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Laevifonol: A Unique Dimer Oligostilbene from the Stem Bark of *Vatica odorata*

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Abstract: The isolation of laevifonol (dimerstilbene) in *Vatica odorata* is the second time for its present in *Vatica sp*. This compound is a unique oligostilbene formed from a condensation between ε-viniferin and ascorbic acid and was firstly isolated from *Shorea laevifonia* and recently from *Vatica umbonata*. The structure of laevifonol was established on the basis of their spectral data, including UV, IR and NMR spectra and also in comparison with the previously reported data. Cytotoxic properties was evaluated against murine leukimia P-388 cells and *Artemia salina* which resulting not strongly inhibited with IC_{50} values of 60.5 μM and > 796.2 μM, respectively. Antibacterial activity also was screened against two gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aeureus*) and one gram negative bacteria (*Escherichia coli*). The antibacterial testing was carried out by using the disc diffusion method. Blanc disc of 6mm diameter was loaded with 1000 μg/ml of the compound applied to the inoculate plate. The compound showed moderate activity against all the bacteria with inhibition zones of 0.5 mm against *E.coli* and *B.subtilis* and 0.1 mm against *S. aerues* compared to positive control (Erythromycin 60 μg) with 0.13 mm, 0.03 mm and 0.1 mm each. The present investigation is apart of our ongoing studies on the oligostilbene of Malaysian Dipterocarpacaeae in which no phytochemical data was recorded on *V. odorata*.

Key words: Oligostilbene · Dipterocarpaceae · *Vatica odorata* · Laevifonol · Biological activity

INTRODUCTION

Stilbene (3,4',5-trihydroxystilbene) is a phenolic compound are occuring in the particular families such as Dipterocarpaceae, Vitaceae, Cyperaceae, Leguminoseae and Gnetaceae [1]. *Vatica* is a relatively large genus belonging to the family Dipterocarpaceae and is distributed mainly in Southest Asia [2]. Genus *Vatica*, *Shorea*, *Hopea* and *Vateria* have proven to be a rich source of oligostilbene compounds derived from a stilbene [3]. As a phytoestrogen, oligostilbene has recently been drawn to attentions because of its various biological properties that may account for its possible cardioprotective action, including of smooth muscle cell proliferation and platelet aggregration [4]. Moreover, this compound shows anti-inflammatory [5] and anticancer activities [6]. Because of its pharmacological functions, oligostilbene is now gaining scientific attention as a longevity promoter.

MATERIALS AND METHODS

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

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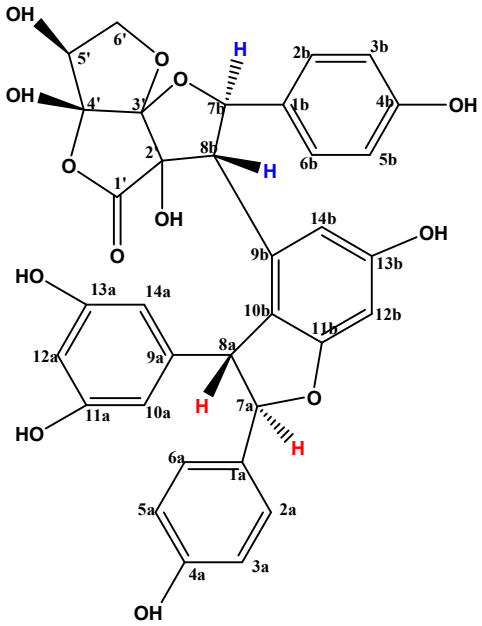

Fig. 1: Laevifonol

Extraction and Isolation: The dried powdered tree bark (0.45 kg) of *V. odorata* was macerated with acetone (3 x 4L) followed by methanol (3 x 4L) and each extract was evaporated under reduced pressure to give dark brown residues. The MeOH (75g) extract was subjected to fractionation using VLC (silica gel, *n*-hexane-EtOAc = 10:0, 8:2, 6:4, 4:6 and 2:8) into five major fractions F-J. Fractionation of fraction J (6g) was done by flash column chromatography (silica gel, *n*-hexane-EtOAc = 10:0, 8:2, 6:4, 4:6 and 2:8 and EtOAc-MeOH = 10:0 to 7:3) into six subfractions J1-J6. Purification of subfraction J3 by radial chromatography (silica gel, $CHCl_3$-MeOH = 10:0, to 1:1) yielded laevifonol (40mg).

(-)-Laevifonol (Figure 1)
$[\alpha]_D$:-175°(*c* 0.1, MeOH).
Rf: 0.58 (100% EtOAc).
IR (KBr): 3364, 2913, 1789, 1614, 1516, 1454, 1257, 835 cm^{-1}.
UV/Vis λ_{max} (MeOH) nm: 203, 226, 278, 284, 298.

^{1}H NMR (400 MHz, CD_3COCD_3: 6.76 (2H, m, H-2a/H-6a), 6.74 (2H, m, H-3a/H-5a), 5.03 (1H, br d, *J* = 7.3 Hz, H-7a), 3.26 (1H, d, *J* = 7.4 Hz, H-8a), 5.90 (2H, s, H-10a/H-14a), 6.15 (1H, t, *J* = 2.2 Hz, H-12a), 6.96 (2H, d, *J* = 8.4 Hz, H-2b/H-6b), 6.73 (2H, m, H-3b/H-5b), 5.27 (1H, d, *J* = 10.6 Hz, H-7b), 3.30 (1H, ovlp., H-8b), 6.17 (1H, d, *J* = 2.2 Hz, H-12b), 7.13 (1H, br s, H-14b), 4.41 (1H, br s, H-4') 4.21 (1H, m, H-5'), 3.97 (1H, dd, *J* = 4.4, 10 Hz, H-6'a), 4.05 (1H, dd, *J* = 2.2, 10.3 Hz, H-6'b).

^{13}C NMR (100 MHz CD_3COCD_3): 130.7 (C-1a), 128.0 (C-2a/C-6a), 114.9 (C-3a/C-5a), 157.6 (C-4a), 93.2 (C-7a), 55.1 (C-8a), 144.8 (C-9a), 106.1 (C-10a/C-14a), 159.9 (C-11a/C-13a), 101.4 (C-12a), 128.6 (C-1b), 127.1 (C-2b/C-6b), 114.1 (C-3b/C-5b), 157.3 (C-4b), 88.8 (C-7b), 55.1 (C-8b), 131.1 (C-9b), 121.7 (C-10b), 160.0 (C-11b), 95.9 (C-12b), 157.9 (C-13b), 110.0 (C-14b), 171.2 (C-1'), 80.0 (C-2'), 117.7 (C-3'), 88.1 (C-4'), 73.4 (C-5'), 74.6 (C-6').

HRMS-FAB: m/z $[M-H]^+$ calcd for $C_{34}H_{28}O_{12}$: 627.1318; found: 627.1408.

Cytotoxicity Assay: Laevifonol was tested with brine shrimp lethality test which carried out according to Said *et al*., 1990. Meanwhile, cytotoxic test on compound against murine leukimia P-388 cells was carried out according to the method described previously [8].

Antibacterial Assay: The bacteria used for the tests were obtained from Institute Medical Research (IMR), Malaysia which included both gram-positive (*B.subtilis* and *S.aeureus*) and gram-negative bacteria (*E.coli*). A 100 µL of bacterial suspension was spread on nutrien agar (NA) plate. Laevifonol (1000 µg/mL) as dissolved in methanol on 6 mm sterile filter paper disc and placed on inculated agar. Paper disc with methanol solvent was used as negative control meanwhile for positive control Erythromycin 60 µg was used. The plates were incubated at 37°C for 18-24h. After incubation time, zone of inhibition was measured. This method was referred to Barry *at el*. [9].

RESULTS AND DISCUSSION

Laevifonol was isolated as white crystals, which has a melting point at 300°C and optical rotation $[\alpha]_D$ =-175(c=0.1; MeOH). The UV spectrum showed the maximum absorptions at 203, 226, 284 and 284 nm which suggesting the presence of stilbene skeletal in laevifonol. The IR spectrum showed an absorption band for hydroxyl at 3364 cm^{-1}, a stretching vibration for carbon aliphatic at 2913 cm^{-1}, a stretching absorption for carbon aromatic at 1614, 1587, 1516, 1454 cm^{-1}, a stretching absorption for tetra-substituted ring at 835 cm^{-1} and aryl-O at 1257cm^{-1}. Beside that, there was a special characteristics for adsorption of laevifonol which was an absorption band at 1789cm^{-1} for lactone carbonyl.

The ^{1}H NMR spectrum for laevifonol showed the signals was for dimer resveratrol. This data was proved by the presence of one multiplet signals at δ6.73-6.70 integrated as three equivalent revealed at H-2a/H-6a, H-3a/5b. Meanwhile one doublet signals at δ 6.96 (*J* = 8.4 Hz, H-2b/H-6b) assigned as two unit 4-hydroxyphenyl.

Table 1: NMR data of Laevifonol in acetone d6 (400 MH_Z)

No	δH (multiplicity, *J* in Hz)	δC
1	-	13.07
2a,6[a]	6.76 (m)	128.0
3[a],5[a]	6.74(m)	114.9
4[a]	-	157.6
7[a]	5.03(brd, 7.3)	93.2
8[a]	3.26	(d,7.4)
9[a]	-	144.8
10[a]	5.90(s)	106.1
11[a]	-	159.9
12[a]	6.15(t,2.2)	101.14
13[a]	-	159.9
14[a]	5.90(s)	106.1
1b	-	128.6
2b,6b	6.96(d.8.4)	127.1
3b,5b	6.73(m)	114.7
4b	-	157.3
7b	5.27(d,10.6)	88.8
8b	(3.30(overlapped)	55.1
9b	-	131.1
10b	-	121.7
11b	-	160.0
11b	-	160.0
12b	6.17(d,2.2)	95.9
13b	-	157.9
14b	7.13(brs)	110.0
1’	-	171.2
2’	-	80.0
3’	-	117.7
4’	4.41(br s)	88.1
5’	4.21(m)	73.4
6’	3.97(dd,4.4,10.0)	74.6
	4.05(dd,2.2,10.3)	74.6

Table 2: Inhibition zone of bacteria treated with Laevifonol, Erythromycin 60 µg and methanol

Bacteria	Inhibition zone (mm)		
Gram positive	Laevifonol	Erythromycin (60 µg) (Positive control)	Methanol (Negative control)
Bacillus subtilis	0.5	0.03	0
Staphylococcus aeureus	0.1	0.10	0
Gram negative			
Escherichia coli	0.5	0.13	0

The presence of 1,3,5-trisubstituted ring group was assigned by the triplet signals at δ 6.15 (*J* = 2.2 Hz, H-12a) and multiplet signals at δ 5.90 integrated as two equivalent protons at H-10a and H-14a. The signals for one set aromatic proton with meta signals at δ 6.17 (1H,d, J=2.2Hz) and δ 7.13 (br s, 1H) each was revealed signals at H-12b and H-14b assigned as 1,2,3,5-*tetra*-substituted ring group.

Two signals for aliphatic proton in the dihydrobenzofurane integrated as dublet at δ 5.03 (br d, *J* = 7.3 Hz, 1H, H-17a) and δ 3.26(H-8a). ^{1}H NMR spectrum also revealed that that were two signals for aliphatic proton at δ 5.27(d, *J* = 10.6 Hz,1H, H-7b) and at δ 3.30(1H, H-8b). However two signals at δ 3.26 and δ 3.30 for H-8a and H-8b could not count the multiplicity due to it was hidden in the water peak. (δ 3.28-δ 3.31).

Laevifonol compound was in same group with ε-viniferin [10]. This type of dimer stilbene has a ring of 2,3-dihydrobenzofurane. However, laevifonol no longer has this skeletal stilbene as ε-viniferin. Vinyl group from stilbene skeletal has been broken and combined with ascorbic acid unit [11]. The presence of ascorbic acid unit was assigned with integration of four signals for aliphatic proton at δ 4.41 (br s, 1H, H-4’), δ 4.21(m, 1H,H-5’, δ3.97 (dd, *J*==4.4Hz, 10.0Hz, H-6a’) and δ4.05 (dd, *J*==2.2Hz, 10.3, 1H, H-6b’).

The ^{13}C NMR spectrum gave a 27 signals represented for 34 carbons, seven of them are equivalent. Seven equivalent carbon assigned at δ128.0 (C-2a/6a), δ 114.9 (C-3a/5a), δ 55.1(C-8a/8b), δ 106.1 (C-10a/14a), δ 159.9 (C-11a/13a), δ 127.1(c-2b/6b) and δ 114.7(C-3b/5b). There was one signals shifted at downfield (δ171.2) revealed a characterictic signal for carbonyl carbon which support IR data for lactone carbonyl band absoption. Five signals of oxyaryl carbon assigned at δ157.3-159.9 revealed that laevifonol was a dimer stilbene. Other signals were signals for nine aliphatic carbon at δ55.2-117.7 and four aromatic carbon at δ95.9-144.8.

Cytotoxicity Activity: A preliminary toxicity test of the laevifonol isolated revealed that it was not strongly inhibited to *Artemia salina* with IC_{50} value of >796.2 μM and cytotoxic evaluation against P-388 cells inidicated IC_{50} values of 60.5 μM.

Antibacterial Activity: As can be seen in Table 2 above, laevifonol showed strongly inhibited against *B. subtilis* followed by *E.coli* which compared with positive control as standard. Meanwhile for *S. aeureus* it showed the same activity with standard. The negative control (methanol) did not inhibit any bacterial growth.

CONCLUSION

The presence of laevifonol in *V. odorata* could be the important chemical characters for the chemotaxonomical analysis of *Vatica*. Moreover, the occurence of laevifonol which is a unique oligostilbenoid formed from a condensation of **ε**-viniferin and ascorbic acid highlights the relationship between *Vatica* with *Shorea*.

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