

## MONOMER STILBENOID GLUCOSIDES FROM *Vatica pauciflora* AND *Vatica lowii* (DIPTEROCARPACEAE)

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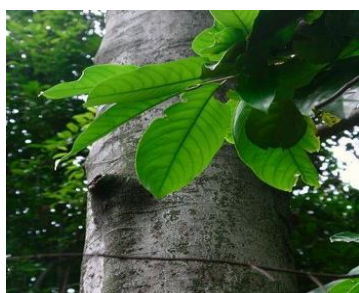
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### Graphical abstract



### Abstract

Phytochemical studies on the woods of *Vatica pauciflora* and *Vatica lowii* have been conducted. The woods of *V. pauciflora* were extracted with methanol by using Soxhlet apparatus while the woods of *V. lowii* were extracted with acetone at room temperature. The methanolic extract of *V. pauciflora* was semi-purified by using vacuum liquid chromatography (VLC) and further purified by high performance liquid chromatography (HPLC) to obtain a pure compound **(1)**. The same technique of semi-purification has been employed on *V. lowii* acetone extract which was further purified by radial chromatography to give a pure compound **(2)**. Based on the UV, IR, NMR and mass spectral data, the isolated compounds were characterized as (-)-*cis*-resveratrol-10-C-β-D-glucopyranoside and (-)-piceid respectively.

Keywords: *V. pauciflora*, *V. lowii*, (-)-*cis*-resveratrol-10-C-β-D-glucopyranoside, (-)-piceid

### Abstrak

Kajian fitokimia ke atas bahagian kayu *Vatica pauciflora* dan *Vatica lowii* telah dijalankan. Bahagian kayu *V. pauciflora* telah diekstrak dengan metanol menggunakan Soxhlet manakala bahagian kayu *V. lowii* diekstrak dengan aseton pada suhu bilik. Ekstrak metanol *V. pauciflora* telah dipisahkan separa dengan kromatografi cecair vakum (VLC) dan selanjutnya ditulenkan dengan kromatografi cecair prestasi tinggi (HPLC) bagi menghasilkan sebatian tulen **(1)**. Teknik yang sama telah digunakan ke atas ekstrak aseton *V. lowii* yang seterusnya dipisahkan menggunakan kromatografi jejarian bagi menghasilkan sebatian tulen **(2)**. Berdasarkan data spektrum UL, IM, RMN dan MS, sebatian yang diasingkan masing-masing dicirikan sebagai (-)-*cis*-resveratrol-10-C-β-D-glukopiranosida dan (-)-piseid.

Kata kunci: *V. pauciflora*, *V. lowii*, (-)-*cis*-resveratrol-10-C-β-D-glukopiranosida, (-)-piseid

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## 1.0 INTRODUCTION

Dipterocarpaceae family is not only known to contain abundant sources of oligostilbenoids but also an important source in timber production [1, 2]. The family members of Dipterocarpaceae usually found in lowland area as well as on the hill. Based on the research by Cronquist, Borneo and Peninsula Malaysia are rich in these species with 16 genera and almost 600 species were recorded in these areas [3].

The *Vatica* genus belongs to the largest subfamily in Dipterocarpaceae which is Dipterocarpoideae. The species from this genus which is mainly found in Borneo and Malay Peninsula usually occur in the lowland area below 1000 feet, hill-forest between 2000 and 3000 feet and peat swamp forests [4, 5].

Most of *Vatica* species are relatively smaller compared to other genus in Dipterocarpaceae family such as *Shorea*, *Hopea* and *Neobalanocarpus*. As the wood is undurable, this genus has less commercial importance and is usually used in temporary constructions [5]. However, this genus can produce resin in small and large quantity depending on the species [4, 5].

*V. pauciflora* is one of the most species used as *laru* to prevent fermentation of toddy [5 – 7]. The trees are small medium-sized and produced yellowish dammar which can be found in the branches. The stipules are oblong while the leaves are usually elliptic and oval. The flowers are creamy white in colour with some redness at the bottom of the petals. The fruits are in various sizes with pale pinkish brown colour [4].

*V. lowii* is a species found on the hill with 2000 feet from the sea level. The trees reached 60 to 80 feet high and the size of the stem is 18 to 24 inches. The leaves are small and glossy green while the colours of the flowers are white. However, very little information is reported on the usage of *V. lowii* [4].

The previous study by Ito and co-workers on the stem bark of *V. pauciflora* originated from Indonesia have successfully isolated a non-oligomer compound and 26 stilbene oligomers which eight of them are new compounds namely pauciflorols A-E; isovaticanols B and C and pauciflorosides A-C. The other 16 known stilbene oligomers reported are from monomer to tetramer stilbenoids [1].

Subsequent work done by the same researchers [8] has successfully isolated three new oligomer stilbenoids which one of them is a heptamer stilbenoid. Meanwhile, *V. lowii* has not been studied by any researcher yet.

Many studies done on the species from this family have revealed significant bioactivities results that may contribute to the development of drugs and natural antioxidant [9]. Biological studies done on some of these isolated chemical constituents of *V. pauciflora* revealed significant cytotoxic activities against murine leukemia P-388 [10, 11], colon cancer SW480, leukemia HL60 cell lines [9] as well as chemoprevention effect on liver Chang cells [12].

In searching for the potential natural and pharmaceutical products for medicinal purposes, the

isolation on Malaysia's *V. pauciflora* and *V. lowii* have been done to isolate the potential oligostilbenoids from these species. Apart from that, this study is also done to compare the chemical constituents in the same species but from different origin.

## 2.0 EXPERIMENTAL

### 2.1 Analysis

The structures of compound **(1)** and **(2)** were elucidated by means of infrared (IR), ultraviolet-visible (UV-VIS), mass (MS) and one and two dimensioned nuclear magnetic resonance spectroscopy (1D and 2D NMR), optical rotation analysis and direct comparison with literature data. Chemical shift,  $\delta$ , ppm recorded in CD<sub>3</sub>OD and CD<sub>3</sub>COCD<sub>3</sub>.

### 2.2 Plant Materials

*V. pauciflora* was collected from Gunung Pantii, Pontian, Johor, Malaysia in August 2006 while *V. lowii* was collected from Lata Belatan, Jertih, Terengganu, Malaysia in March 2007. The voucher specimens (WYP 15 and WYA 116 respectively) were deposited at the Herbarium of Universiti Kebangsaan Malaysia.

### 2.3 Extraction and Isolation

The woods of *V. pauciflora* were dried and ground to form powder. The powder of the woods (400 g) was then extracted with *n*-hexane, ethyl acetate and methanol by using Soxhlet apparatus. The extractions were done for 8 hr and were repeated for three times. The extracts were dried by using rotary evaporator to give crudes extracts VHB (3.00 g), VEB (9.48 g) and VMB (16.73 g). The crude methanol extract was then fractionated by Vacuum Liquid Chromatography (VLC) with a mixture of *n*-hexane-ethyl acetate in increasing polarity to give 14 fractions (VMB01-14). Fraction VMB15 (798.6 mg), which was a polar fraction was further purified by high performance liquid chromatography (HPLC) with gradient eluent of acetonitrile, trifluoroacetic acid (TFA) and methanol to give nine fractions (VMB151-159) which one of them was a pure compound **(1)** (0.3 mg). The pure compound was obtained as brownish viscous oil. The quasi-molecular ion [M+Na]<sup>+</sup> peak was recorded at *m/z* 413 (100%). The absorption of UV spectrum in methanol was recorded at  $\lambda_{max}$  212, 284 nm.

The woods of *V. lowii* were dried and ground to form powder. The powder of the woods (2.3 kg) was then extracted with acetone at room temperature followed by ethanol for 24 hours and repeated three times. The extracts were dried by using rotary evaporator to give crudes extracts VLBA (60.45 g) and VLBT (69.88 g). The crude acetone extract was subjected to VLC with a mixture of *n*-hexane-ethyl

acetate in increasing polarity to give six fractions (VLBA1-6). Fraction VLBA6 (1.2 g) was further purified by radial chromatography (RC) using gradient eluent of chloroform and methanol (8:2) to give six fractions (VLBA61-66) which one of them was pure **(2)** (11.1 mg). The pure compound was obtained as brownish needle. The ESI-MS spectrum recorded the quasi-molecular ion  $[M-H]^+$  at  $m/z$  389. The absorption of UV spectrum in methanol was recorded at  $\lambda_{max}$  225, 298 nm. When the UV spectrum was recorded in the presence of NaOH, bathochromic shift was observed in which  $\lambda_{max}$  225 shifted to 237 nm and  $\lambda_{max}$  298 to 314 nm.

### 3.0 RESULTS AND DISCUSSION

Compound **(1)** was obtained from the wood extract of *V. pauciflora* as brownish viscous oil. The quasi-molecular ion  $[M+Na]^+$  peak indicated a molecular formula of  $C_{20}H_{22}O_8$ . The UV spectrum showed a characteristic of an aromatic and phenol. This absorption is a typical signal of oligostilbenoid with *cis*-stilbene skeleton [13, 14]. The presence of aromatic and phenol are supported by the IR absorption at 3334 (O-H); 1611 and 1453  $cm^{-1}$  (C=C Ar). Absorption at 1200 and 1002  $cm^{-1}$  suggested the presence of ether (C-O-C).

The  $^1H$  NMR spectrum of **(1)** showed the presence of four aromatic protons at  $\delta_H$  7.05 – 6.13. Two doublets ( $J = 8.8$  Hz) at  $\delta_H$  7.05 and 6.57 indicated the presence of 1,4-disubstituted aromatic ring. Two *meta*-coupled aromatic signals ( $J = 2.5$  Hz) at  $\delta_H$  6.21 and 6.13 showed the presence of another tetra-substituted aromatic ring. Two mutually coupled doublets ( $J = 12.1$  Hz) at  $\delta_H$  6.13 and 6.15 indicated the presence of olefinic protons which are *cis* to each other. Other signals at  $\delta_H$  4.68 – 3.17 are typical for glycoside protons. A broad singlet at  $\delta_H$  4.68 exhibited the presence of anomeric proton while another broad singlet at  $\delta_H$  3.73 and doublet at  $\delta_H$  3.71 assigned to methylene protons of glucoside molecule.

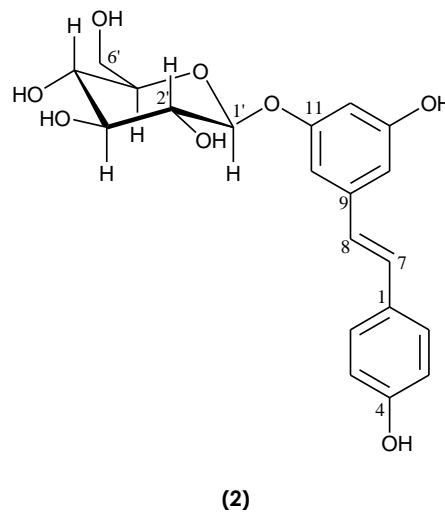
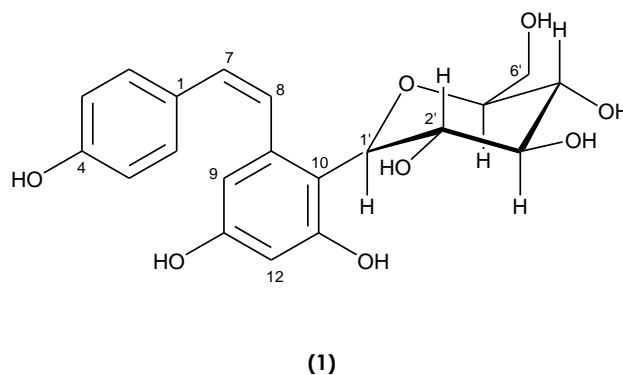
Since only a minute amount of this compound obtained, the  $^{13}C$  – APT NMR spectrum was not recorded. However, the signals shown in  $^1H$  NMR and COSY spectra of **(1)** are consistent with the literature [15] and is characterized as (-)-*cis*-resveratrol-10-C- $\beta$ -D-glucopyranoside **(1)**.

Compound **(2)** was obtained from the wood extract of *V. lowii* as brownish needle. The ESI-MS spectrum recorded the quasi-molecular ion  $[M-H]^+$  corresponding to a molecular formula of  $C_{20}H_{22}O_8$ . The UV spectrum showed the presence of aromatic and phenolic system. When the UV spectrum was recorded in the presence of NaOH, bathochromic shifts were observed with the value  $\lambda_{max}$  237 and 314 nm which indicated the presence of *trans*-stilbene skeleton in the molecule [13, 14]. The IR spectrum supported the presence of phenol ring at 3379 (O-H) and 1655  $cm^{-1}$  (C=C Ar). The absorption at 1032  $cm^{-1}$

suggested that this compound contain functional group of ether (C-O-C).

The  $^1H$  NMR spectrum of **(2)** displayed five aromatic signals at the range of  $\delta_H$  7.42 – 6.45. Two doublets ( $J = 8.4$  Hz) at  $\delta_H$  7.42 and 6.83 indicated the presence of 1,4-disubstituted aromatic ring. Three triplets ( $J = 1.8$  Hz) at  $\delta_H$  6.80, 6.66 and 6.45 indicated the presence of 1,3,5-trisubstituted aromatic ring. Two doublets ( $J = 16.4$  Hz) at  $\delta_H$  7.10 and 6.92 indicated the presence of two olefinic protons which are *trans* to each other. The signals at  $\delta_H$  4.93 – 3.45 were typical signals for glycoside protons where a doublet ( $J = 7.7$  Hz) at  $\delta_H$  4.93 indicated the anomeric proton in  $\beta$ -configuration. Other prominent signals are a doublet ( $J = 11.7$  Hz) at  $\delta_H$  3.92 and a double of doublets ( $J = 11.7, 1.8$  Hz) at  $\delta_H$  3.72 were typical of methylene protons of a glucopyranoside.

The  $^{13}C$  – APT NMR spectrum of **(2)** exhibited eighteen signals which represent twenty carbons. The presence of oxyaryl carbons were shown by the signals at  $\delta_C$  158.3, 159.4 and 160.3. Additionally, two quaternary carbons observed at  $\delta_C$  129.9 and 140.8 and seven aromatic carbons at  $\delta_C$  103.8, 106.4, 108.2, 116.4, 116.4, 128.8 dan 128.8. The remaining signals represent two olefinic carbons at  $\delta_C$  126.4 and 129.6 and six glucoside carbons at  $\delta_C$  62.7, 71.4, 74.7, 77.9, 78.1, 102.0. The data comparison of the  $^1H$  and  $^{13}C$  – APT NMR were consistent with the literature [16] and is characterized as (-)-piceid **(2)**.



The isolation of Malaysia's *V. pauciflora* found that only three oligostilbenoid compounds are similar to the Indonesian species namely (-)- $\epsilon$ -viniferin, (+)-vaticanol G and (-)-vaticanol B [1, 12]. However, (-)-*cis*-resveratrol-10-C- $\beta$ -D-glucopyranoside (**1**) is only present in Malaysian species. The difference on the presence of these compounds is due to the locality of the species which might be influenced by the soil as well as the environment of the species.

#### 4.0 CONCLUSION

In conclusion, the woods of *V. pauciflora* and *V. lowii* were found to contain monomer stilbenoid glucosides namely (-)-*cis*-resveratrol-10-C- $\beta$ -D-glucopyranoside (**1**) and (-)-piceid (**2**) respectively. Both monomer stilbenoid glucosides are reported for the first time for each species respectively. The isolation of Malaysia's *V. pauciflora* exhibited slightly different compounds compared to the Indonesian species. (-)-*cis*-resveratrol-10-C- $\beta$ -D-glucopyranoside (**1**) was only found in Malaysian species while other oligostilbenoids namely (-)- $\epsilon$ -viniferin, (+)-vaticanol G as well as (-)-vaticanol B are among the compounds that are present in both species [1, 12].

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