

PRENYLATED DIHYDROSTILBENES FROM *Macaranga heynei* (EUPHORBIACEAE)

(Dihidrostilbena Terprenil daripada *Macaranga heynei* (Euphorbiaceae))

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Abstract

Macaranga is the largest genus in Euphorbiaceae which consists of approximately 300 species and are widely distributed in New Guinea, Borneo and from West Africa to the south Pacific islands. Phytochemical study on the leaves of *Macaranga heynei* has been conducted. The powder of the leaves of *M. heynei* was macerated in methanol at room temperature for 24 hours and repeated three times. The crude extract obtained was dissolved in aqueous methanol (80:20) and partitioned with hexane and ethyl acetate. The ethyl acetate crude extract was fractionated by vacuum liquid chromatography (VLC) with hexane:ethyl acetate in increasing polarity to give seven combined fractions (MH 1-7). Fraction MH 2 (1000 mg) was further purified by radial chromatography (RC) with hexane:ethyl acetate in increasing polarity to give six combined subfractions (MH 21-26). Fraction MH 23 (693.7 mg) was further purified by the same chromatographic technique but with different eluent which was chloroform:ethyl acetate in increasing polarity to afford two pure compounds (**1**) (10 mg) and (**2**) (12 mg). Based on spectroscopy analysis, the pure compounds were characterized as laevifolins A (**1**) and B (**2**).

Keywords: Laevifolin A, laevifolin B, leaves, *Macaranga heynei*

Abstrak

Macaranga merupakan genus terbesar dalam famili Euphorbiaceae yang mengandungi kira-kira 300 spesies dan taburannya meliputi New Guinea, Borneo dan dari Afrika Selatan ke utara kepulauan Pasifik. Kajian fitokimia ke atas daun *Macaranga heynei* telah dijalankan. Serbuk daun *M. heynei* direndam dalam metanol pada suhu bilik selama 24 jam dan pengeskrakan diulang sebanyak tiga kali. Ekstrak mentah yang diperolehi telah dilarutkan dalam larutan metanol (80:20) dan dipisahkan dengan heksana dan etil asetat. Etil asetat mentah difraksinasi menggunakan kromatografi cecair vakum (KCV) dengan heksana:etil asetat mengikut peningkatan kekutuban menghasilkan tujuh fraksi gabungan (MH 1-7). Fraksi MH 2 (1000 mg) selanjutnya ditulenkan dengan menggunakan kromatografi radial (RC) dengan heksana:etil asetat mengikut peningkatan kekutuban menghasilkan enam subfraksi gabungan (MH 21-26). Fraksi MH 23 (693.7 mg) seterusnya ditulenkan dengan menggunakan teknik kromatografi yang sama tetapi menggunakan eluen yang berbeza iaitu kloroform:etil asetat mengikut peningkatan kekutuban menghasilkan dua sebatian tulen (**1**) (10 mg) dan (**2**) (12 mg). Berdasarkan analisis spektroskopi, sebatian tulen tersebut dicirikan sebagai laevifolin A (**1**) dan B (**2**).

Kata kunci: Laevifolin A, laevifolin B, daun, *Macaranga heynei*

Introduction

Macaranga which comprises a large genus with 300 species [1] is widely distributed in New Guinea, Borneo and from West Africa to the south Pacific islands [2]. Generally, these species are found in village-thickets, wastelands and swampy forests [3]. These species are commonly distributed in the forests as well as in disturbed areas. However, most of the species are found to be well-flourished in developed or disturbed vegetation areas compared to the natural forest [4]. Apart from being exploited as plywood and match splints, this genus is widely used for traditional remedies by the old folks. The decoction root of *M. tanarius* has been used to treat fever and as antitusive for coughing. In addition, the dried root is used as emetic agent while the fresh leaves have been used to cover wounds which act as an anti-inflammatory [5]. In Sumatra, the fruit of this species was used to improve the quality of the sugar produced while in Philippines, the bark and leaves are used in the preparation of fermented drink made from sugarcane [6].

Phytochemical studies on the species from *Macaranga* have shown that this genus has abundant sources of flavonoids especially prenylated flavonoids [7, 8]. In addition, this genus does contain stilbenoids, tannins, as well as terpenoids [9, 10, 11]. The flavonoid compounds isolated from *Macaranga* species possessed broad spectrum of pharmacological properties such as cytotoxicity, antiviral, antimicrobial as well as acetylcholinesterase activities [12, 13, 14]. Similarly, flavonoids displayed significant role in dietary antioxidant by reducing the oxidative stress in the body [15]. Meanwhile, the stilbenoids namely laevifolins A and B which were isolated from *M. javanica* possessed significant activities against *S. aureus* with MIC values 35 μ M [16].

M. heynei or being called as mahang biru by the locals is widely distributed in Peninsular Malaysia and Thailand. This species was found to occur on open places up to 1000 metres height as well as on exposed subsoil. *M. heynei* is classified by the folks as medicinal plants due to its potential in treating several illness [17]. Due to the promising bioactivities of compounds isolated from *Macaranga* species, *M. heynei* was selected for the phytochemical study.

Materials and Methods

The leaves of *M. heynei* was collected from Kuala Kangsar, Perak in December 2015 and the voucher specimen SK2875/15 was deposited at the herbarium of Universiti Putra Malaysia. The plant was identified by Dr. Shamsul Khamis, a botanist from Universiti Putra Malaysia.

Extraction and isolation

The leaves of *M. heynei* was dried and ground to form powder. The powder of the leaves (2 kg) was then macerated in methanol at room temperature. The extraction was done for 24 hours and repeated for three times. The extract was dried by using rotary evaporator to give methanolic crude extract (300 g). The methanolic crude extract was dissolved in the mixture of 80% methanol: 20% water followed by partition with hexane and ethyl acetate. The ethyl acetate crude extract (150 g) was fractionated by using vacuum liquid chromatography (VLC) with a mixture of hexane-ethyl acetate in increasing polarity to give seven combined fraction (MH 1-7). Fraction MH 2 (1000 mg) was further purified by radial chromatography (RC) with hexane: ethyl acetate in increasing polarity to give six combined subfractions (MH 21-26). Fraction MH 23 (693.7 mg) was further purified by the same chromatographic technique but with different eluent which was chloroform:ethyl acetate in increasing polarity to afford two pure compounds (**1**) (10 mg) and (**2**) (12 mg).

Analysis

The isolated compounds (MH 233 and MH 235) were elucidated by means of ultraviolet-visible (UV-VIS), infrared (IR), mass (MS) and one and two dimensioned nuclear magnetic resonance (NMR) spectroscopy, as well as comparison with literature data. The proton NMR was recorded in 600 MHz while the carbon-APT NMR in 150 MHz. Chemical shift, δ , ppm recorded in CDCl₃.

Results and Discussion

Compound (**1**) as shown in Figure 1 was obtained as brownish viscous oil from the isolation of the leaves of *M. heynei*. The TOFMS in positive mode recorded a pseudo-molecular ion $[M+H]^+$ peak indicated the molecular

formula of $C_{29}H_{38}O_4$. The UV absorptions (λ 208, 257 nm) showed the characteristic of typical aromatic and phenol. It is supported by the IR absorptions at 3447 (O-H); 2923(alkyl); 1606 (C=C aromatic) and 1216, 1012 (ether) cm^{-1} . The 1H NMR spectrum of (**1**) exhibited three aromatic protons at δ_H 6.75 – 6.31. One singlet signal at δ_H 6.31 of aromatic proton, a doublet signal at δ_H 3.34 (4H), a triplet of multiplet signal at δ_H 5.10 (2H) as well as two broad singlet signals at δ_H 1.80 (6H) and 1.73 (6H) showed that the aromatic proton of ring A pairs with two prenyl groups which is in symmetrical structure. Meanwhile, there are two doublet signals at δ_H 6.75 and 6.68 indicated the presence of *ortho*-coupled aromatic protons which pair with a prenyl group at δ_H 5.12, 3.39, 1.80 and 1.74 of ring B. In addition, there are two multiplet signals at δ_H 2.86 and 2.73 exhibited the presence of methylene protons which are connected to ring A and B which suggested that the compound is a dihydrostilbene derivative.

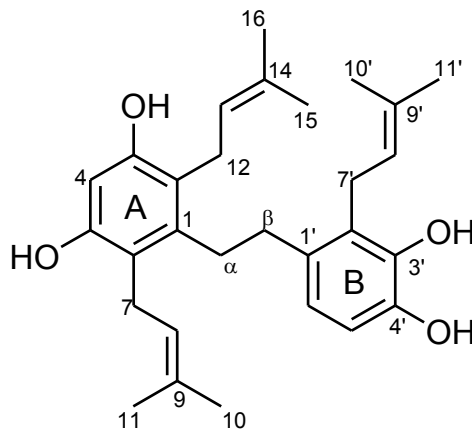


Figure 1. Chemical structure of compound (**1**)

The ^{13}C – APT NMR spectrum of (**1**) exhibited twenty two signals which represent twenty seven carbons suggested that the dihydrostilbene structure contains of four hydroxyl substituents and three phenyl groups. It was displayed by the oxyaryl signals at δ_C 153.5, 142.4 and 142.3 while the signals at δ_C 139.7, 134.6, 133.8, 132.2, 125.9 and 118.2 represents eight quaternary carbons. The signals at δ_C 123.0, 122.1, 121.1, 112.8 and 102.0 represents six methine carbons while five methylene carbons were represented by the signals at δ_C 33.9, 31.5, 26.6, 25.7 and 25.6. Three methyl carbon was exhibited by the signals at δ_C 25.8 and 17.9.

The long range correlations between proton and carbon could be observed from HMBC spectrum. The doublet signal at δ_H 3.34 (4H) showed correlation with carbon signals at δ_C 139.7 (C-1), 118.2 (C-2/6) and 153.5 (C-3/5) which confirmed the position of two prenyl groups at C-2 and C-6 at ring A. Meanwhile, another correlation was observed between the doublet signal at δ_H 3.39 (2H) with carbon signals at δ_C 132.2 (C-1'), 125.9 (C-2') and 142.3 (C-3'), confirming the position of one prenyl group at C-2' at ring B (Figure 2). The signals shown in 1H , ^{13}C – APT and HMBC NMR spectra are consistent with the literature [18] and characterised as laevifolin A (**1**).

Compound (**2**) as shown in Figure 3 was obtained as brownish viscous oil from the isolation of the leaves of *M. heynei*. The pseudo-molecular ion $[M+H]^+$ peak indicated the molecular formula of $C_{29}H_{38}O_4$. The UV absorptions (λ 208, 260 nm) exhibited the presence of phenol and aromatic system. The IR absorptions at 3412 (O-H); 2923(alkyl); 1599 (C=C aromatic) and 1217, 1017 (ether) cm^{-1} supported the presence of phenolic system in the structure. The 1H NMR spectrum of (**2**) is close to the compound (**1**) except for two doublet signal at δ_H 6.61 and 6.54 which showed the presence of *meta*-coupled aromatic protons in ring B. A broad singlet signal at δ_H 6.31 indicated the presence of aromatic proton in ring A. The presence of three prenyl groups was displayed by the signals at δ_H 5.35 – 1.74 which two of them are attached to ring A while the remaining is attached to ring B.

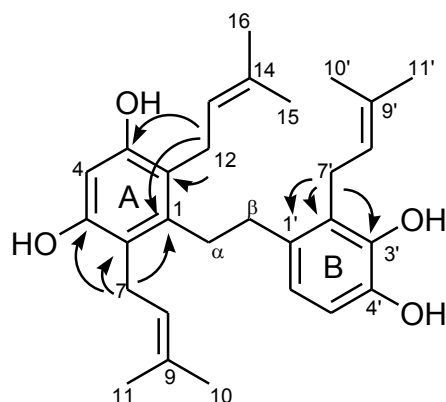


Figure 2. Selected $^1\text{H} - ^{13}\text{C}$ HMBC found in compound **(1)**

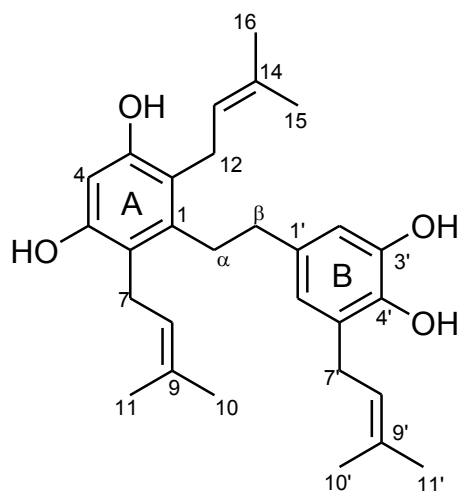


Figure 3. Chemical structure of compound **(2)**

The ^{13}C – APT NMR spectrum of **(2)** exhibited twenty two signals which represent twenty seven carbons which are similar to those shown in the spectrum of compound **(1)**. There are four signals representing oxyaryl carbons at δ_{C} 153.4, 153.1, 143.8 and 140.4 while five signals indicating the presence of eight quaternary carbons at δ_{C} 139.6, 134.2, 133.1, 127.6 and 118.2. Meanwhile, six methyne carbons were exhibited by the presence of five signals at δ_{C} 123.5, 122.1, 121.4, 113.0 and 102.8. The methylene carbons were represented by the signals at δ_{C} 35.7, 31.7, 29.5 and 25.2 while six methyl carbons were displayed at δ_{C} 26.7, 26.6, 18.0 and 17.9.

The long range correlations between proton and carbon could be observed from HMBC spectrum. A doublet signal of methylene protons at δ_{H} 3.37 (2H) exhibited correlation with 140.4 (C-4'), 127.6 (C-5') and 122.1 (C-6') confirming the location of one prenyl group at C-5' at ring B (Figure 4). Meanwhile, the position of two prenyl groups at ring A are similar to that of laevifolin A **(1)**. Based on the comparison of ^1H , ^{13}C – APT and HMBC NMR spectra with the literature [18], the compound was characterised as laevifolin B **(2)**.

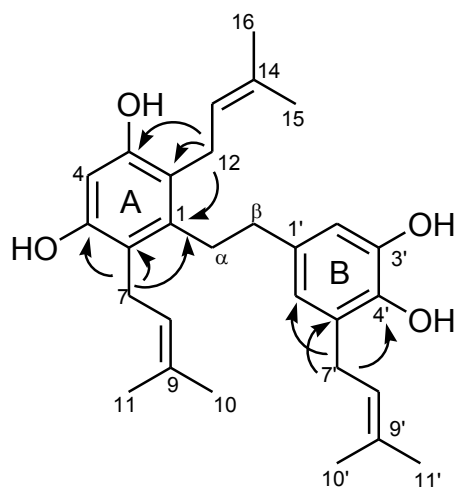


Figure 4. Selected $^1\text{H} - ^{13}\text{C}$ HMBC found in compound (2)

Conclusion

In conclusion, the phytochemical study on the leaves of *M. heynei* has successfully isolated two prenylated dihydrostilbenes namely laevifolins A (**1**) and B (**2**). This is the first report of the compounds in this species.

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