

# Spectroscopic Studies of Oligostilbenes from Malaysian Dipterocarpaceae

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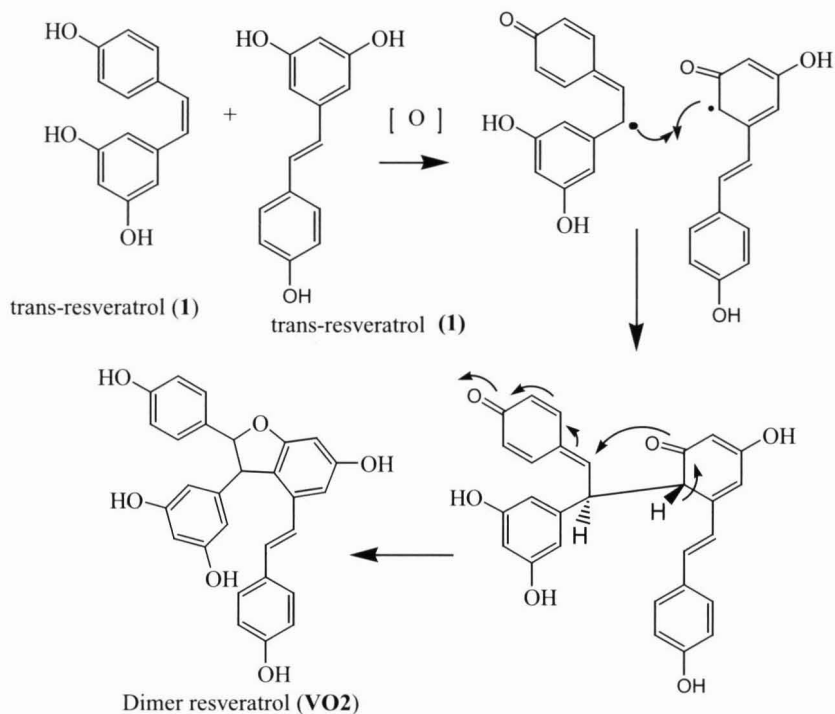
## ABSTRACT

*Oligostilbenoid compounds derived from stilbenes are found to be the major constituents of Dipterocarpaceae. These oligostilbenes comprise of a series of oligomers such as resveratrol dimers, trimers, tetramers, hexamers, and octamers. This paper, reports the structural elucidation of oligostilbenes from one of the Malaysian Dipterocarpaceae species.*

**Keywords:** *Dipterocarpaceae, Oligostilbenes, Oligomer NMR, Vatica odorata*

## Introduction

The Dipterocarpaceae is a relatively large plant family comprising 16 genera and about 600 species. It is found mainly in the tropical region (Cronquist, 1981). The main genus of this family is *Shorea* or locally known as Meranti. It is one of the economically important plants for paper and timber industries. Phytochemically, Dipterocarpaceae plants are a rich source of oligomeric stilbenes, in which most are bioactive, i.e as inhibitors of  $5\alpha$ -reductase (Hirano, 2001) and cytotoxic (Seo, 1999). The basic unit (monomer) of oligostilbenes is resveratrol or 3, 5, 4'-trihydroxy-*trans* – stilbene (**1**). The resveratrol units are joined together by phenolic oxidative coupling reactions at several different active sites resulted in the formation of complicated oligostilbenes (dimer, trimer, tetramer and etc.) (Scheme 1). This paper, reports the basic guidance in determining the structure of oligostilbene compounds (monomer and dimer) based on their spectroscopic evidence.



Scheme 1: Biogenetic Pathway of Dimer Resveratrol (VO2)  
(Sotheeswaran, 1993)

Presently, a vast variety of oligostilbenoid compounds have been increasingly isolated and many of their structures have been identified. Therefore, new spectroscopic data such as UV, IR, Mass, 1D and 2D NMR are added. The first perception could be seen from the UV spectrum.

The UV spectrum of oligostilbenes consists of absorption bands at max 300-320 (Band I), 280-290 (Band II) and 220-230 nm (Band III). Band II and III which are present in all oligomer stilbenoid indicate aromatic (phenolic) group. In other words, Band I which is attributable to a conjugated trans double bond, reveals reveratrol unit is conserved in the structure of oligostilbene.

The IR spectral data reveal the stretching absorption bands at 3500 -3000  $\text{cm}^{-1}$  and 2900  $\text{cm}^{-1}$  diagnostic for hydroxyl and CH- aliphatic respectively. In addition, bands at 1600-1400  $\text{cm}^{-1}$  and 830  $\text{cm}^{-1}$  indicate the C=C stretching and a *para*-substituent aromatic respectively. However,

the UV and IR data could not distinguish the oligostilbene in which group either in monomer, dimer, trimer and etc.

The presence of oligomer stilbenoid can be distinguished based on several additional  $^1\text{H}$  NMR signals. The dimeric, trimeric and tetrameric oligostilbenoids can be classified based on the number of aromatic signals which are directly proportional to the number of aromatic ring present in oligostilbenoid. If four aromatic rings are present, it indicates oligostilbenoids are dimer. For instance, the presence of two proton aromatic doublets which are symmetrical each at  $\delta$  6.0 and 7.5 ppm, with a coupling constant value of 8.5 Hz, reveals the *para*-substituted phenolic moiety. The existence of 1,3,5-trisubstituted aromatic ring will be revealed by the presence of three aromatic protons at  $\delta$  6.3-6.7 with a coupling constant of 2 Hz. Another significant  $^1\text{H}$  NMR characteristic of the stilbene is the presence of a pair of doublets at  $\delta$  6.70 and  $\delta$  7.04 with a coupling constant of (16.3 Hz respectively), which reveals a pair of vinylic protons in a *trans*-configuration as observed. In addition, there are additional signals for a pair of aliphatic protons occurring at  $\delta$  5.41 &  $\delta$  4.47 (each d) assigned to a dihydrobenzofuran moiety, a special characteristic for oligostilbenoids.

The  $^{13}\text{C}$  NMR experiment reveals the specific type and number of carbons of oligostilbenes which further identifies the amount of stilbene unit in one particular oligostilbene. Thus, signals at  $\delta$  150-160 are diagnostic of oxyaryl carbons,  $\delta$  95-150 ppm for aromatic carbon and  $\delta$  35-56 ppm for aliphatic carbon signals respectively. The aliphatic carbon which will occur when dimer, trimer and etc. contains benzofuran moiety. Oligostilbenes also have several chiral centres, thus, determination of their stereochemistry is necessary. The relative stereochemistry of the chiral centres could be determined by the 2-D NMR experiments, but the absolute molecular structure of oligostilbenes need the aid of X-ray diffraction (XRD) and chiral dichroism (CD) analysis. In some cases, where these two methods are unavailable, the optical rotation and optical rotation dispersion (ORP) are found useful to predict the relative molecular structure of oligostilbenoids. The mass spectrometry data show molecular and fragmentation ions that reveals the amount of oligostilbenes units.

## Materials and Methods

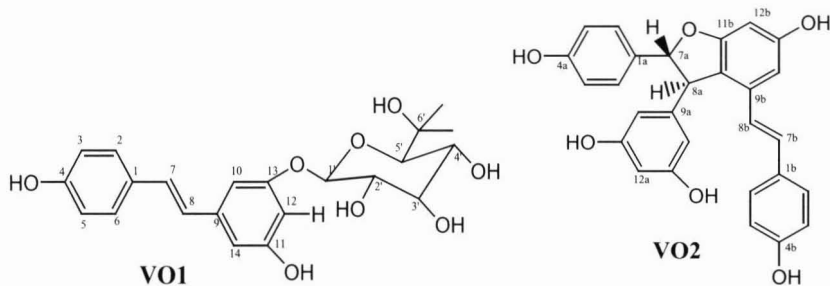
The isolation of stilbenoids were carried out by several chromatographic methods (column, vacuum liquid and radial chromatography) on silica

gel eluted with various solvent systems. The structures of the compounds were established on the basis of their spectral data, including UV, IR and NMR spectra, as well as molecular optical rotations, in comparison with the previously reported data and by direct comparison with the authentic samples.

## General Experimental Procedure

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Varian Cary 100 Bio UV-visible spectrophotometer in MeOH at 0.01 mg/ml concentrations. FT-IR spectra were obtained on a salt plate using a Perkin-Elmer Spectrum. Melting points were obtained on Mikro Fisher John. All NMR experiments were performed on JEOL ECP 400 NMR spectrophotometer with frequency of 400 MHz ( $^1\text{H}$ ) & 100.56 MHz ( $^{13}\text{C}$ ) using the solvent as a secondary reference standard in Acetone- $\text{d}_6$ . Chemical shifts,  $\delta$  in ppm.

## Results and Discussion



Scheme 2: Comparison of Monomer (VO1) and Dimer (VO2) Structure

Compound VO1 was isolated as amorphous white solid, which melted at 184-187°C. The UV spectrum exhibited absorptions (Band I- $\lambda_{\text{max}}$  318 nm, Band II- $\lambda_{\text{max}}$  306 and Band III  $\lambda_{\text{max}}$  – 230 nm) typical for a stilbenoid chromophore that have trans configurations (Mattivi, 1996). The bathochromic shift on addition of NaOH revealed that the compound have phenol groups. The IR spectrum of VO1 showed the stretching absorption band for hydroxyl ( $3422\text{ cm}^{-1}$ ), a stretching vibration for carbon aliphatic at  $2920\text{ cm}^{-1}$ , a stretching absorption for carbon aromatic at  $1606\text{--}1447\text{ cm}^{-1}$  and a stretching absorption for *p*-hydroxyphenyl groups at  $834\text{ cm}^{-1}$ .

The  $^1\text{H}$  NMR spectrum shows the presence of stilbene unit that resonates at  $\delta 7.04$  (d,  $J = 16.3$  Hz) suggesting a pair of vinylic protons in a trans-configuration as observed. These protons were assigned as H-7 and H-8. One set of ortho-coupled aromatic protons assignable to one 4-hydroxyphenyl group was observed as resonance at  $\delta 7.40$  (d,  $J = 8.6$  Hz) and  $\delta 6.80$  (d,  $J = 8.6$  Hz). This proton was revealed as H-2/6 shifted downfield compared to H-3/5 and both signals were integrated as two protons.

The signals at  $\delta 6.76$  (br s),  $\delta 6.48$  (t,  $J = 2.0$  Hz) and  $\delta 6.65$  (t,  $J = 1.8$  Hz) were assigned as meta protons observed on 1,3,5-tetra substituted ring group. All proton signals reveal that VO1 is in the group of monomer resveratrol. The remaining signal shows a series of signals attributable to a glucosyl moiety, which was deduced to be a *O*-glucoside based on the appearance of a doublet for the anomeric proton at  $\delta 4.94$  (d,  $J = 7.7$  Hz).

The  $^{13}\text{C}$  NMR spectrum of VO1 exhibited 12 carbons resonance representing for 14 carbons, instead of 2 anticipated for a symmetrical structure. Three signals observed between  $\delta 157.1$  -158.9 diagnostic for oxyaryl carbon supported structured VO1 being in a group of monomer resveratrol.

The compound VO2 was also isolated as amorphous white solid, which melted at  $220^\circ\text{C}$  - $222^\circ\text{C}$ . The UV spectrum exhibited absorptions (Band I- $\lambda$  max 322 nm, Band II- $\lambda$  max 248 nm and Band III- $\lambda$  max -226 nm typical for a stilbenoid chromophore that have trans-configurations (Mattivi, 1996), which shows bathochromic shift on addition of NaOH. The IR spectrum of VO1 shows the stretching absorption band for hydroxyl ( $3422\text{ cm}^{-1}$ ), a stretching vibration for carbon aliphatic at  $2920\text{ cm}^{-1}$ , a stretching absorption for carbon aromatic at  $1606$ - $1447\text{ cm}^{-1}$  and a stretching absorption for *p*-hydroxyphenyl groups at  $834\text{ cm}^{-1}$  groups. All the absorption characteristics are the same as VO1.

The  $^1\text{H}$  NMR shows the presence of stilbene unit that resonates at  $\delta 6.91$  (d,  $J = 16.5$  Hz) and  $\delta 6.70$  (d,  $J = 15.0$  Hz) which can be assigned as H-7b & H-8b, that is having the same features as VO1. In the VO2 spectrum, there were additional signals for a pair of aliphatic protons occurring at  $\delta 5.41$  &  $\delta 4.47$  (each d) observed for H-7a & H-8a assigned to a dihydrobenzofuran moiety, a special characteristic for a resveratrol. The signal of H-7b/8b is more shifted downfield compare to signal H-7a & H-8a.

Two sets of ortho – coupled aromatic protons assingnable to two 4-hydroxyphenyl groups were observed in VO2 spectrum. However, in

VO1 classified as a monomer resveratrol group, only one set was observed. These protons were observed as resonance at  $\delta$  7.20 (d,  $J$  = 8.4 Hz),  $\delta$  7.17 (d,  $J$  = 8.4 Hz),  $\delta$  6.83 (d,  $J$  = 8.8 Hz) and  $\delta$  6.73 (d,  $J$  = 8.4 Hz) which can be assigned to H-2a/6a, H-2b/6b, H-3a/5a and H-3b/5b.

A pair of protons resonating at  $\delta$  6.32 (d,  $J$  = 2.2 Hz) and  $\delta$  6.72 (d,  $J$  = 2.2 Hz) were assigned as meta protons for H-12b & H-14b observed for 1,3,5 tetra substituted ring group same as in the VO1 spectrum. The remaining signals are singlets at  $\delta$  6.24 which were assigned as H-10a/12a/14a for 3,5-dihydroxyphenyl group in meta position. These signals were not shown in the VO1 spectrum. The latter was assigned to the five hydroxyl groups in a strongly high downfield.

The  $^{13}\text{C}$  NMR of VO2 exhibited 21 carbon resonance representing 28 carbons, instead of the seven anticipated for a symmetrical structure. Six signals were displayed between  $\delta$  158.2-162.4 for oxyaryl carbons revealing that VO2 is in a group of dimer resveratrol.

## Conclusion

Two major chemical components have been identified as monomer (VO1) and dimer (VO2) oligostilbenoids structure based on spectroscopic evidence.

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