

ORIGINAL ARTICLE

The Isolation of Hopeaphenol, a Tetramer Stilbene, from *Shorea ovalis* Blume

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Blume:

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ABSTRACT

Hopeaphenol, a tetramer stilbene, one of the derivatives of oligomeric stilbene compounds was successfully isolated and identified from acetone extract of stem bark of *Shorea ovalis* Blume (Dipterocarpaceae). The isolation of this tetramer stilbene from this *Shorea* plant has never been done previously. The purification methods included extraction, partition, and fractionation with vacuum liquid chromatography and then followed by gravity column chromatography. The structure of the compound isolated was determined based on the analyses of physical data, UV and IR spectroscopies and compared to the standard compound of hopeaphenol.

Key words: Hopeaphenol, Dipterocarpaceae, *S. ovalis* Blume

Introduction

Indonesia, the second richest country in the biological diversity after Brazil, is a top country in the tropical forest. However, with this abundant resource, it has, so far, not been fully investigated, although the presence of the tropical forest as the natural resources of chemical substances is invaluable and potentially to be much more developed (Achmad, 1995)

The family plant of Dipterocarpaceae is one of the biological diversities of Indonesian plants and it is widely distributed in all parts of Indonesia. Dipterocarpaceae plant groups, locally known as Meranti, Keruing or Tengkwang, produce variety of compounds such as volatile oil, triterpenoid, flavonoid, arylpropanoid, and oligomeric resveratrol (Heyne, 1987; Sotheeswaran and Pasupathy, 1993). Dipterocarpaceae is relatively large family comprising of 16 genus and about 600 species (Cronquist, 1981; Heyne, 1987). The two biggest genus of Dipterocarpaceae family are *Shorea* dan *Dipterocarpus* having 150 and 75 species, respectively.

These plants are mainly growth in tropical areas and are known as a rich source of oligomeric stilbene (Sotheeswaran and Pasupathy, 1993). The oligomeric stilbenes have been shown have many interesting biological activities such as: as inhibitor of 5 α -reductase (Hirano *et al.*, 2001), scavenger of superoxide (Tanaka *et al.*, 2000) and cytotoxic agents (Seo *et al.*, 1999). The chemical research on these plants has long been done (Dai *et al.*, 1998; Ito *et al.*, 2000; Noviany *et al.*, 2002; Saraswathy, *et al.*, 1992; Sultanbawa *et al.*, 1987; Zheng *et al.*, 1994), but for such big family plants, the chemistry of Dipterocarpaceae is still interesting to be explored, due to the fact that the initial researches on Dipterocarpaceae was mainly focused on chemical compounds of their resins such as terpenoid, sesquiterpen and triterpen.

There were so far 17 *Shorea* species of Dipterocarpaceae which have been researched on, about 11 of them have been mainly researched in Indonesia (Aminah *et al.*, 2001; Aminah *et al.*, 2002; Hakim, 2002; Muharini *et al.*, 2001; Noviany, 2002; Noviany *et al.*, 2002; Noviany and Hadi, 2008). From the previous researches carried out, the results showed that the phenolic compounds isolated were generally those of oligomeric stilbenes. Based on this knowledge, the chemical research on *Shorea* genus in Indonesia which has not previously been done must be continued as they have high potential as sources of modern medicines. In this research, *Shorea ovalis* Blume is chosen, as the chemical contents of this species has not been previously reported.

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Materials and Methods

General experiment procedure

Vacuum liquid chromatography (VLC) was carried out using Merck Si-gel 60, TLC analysis on pre-coated Si-gel plates (Merck Kieselgel 60 F₂₅₄, 0.25 mm). The UV lamp of Spectroline, Model ENF-240 C/F was used to see the spot in TLC. Melting points were determined on a Fisher Johns micro-melting point apparatus and are uncorrected. UV and IR spectra were measured with Varian Cary 100 Conc. and Shimadzu FT-IR 8501 Scientific spectrophotometers respectively.

Plant Material

Samples of the stem bark of *S. ovalis* Blume were collected in April 2004 from Way Kambas National Park, East Lampung, Indonesia and were identified by the staff at the Herbarium Bogoriense, Research Centre for Biology, Indonesia Institute of Sciences Bogor, Indonesia and a voucher specimen has been deposited at the herbarium.

Extraction, Isolation and Purification

Dried and ground stem bark of *S. ovalis* Blume (3 kg) was macerated with acetone for 3 x 24 hours. After the removal of acetone under reduced pressure, the gummy brown residue of acetone extract (80 g) was obtained. The acetone extract was dissolved in methanol then partitioned with *n*-hexane. Upon evaporation of the solvent, the methanol extract (50 g) as brown residue was obtained. The methanol extract was then again dissolved in acetone and fractioned twice using VLC (200 g, 7 cm x 10 cm) by increasing polarity (*n*-hexane, *n*-hexane-ethyl acetate, ethyl acetate, methanol) to give 26 fractions which were grouped into 8 major fractions by combining fractions with similar TLC profiles. The main fraction obtained by combining fraction 22-25 (9 g) on repeated chromatographic purification, produced pale yellow crystalline (α -viniferin, 8 mg) mp 231-233 °C (Noviany and Hadi, 2008). The second main fraction obtained by combining fraction 10-18 (12 g) was further fractioned with gravity column chromatography and was eluted with mixture of *n*-hexane, dichloromethane (CH₂Cl₂)-methanol (9:1), CH₂Cl₂, methanol in increasing polarity. On repeated chromatographic purification, light yellow powder (compound **B**, 10 mg) mp 276-278 °C was obtained. The TLC of this compound showed a single spot when it was eluted with three different solvent systems, *i.e.* *n*-hexane - acetone (1 : 1); chloroform : methanol (75 : 25) and dichloromethane : acetone (1 : 1) with Rf of 0.3, 0.4, and 0.5 respectively.

Results and Discussion

The isolated compound **B** was obtained as light yellow powder with melting point of 276-278 °C (decomposed) and optical rotation of -313° (c.0.1 MeOH). The UV spectrum of the isolated compound showed λ_{\max} (MeOH) (log ϵ): 203 (1.17), 231 (0.49), 280 (0.23) nm which indicated phenolic chromophores which did not significantly shift upon addition of NaOH giving λ_{\max} (MeOH+NaOH) (log ϵ): 205(2.10), 251 (0.49), 281 (0.36) (Mattivi *et al.*, 1996). The IR spectrum (in KBr) showed vibration bands on 3335, 2910, 1615, 1600, 1516, 1456, 832.8 cm⁻¹ and in the finger prints area. The stretch vibration at 3335 cm⁻¹ is the typical of hydroxyl (OH) stretch, vibration at 2910 cm⁻¹ is characteristic of aliphatic stretch, while absorptions at 1615, 1600, 1516 and 1456 cm⁻¹ are due to presence of aromatic group of **B** (Table 1), and adsorption at 832.8 cm⁻¹ is indication by the presence of 1,4-disubstituted benzene. These UV and IR data are typical and characteristic of absorption of phenolic derivative compound, especially oligomeric stilbenes (Mattivi *et al.*, 1996).

The spectroscopies data of the isolated compound **B** are similar to that of compound which has previously been identified from some different genus of *Shorea*, consequently the structure determination of the isolated compound **B** was made by comparing to the UV and IR data as shown in Table 1 and 2, respectively and also by matching the UV and IR spectra as in Figure 1 and 2, respectively. To strengthen the structure determination of the compound **B**, the TLC of the isolated compound **B** was checked by eluting with three different solvent systems *i.e.* *n*-hexane : acetone (1 : 1); chloroform : methanol (75 : 25) and dichloromethane : acetone (1 : 1) where in all cases a homogeneous spot were obtained and the Rf values were compared to

the Rf of the standard compound, hopeaphenol, which has been previously isolated and reported from *S. selanica* Blume. The results on three different solvents showed the same Rf values, with Rf values of 0.3, 0.4, and 0.5 respectively (Tukiran *et al.*, 2001).

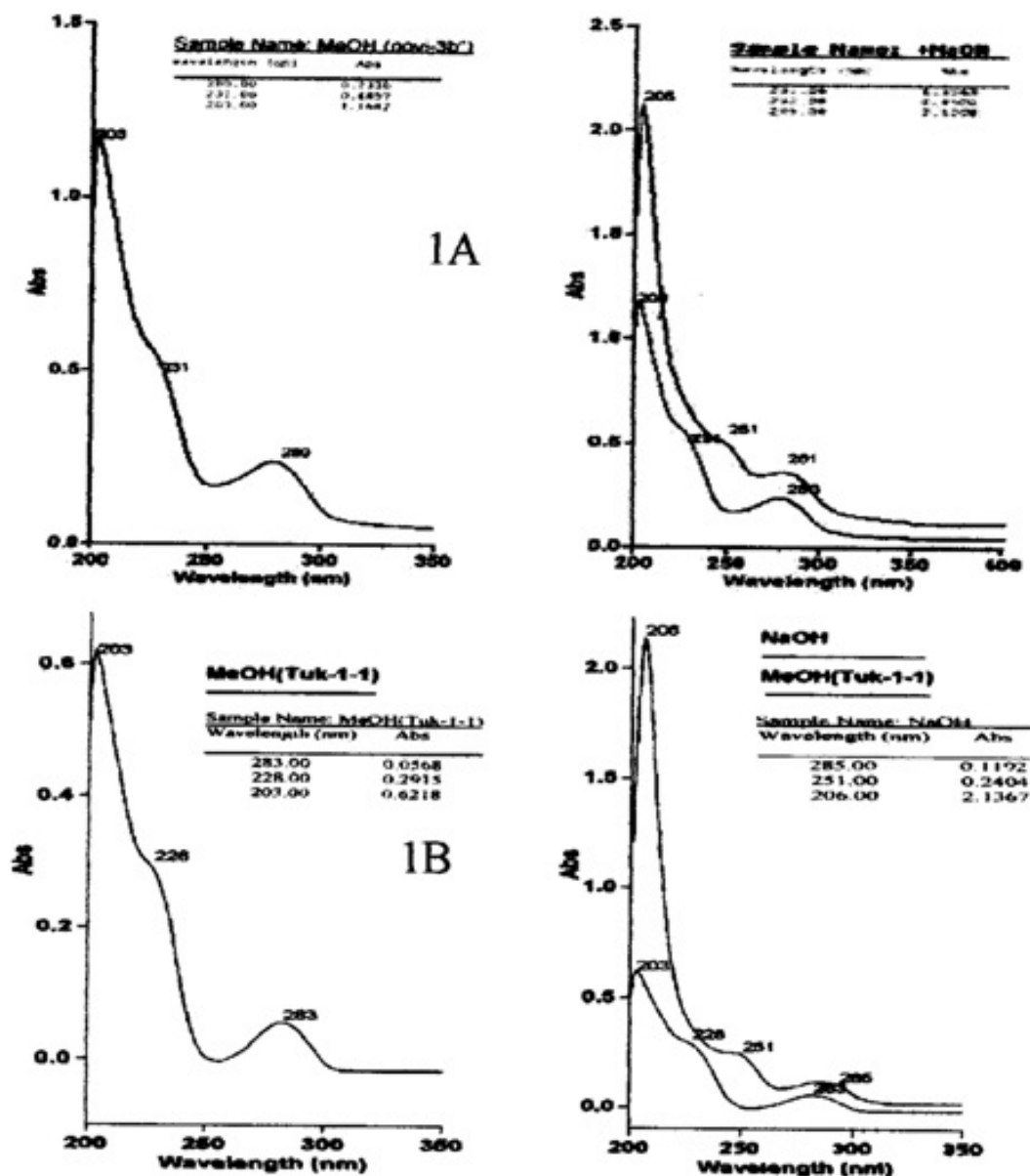


Fig. 1: The comparison of UV Spectra between the isolated compound (1A) and standard hopeaphenol (1B)

Based on the spectroscopies data above and the physical data in Table 3, the values obtained are similar to hopeaphenol previously reported by (Tukiran *et al.*, 2001), therefore it was suggested that the compound **B** is hopeaphenol (Table 1, 2 and 3) with the molecule structure as shown in Figure 3. Hopeaphenol, a tetramer stilbene, similar to α -viniferin, a trimer stilbene (Noviany and Hadi, 2008), is oligomeric stilbene which is also sometimes present in all *Shorea* genus. Thus based on chemotaxonomy, *S. ovalis* Blume also produced oligomeric stilbene compound as expected like other species of *Shorea*.

The biological activity of the compound isolated (hopeaphenol) has been tested against brine shrimp *Artemia salina* assay (Meyer *et al.*, 1982) and the lethal concentration 50% (LC₅₀) was found to be 191.6 mg/ml. The activity against Leukaemia tumour cell P-388 has IC₅₀ of 2.91 mg/ml (Tukiran *et al.*, 2001).

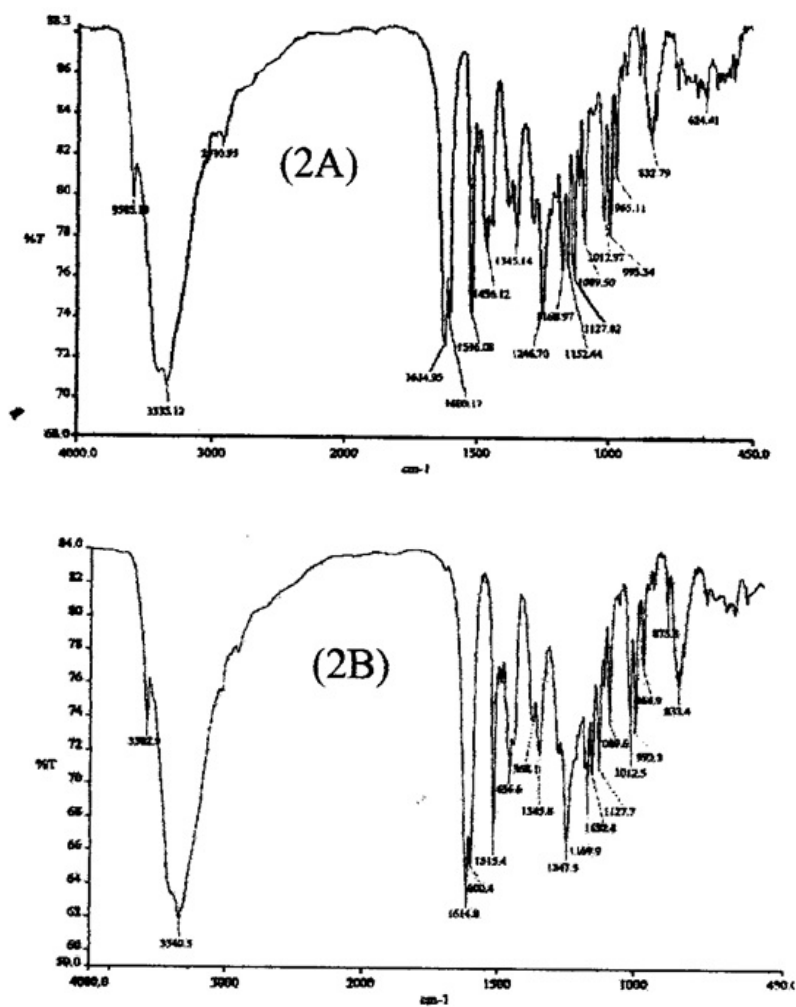


Fig. 2: The comparison of IR spectra between the isolated compound (2A) and standard hopeaphenol (2B)

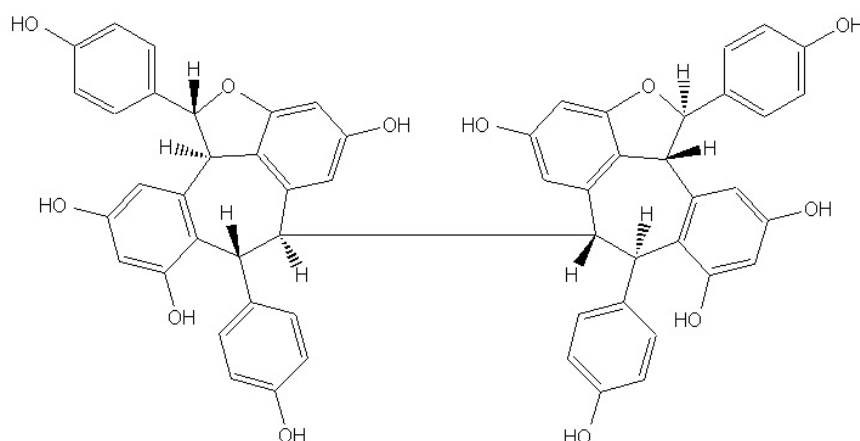


Fig. 3: The Structure of Hopeaphenol

Table 1: The comparison of UV Spectra data between the compound isolated and standard hopeaphenol

Isolated Compound		Standard hopeaphenol	
λ_{max} (MeOH)	λ_{max} (NaOH+MeOH)	λ_{max} (MeOH)	λ_{max} (NaOH+MeOH)
203 nm	205 nm	203 nm	206 nm
231 nm	250 nm	228 nm	251 nm
280 nm	281 nm	283 nm	285 nm

Table 2: The comparison of IR spectra data between the isolated compound and standard hopeaphenol

No.	Vibration	Isolated compound (cm ⁻¹)	Standard hopeaphenol (cm ⁻¹)
1	OH Strech	3335	3340
2	-CH aliphatic	2910	2912
3	C=C aromatic	1615,1600, 1516,1456	1614.9, 1600.4, 1515.4,1456
4	1,4 disubstituted benzene	832.8	833.4

Table 3: The comparison of physical data between the isolated compound and standard hopeaphenol

Physical data	Isolated compound	Standard hopeaphenol
Physical character	Light yellow powder	Light yellow powder
Melting point	276-278°C (decomposed)	290-292°C (decomposed)
$[\alpha]_D^{20}$ (c 0,1 MeOH)	-313°	-420°

Conclusion

Hopeaphenol, a tetramer stilbene, has successfully been isolated from *Shorea ovalis* Blume. From chemosystemic point of view, *S. ovalis* Blume has also produced the oligomeric stilbene similar to other species of *Shorea*. Thus we have clarified the close relationship of *S. ovalis* Blume with other *Shorea* genus and this report also provides another new compound obtained from *S. ovalis* Blume.

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