Stilbene Monomer Glucoside Isolated from *Vatica Odorata* (Dipterocarpaceae)

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ABSTRACT

An acetone extract of Vatica odorata was examined as a part of an ongoing search for much higher condensed stilbenoid In this paper, the structure elucidation of resveratrol-13-O-β-D-glucoside is discussed based on physical and spectroscopy analyses. Cytotoxic properties of the isolated compound was evaluated against murine leukimia P-388 cells and Artemia salina, resulting resveratrol-13-O-β-D-glucoside as inactice compound.

Keywords: *cytotoxic properties, isolated compound, stilbenoid*

Introduction

Stilbene and its oligomers including their glucosides occur in the particular families such as *Dipterocarpaceae*, *Vitaceae*, *Cyperaceeae*, *Leguminosae* and *Gnetaceae* (Ito et al., 2003). *Vatica* is a relatively large genus belonging to the Dipterocarpaceae family and is distributed mainly in Southest Asia (Symington, 1974). This genus, as well as other dipterocarp genera such as *Shorea*, *Hopea* and *Vateria*, has proven to be a rich source of oligostilbene compound that is derived from a stilbene (Sotheeswaran and Pasupathy, 1993). Resveratrol (trans-3,5,4'-trihydroxystilbene), one of the stilbenes, has recently been drawn to attention because of its various biological properties such as anti-ovidative, antimutagenic and an inducer of phase II drug metabolic enzymes (Ito et al., 2003). This paper reports resveratrol-13-O-β-D-glucoside from *Vatica odorata* (resak ranting kesat) of which no report has appeared in the literature describing the phytochemistry or biological activity of this plant.

Experimentals

General Experimental Procedures

UV spectra were measured with a Varian Conc. 100 instrument. IR spectra were determined with a Perkin Elmer FTIR Spectrum One spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL ECP400 operating at 400 (^{1}H) and 100 (^{13}C) MHz using residual and deuterated solvent peaks as reference standards. Vacuum liquid (VLC) and column chromatography were carried out using Merck silica gel 60 GF_{254} and silica gel G60 35-70 mesh. For TLC analysis, precoated silica gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm) were used.

Plant Materials

Samples of tree barks of *Vatica odorata* were collected from Pulau Mata Kail, Belum Forest Reserve, Perak, Malaysia. The plant was identified by a botanist from Universiti Putra Malaysia and a voucher specimen was deposited in the herbarium (SK 616/03).

Extraction and Isolation

The dried powdered tree bark (0.45 kg) of *V. odorata* was macerated with acetone (3 × 4L) followed by methanol (3 × 4L), and each extract was evaporated under reduced pressure to give dark brown residues. The dried acetone extract (27.3 g) was subjected to vacum liquid chromatography (silica gel, *n*-hexane-EtOAc = 4.5:5.5) into five major fractions (A-E). Fraction E (5 g) was further fractionated by flash column chromatography (silica gel, $CHCl_3$-MeOH 10:0 to 1:1) to give three semipurified fractions E1-E3. Repetitive purification of fraction E1 (1.2 g) by radial chromatography (silica gel, EtOAc-MeOH = 10:0 – 8:2 & $CHCl_3$-MeOH = 95:5) gave resveratrol-13-O-β-D-glucoside (27 mg).

Cytotoxicity Assay

The compound was tested with brine shrimp lethality test which was carried out according to (Mc Laughlin et al.,1991). Meanwhile, cytotoxic test on compound against murine leukimia P-388 cells was carried out according to the method described previously (Sahidin et al., 2005)

Results and Discussion

Compound VOA5122 was isolated as white crystals, which has a melting point at 220 – 220°C and optical rotation $[\alpha]_D$ = -45. The UV spectrum showed the maximum absorptions at 205, 215, 306, 316 nm suggesting the presence of stilbene skeleton in compound VOA5122.. The IR spectrum showed an absorption band for hydroxyl at 3401 cm^{-1}, a stretching vibration for carbon aliphatic at 2920 cm^{-1}, a stretching absorption for carbon aromatic at 1606, 1587, 1514, 1410 cm^{-1} and a stretching absorption for tetra-substituted ring at 841 cm^{-1}.

The ^1H NMR spectrum for VOA5122 showed the signals for substituted resveratrol compound. A doublet signals at δ 7.04 (d, J = 16.3 Hz, H-7) and δ 6.86 (d, J = 16.3 Hz, H-8), suggested the presence of *trans*-olefinic protons connecting two benzene rings (A and B ring) in resveratrol skeleton. One set of ortho–coupled aromatic protons assignable to one 4-hydroxyphenyl group was observed as resonance at δ 7.40 (d, J = 8.6 Hz) and 6.80 (d, J = 8.6 Hz). This proton was revealed as H-2/6 shifted downfield compared to H-3/5 and both signals integrated as two protons. The signals at δ 6.76 (br s), δ 6.48 (t, J = 2.0 Hz) and δ 6.65 (t, J = 1.8 Hz) were assigned as meta protons observed on 1,3,5-tetra substituted ring group (B ring).

The following characteristic signals of glucoside molecule confirmed the presence of glucoside molecule as one of the substituent groups. A doublet at δ 4.94 (J = 7.7 Hz, H-1') was assigned for anomeric proton, meanwhile, another signals at δ 3.92 (br d, H-6a'), δ 3.70 (d, H-6b') and δ 3.57-3.42 (m, H-2', H-3', H-4', H-5') were due to the hydrogen atom signals which bonded to glucoside carbon that had hydroxyl group. Based on the signal pattern for glucopyronoside proton, this glucose unit was suggested to be bonded at C-13. The aromatic protons in resveratrol unit and glucose position were confirmed from the correlations coupling systems in the ^1H-^1H COSY spectrum (Figure 1a). The presence of glucose molecule was also confirmed by a broad absorption at 3401 cm^{-1} in IR spectrum.

The ^{13}C NMR spectrum gave a total of 20 carbons. Three oxyaryl carbon signals were observed at δ 157.1 (C-4), δ 158.3 (C-13) and δ 158.9 (C-11), hence, confirming that VOA5122 was the resveratrol monomer. Meanwhile, methine carbon signals at δ 127.5 (C-2a/6a), δ 115.3 (C-3a/5a), and three carbon signals at δ 102.5 (C-10), δ 102.6 (C-12) and δ 106.9 (C-14) were due to the 1, 3, 5 – meta substitution ring. Other, methine carbon signals appeared at δ 73.4 (C-2'), δ 76.5 (C-3'),

δ 70.1 (C-4'), δ 76.7 (C-5') and δ 61.4 (C-6') belonged to the carbon atom in glucoside unit. Two quaternary carbon signals at δ 128.4 and δ 139.5 were assigned for C-1 and C-9, respectively. All correlations of these methine carbons were confirmed based on the interaction of $^{1}J_{C-H}$ that had been assigned in ^{1}H-^{13}C HMQC spectrum.

The bonding of glucopyroside ring to resveratrol unit was confirmed by 2D ^{1}H-^{13}C HMBC analysis (Figure 1b, Chart 1). A correlation between anomeric proton (H-1') at d 4.93 with carbón signal at δ 158.3 suggested that glucopyroside ring is attached at C-13 of resveratrol unit by ether bonding. Figure 1b shows some of the important interactions of $^{3}J_{C-H}$ of VOA5122.

The data analysis and NMR spectroscopy analysis shows the white crystals of VOA5122 were identified as reveratrol-13-O-β-D-glucoside. The comparison of these data was reported earlier by Hanawa et al. (1992).

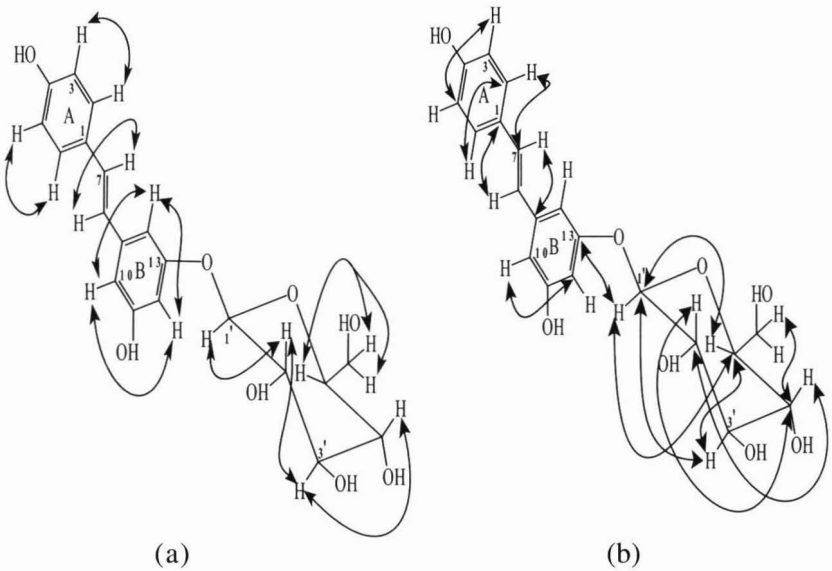

Figure 1: Proton Correlation Coupling (a) and Heteroatom Interactions (b) Assigned in ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC Spectrum of VOA5122

Table 1: $^{1}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ Interaction Assigned in HMQC and HMBC Spectrum of VOA5122

Proton	HMQC	HMBC
H-2/H-6	127.5 (C-2a/6a)	157.1(C- 4), 128.3(C-7)
H-3/H-5	115.3 (C-3/5)	128.4 (C-1)
H-7	128.3 (C-7)	139.5 (C-9), 127.5 (C-2/6)
H-8	125.1 (C-8)	105.2 (C-10), 106.9 (C-14)
H-10	105.2 (C-10)	125.1(8), 102.6(12),106.9(14)
H-14	106.9 (C-14)	125.1(8), 102.6(12),105.2(10)
H-12	102.6 (C-12)	105.2(10),106.9(14)
H-1'	100.7 (C-1')	158.9(13),76.5(C-3'),76.7(5')
H-2'	73.4(C-2')	Interaction/overlap signal
H-3'	76.5 (C-3')	Interaction/ overlap signal
H-4'	70.1 (C-4')	Interaction/ overlap signal
H-5'	76.7 (C-5')	Interaction/ overlap signal
H-6'	61.4 (C-6')	Interaction/ overlap signal

From the literature, it is found that VOA5122 has very limited distribution in dipterocarp plants and this is the first report for the *vatica* genus. VOA5122 was tested against murine leukemia P-388 cells and *Artemia salina*, and it was found that it is an inactive compound.

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