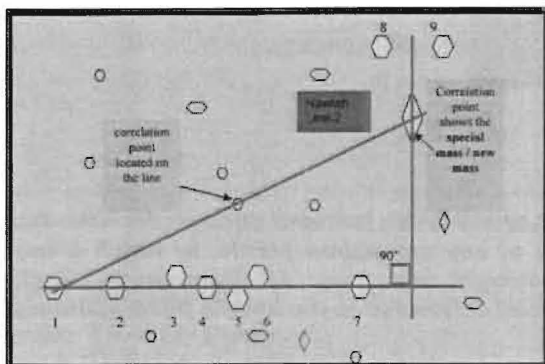


Figure 3 Nawiah 9x45 (2) Theory

1.4 Halo-N 9.2 Homolength Theory

“The Single Compound or Mixed Compounds status of a carbon compound discovered through the Nine Stars Halo-N Theory can be determined based on two homolength reference points forming a triangular shape at the part of the New Mass (Special Mass) and another two homolength reference points forming a triangular shape at the base of the first and second correlation points for the Nine Stars Halo-N Theory.” [4].

Figure 4 shows the status of a compound as a mixed compound. Triangle **A** is formed between the eighth and ninth correlation points with the new mass (special mass). Triangle **B** is formed between the first and second correlation points with the second mass for the Nine Stars Halo-N Theory. If the triangular shape of A is same as the triangular shape of B, thus the status of the compound discovered is mixed compounds. On the contrary, if the triangular shape of B is not the same as the triangular shape of A, thus the status of the compound discovered is single compound. The status of the compound in Figure 4 is mixed compounds because the triangular shape of A is same as the triangular shape of B, in terms of their distance and correlation points.

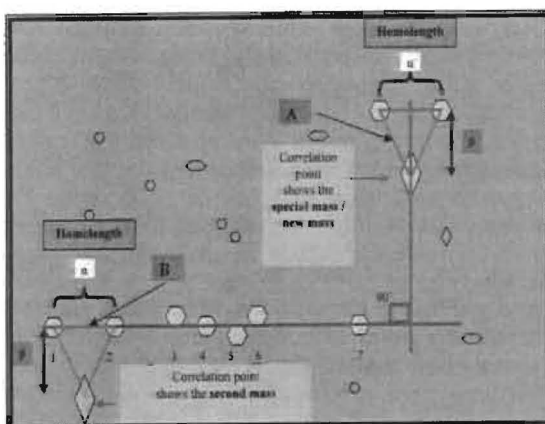


Figure 4 Halo-N 9.2 Homolength Theory shows the status of a compound as mixed compounds

Figure 5 shows the status of the compound as a single compound. The triangular shape of B is not the same as the triangular shape of A, as there is no correlation point at the base of triangle B.

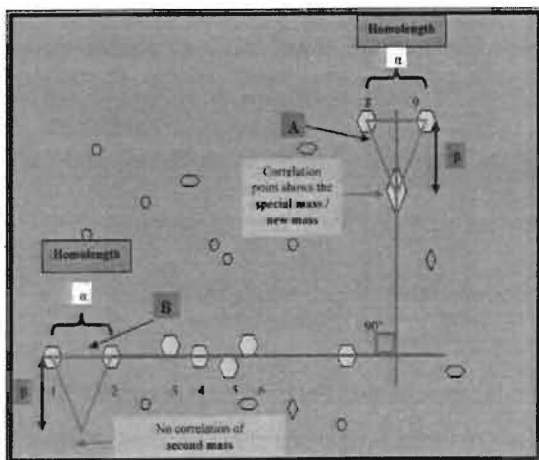


Figure 5 Halo -N 9.2 Homolength Theory shows the status of a compound as single compound.

2. EXPERIMENTAL PROCEDURES

Studies on the validity and authenticity of Dr. Halo-N Theories were done based on the HMBC spectra of the compounds of natural products isolated from Lauraceae species such as *Phoebe tavoyana*, *Phoebe grandis*, *Litsea petiolata* and *Dehaasia longipedicellata*.

2.1 General experimental procedures

Spectroscopic measurements were performed as follows; Optical rotations were determined on Autopol 111 Automatic Polarimeter Machine with methanol and chloroform as solvent. UV spectra were obtained using Shimadzu UV-160 Ultraviolet-Visible Spectrometer. IR spectra were obtained with CHCl_3 on a Perkin Elmer Spectrum 2000-FTIR Spectrometer. HR-ESI-MS were performed on a Shimadzu LC-MS-IT—TOF spectrometer. ^1H NMR (400MHz), ^{13}C NMR (400MHz), DEPT, COSY, HMQC and HMBC spectra were acquired in a Bruker Avance 400 spectrometer using TMS as the internal standard and CDCl_3 as solvent.

3. RESULTS AND DISCUSSION

3.1 Nine Stars Halo-N Theory

3.1.1 Alkaloid (-) Norboldine

Figure 6 is the HMBC spectrum of alkaloid (-) Norboldine which had been matched with the Nine Star Halo-N Theory. Based on the HMBC spectrum, the Nine Stars Halo-N is apparent with the arrangement of 2:4:1:2 - L System, which consists of correlation points connected with the red line. A correlation point which touched the vertical 90° angle straight line is the sought of new / special mass. Whichever HMBC spectrum for a natural product that matches the *Nine Stars Halo-N Theory* will be a sign, that the natural product can be a vaccine. The antimalaria activity of isolated compound (-) Norboldine was determined by the procedure described by [5].