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4′,5′-Dihydroxy-epiisocatalponol, a new naphthoquinone from *Tectona grandis* L. f. heartwood, and fungicidal activity

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ABSTRACT

A new naphthoquinone derivative was isolated from the heartwood of the teak stem. The chemical structure of this new compound, 4′,5′-dihydroxy-epiisocatalponol, was determined using 1-D and 2-D nuclear magnetic resonance experiments, vibrational circular dichroism, HRMS, and optical rotation. We showed that this new naphthoquinone derivative plays a key role in the variability of decay resistance in teak wood. A high negative correlation was found between its concentration and the mass losses of the wood samples after exposure to the brown rot *Antrodia* sp., the fungus that is the most virulent against teak ($R = -0.9$; $\rho < 0.0001$). In-vitro bioassays allowed us to demonstrate that 4′,5′-dihydroxy-epiisocatalponol acted as a fungicide against *Trametes versicolor* (white rot) at 58 mg ml^{-1} (0.22 mM). Overall, our results demonstrated that the concentration of 4′,5′-dihydroxy-epiisocatalponol could be used as a new tool to evaluate teak wood durability.

1. Introduction

Teak (*Tectona grandis* Linn. f.) is a highly valuable timber because of its texture, aesthetics, and high natural durability (Krishnapillay, 2000). It has been selected as the most promising species for sustainable high-quality timber production (Bhat and Priya, 2004). For economic reasons, teak plantations are managed using short rotations that induce intraspecific variability in the teak wood properties, such as colour and natural durability (Kaosa-ard, 2000; Bhat and Florence, 2003; Kokutse et al., 2006). Natural durability, or decay resistance, is an important factor for the environmentally friendly use of exterior timber. The natural durability of teak is ascribed to its extractives (Asamoah and Antwi-Boasiako, 2007), and the identification and quantification of active compounds in teak would be useful for the fast and early evaluation of its natural durability.

Teak has been reported to contain lapachol (Lukmandaru and Takahashi, 2009), 1-hydroxy-2-methyl-anthraquinone, 2-(hydroxymethyl) anthraquinone (Windeisen et al., 2003), tectoquinone (Sumthong et al., 2008; Lukmandaru and Takahashi, 2009),

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pachybasin (Sumthong, 2007), dehydrotectol, tectol (Lukmandaru and Takahashi, 2009), dehydro-α-lapachone, 2-methylquinizarin (Khan and Mlungwana, 1999), deoxylapachol (Windeisen et al., 2003; Sumthong et al., 2008), isodeoxylapachol (Sumthong et al., 2008), 1,4-naphthoquinone (Thulasidas and Bhat, 2007), β-sitosterol (Lukmandaru and Ogiyama, 2005), obtusifolin (Sumthong et al., 2008), squalene (Windeisen et al., 2003; Lukmandaru and Takahashi, 2009), betulinic acid, ferulic acid, gallic acid, caffeic acid (Shalini and Srivastava, 2009; Jaybhaye et al., 2010), and rubber (Sandermann and Simatupang, 1966; Yamamoto et al., 1998).

Both anthraquinones and naphthoquinones are involved in the resistance of teak wood to insects and fungi (Rudman and Gay, 1961; Sumthong et al., 2006). Naphthoquinones, however, are believed to be more toxic against fungi than are anthraquinones (Sumthong et al., 2008). They are also widespread and found in many vascular plant families, such as Droseraceae, Juglandaceae, Nepenthaceae, Plumbaginaceae (Duroux et al., 1998; Babula et al., 2009), and Verbenaceae (Santos et al., 2003). Naphthoquinones and their derivatives exhibit antimicrobial, antibacterial, fungicidal, cytotoxic, antiparasitic, insecticidal, and anti-ulcerogenic activities (Khan and Mlungwana, 1999; Babula et al., 2009).

Our previous studies showed that two new compounds (a hydroxycinnamic acid derivative and P1, an unidentified compound) are related to the heartwood formation process in Malaysian teak wood (Niamké et al., 2011). The hydroxycinnamic derivative was only detected in the sapwood, while P1 was detected exclusively in the heartwood. Moreover, P1 was suspected as one of the biochemical compounds responsible for the decay resistance of teak heartwood without any indication of its chemical structure and biological activities. The present paper focuses on the role of P1 through the study of correlation between mass losses after decay exposure to *Antrodia* sp. and content of P1; the fungicidal activity of P1, which has been identified as a new naphthoquinone derivative isolated from teak heartwood.

2. Materials and methods

2.1. Plant material

Six 5.5–10-yr-old teak trees were harvested in 2004 from the Luasong Forestry Center and the provenance–progeny trial of Taliwas located in Tawau, Sabah, Malaysia, both of which areas were managed by the Yayasan Sabah Group. From each tree, a diametral plank was collected, and two transversal wood slices were taken. The transversal wood slices were air-dried and conditioned in a climate-controlled room (humidity: 65 ± 5%, temperature: 20 ± 1 °C), which led to a 12% wood moisture content. Wood samples were taken from the outer heartwood. For the decay tests, a long slice of the outer heartwood was used to obtain 12 wood samples (25, 15, and 50 mm in radial, transverse, and longitudinal dimensions, respectively), six for the decay test and six for the initial moisture content determination. All of the wood samples were conditioned in a climate-controlled room (relative humidity: 65 ± 5%, temperature: 20 ± 1 °C) again before use.

2.2. Natural decay resistance test

Natural durability bioassays against *Antrodia* sp., strain CTFT 57 A, were assessed according to the European standards EN 350-1 (Afnor, 1994) and EN 113 (Afnor, 1996). This brown-rot fungus was found to be the most virulent against teak heartwood (Kokutse et al., 2006). For each tree, six wood samples from the outer heartwood (a total of 36 samples) were weighed (*M*1), sterilised (gamma ray), and then exposed to the fungus under controlled conditions (21 °C and 70% relative humidity). Ten additional sapwood samples (25, 15, and 50 mm for radial, transverse, and longitudinal dimensions, respectively) from *Pinus sylvestris* were used to control the fungal virulence. After 16 wk of exposure, the mycelia were removed from the wood blocks, and the samples were dried at 103 °C until a constant mass was obtained to determine the oven-dry mass (*M0f*). Six wood samples were heated to 103 °C for 24 h, and their mean moisture content (MC) was calculated and used to calculate the theoretical initial dry mass (*M0i*) of each wood sample. The percentage mass loss (ML) based on the dry weight (*M0i*) was calculated according to Eqs. (1) and (2):

$$M0i = M1 \times 100/(100 + \mathrm{MC}) \quad (1)$$

$$\mathrm{ML} = 100 \times (M0i - M0f)/M0i \quad (2)$$

The mean mass loss was used to determine the natural durability class for each tree. The mean mass loss of the pine sapwood samples was 28%, which was higher than the 20% mass loss recommended by the European standard EN 350-1 (Afnor, 1994). This result validated the decay test, and the relative mass losses (RMLs) were calculated using the following equation: RML = ML/28.

2.3. Extraction and HPLC analysis

The wood samples used for chemical analysis were ground, extracted, and analysed by HPLC according to the method described by Niamké et al. (2011). The content of each compound is expressed as μmol per gram dry weight.

2.4. P1 extraction and isolation

The P1 was isolated from 39.3 g of dried teak heartwood meals (a mixture of meals from the six teak heartwood samples harvested in Malaysia; see section on plant material above). Extraction was conducted using 500 ml of acetone/water (80:20, v/v) at 4 °C. The acetone was evaporated (Buchi R-215 rotary evaporator coupled to a V 850 vacuum controller; Rungis, France), the remaining aqueous phase was lyophilised (Bioblock Christ Alpha 1–2, Paris, France), and 3.9 g of the residue was dissolved in 25 ml of pure methanol. The solution (0.156 g ml^{-1}) was diluted four times and fractionated by preparative HPLC coupled to a fraction collector (Agilent Technologies 1200 series, Paris, France). The separation and collection of the fractions containing P1 were accomplished using a semi-preparative HPLC with a reverse 5-μm C18 column SA (AB250SP1), 250 × 10 mm (Cluzeau, Paris, France). The semi-preparative HPLC conditions were as follows: mobile phase of solvent A = water/acetic acid (99:1, v/v) and solvent B = methanol/acetonitrile (1:1 v/v); elution gradient of 0–13 min with 30% solvent B, 14–15 min with 30–100% B, 16–25 min with 100% B, and 26–28 min with 30% B. The flow rate was set to 2.5 ml min^{-1}, with a maximum pressure of 250 bars and detection at 254 nm. The injection volume was 500 μl of the crude methanolic extract. The P1 was collected at 9.7 min.

2.5. Experimental procedure for P1 characterisation

The identification of P1 was confirmed by both MS and NMR. HPLC–MS-ESI was used to confirm the chemical structures of P1 in this study. LC/MS experiments were conducted in the Laboratoire des Mesures Physiques at Montpellier 2 University using a Water micromass Q-TOF apparatus (Paris, France) equipped with a DAD detector Waters 996 (Paris, France) and one separation module on a C18 Thermo Scientific (Saint Herblain, France) (50 × 2.1 mm, 5-μm pore size) column. The mobile phase consisted of (A) water and formic acid (98:2, v/v), and (B) water, acetonitrile, and formic

acid (18:80:2, v/v). The flow rate was 0.2 mL min^{-1}, and the gradient went from 0% to 90% (B) over 30 min. The full-mass scan spectrum of m/z 0 to 1000 was collected. All mass spectrometry data were acquired using a positive ionisation mode.

NMR experiments were performed on a Varian 400 MHz spectrometer (Varian NMR Instruments, Paris, France) operating at 400 MHz for ^{1}H and 160 MHz for ^{13}C. All spectra were recorded at 25 °C in deuterated acetone. The chemical shifts (δ) are given in parts per million downfield from tetramethylsilane and the coupling constants, J, are in hertz. These spectra were processed and analysed using the ACD/NMR Processor Academic Edition.

The IR and VCD spectra were recorded using a Fourier Bruker Vertex 70 spectrophotometer (Wissembourg, France) coupled to a PMA50. The analytic conditions used were a temperature of 25 °C and room humidity of 18%. The sample was dissolved in CD_3OD, and 12,000 scans were recorded over 3 h for each spectrum with a resolution of 4 cm^{-1}.

Chemical description of (3R, 4R)-4′,5′-dihydroxy-epi-isocatalponol: Slightly brownish product; $[\alpha]_D^{20} = +8.5$ ($c = 0.6$, H_2O); IR ν_{max}: 1675 cm^{-1} (α, β-conjugated C=O) and 1588 cm^{-1} (C=C). ^{1}H and ^{13}C NMR data, see Table 1. ESMS (MeOH, 271 eV) m/z (rel. Int.): 245 $[M + 1 - H_2O]^+$ (68) and 227 $[M + 1 - 2H_2O]^+$ (76); HRMS (MeOH, 20 V) m/z 269.1360 $[M + Li]^+$ (calc. 263.1205).

2.6. Antifungal assay

One white-rot fungus [*Trametes versicolor* (Linnaeus) Quelet (CYB 863-A) *sp.*] was used. The antifungal assays were performed using a broth microdilution (Hadacek and Harald, 2000) with sterile, disposable microtitre plates with 96 U-bottomed wells (Elisa type from Corning Inc., Corning, NY, USA). The unknown compound, P1, was dissolved in water and mixed with 40 g l^{-1} of the malt extract broth. Stock solutions were used at varying concentrations. The commercial biocide tebuconazole [(3*RS*)-5-(4-chlorophenyl)-2,2-dimethyl-3-1*H*-[1,2,4-triazole] methyl-3-pentanol] was used as the positive control (0.14 μM), and water as the negative control. The plates were incubated at 21 °C and 70% humidity. After 72 h, inoculums were transferred to plastic petri dishes (90 cm, Greiner, France) filled with a malt/agar culture medium (40 g l^{-1} malt and 20 g l^{-1} agar). The radial growth rate of the mycelia was monitored for 11 days and evaluated every two days by measuring the diameter growth of the colony in the petri dishes.

$$\text{Radial growth rate} = \text{mycelia growth (cm)} \times 100/90(\text{diameter petri dish in cm}) \quad (3)$$

If mycelia growth is 0 then there is an inhibition of the growth; if mycelia growth is 90 cm then there is no inhibition of the growth.

The fungal toxicity, expressed as the inhibition activity on the mycelial growth, was calculated versus the negative control. The fungal growth inhibition (antifungal index, or AI) was determined as follows:

$$AI = 100 - ((Dr - De) \times 100/Dr) \quad (4)$$

where Dr is the diameter of mycelia in the reference petri dish, and De is this diameter in the test petri dishes (in millimetres). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of P1 that entirely inhibited mycelial growth. The concentration that inhibited 50% of the mycelial growth (IC_{50}) was determined using the graphic fit curve for the dose/response data from all inhibitory activity tests.

2.7. Other statistical analysis

The XLSTAT software package (Paris, France) was used for the logarithmic, nonparametric Spearman correlation between the P1 content and mass loss because of the absence of normality. The non-parametric Mann Whitney test was used to compare both the concentrations and inhibitory effects of P1. Values were considered to be statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Structural identification of P1

The P1 (0.61%; w/w) was isolated as a slightly brownish product, and UV analysis (MeOH) yielded a spectrum with the following peaks: 249 and 289 nm. The specific rotation was measured as $[\alpha]_D^{20} = +8.5$ ($c = 0.6$, H_2O), and mass spectrometry revealed an m/z of 263 ($[M]^+$, 100%) with additional fragment ions at m/z of 245 $[M + 1 - H_2O]^+$ (68) and 227 $[M + 1 - 2H_2O]^+$ (76). High-resolution mass spectrometry analysis yielded an m/z of 262.1205, thereby indicating a product formula of $C_{15}H_{18}O_4$ with $C_{15}H_{16}O_3$ and $C_{15}H_{14}O_2$ for the first and second fragment ions, respectively. In the IR spectrum, this material showed absorptions at 1675 cm^{-1}

Table 1
^{1}H NMR, ^{13}C NMR, COSY, NOESY, and HMBC ($^{1}H \rightarrow {}^{13}C$) data for 4′,5′-dihydroxy-epiisocatalponol (P1).

	^{1}H	^{13}C	COSY[a]	NOESY	HMBC ($^{1}H \rightarrow {}^{13}C$)[a]
1		198.9			
2	a: 2.49 (1H, dd, $J = 17.3, 3.8$ Hz)	40.8	H2b, H3, *H4*	H2b, H3	C1, C3, C4, *C8a*
	b: 2.77 (1H, dd, $J = 17.3, 11.4$ Hz)		H2b, H3	H2a	C1, C3, C4, C1′
3	2.30 (1H, m)	42.1	H2a, H2b, H4, H1′a, H1′b	H4, H2b, H1′b, H2′	C2
4	4.83 (1H, d, $J = 2.8$ Hz)	69.8	*H2a*, H3	H3, H5, H1′b, H2′	C2, C5, *C8a*
4a		147.1			
5	7.51 (1H, d br, $J = 7.5$ Hz)	131.3	H6	H4, H6	C4, C7, C8a
6	7.59 (1H, td, $J = 7.5, 1.4$ Hz)	135.5	H5, H7, *H8*	H5, H7	C4a, C8
7	7.41 (1H, td, $J = 7.6, 1.3$ Hz)	130.0	H6, H8	H6, H8	*C4a*, C5, *C8*, C8a
8	7.86 (1H, dd, $J = 7.8, 1.1$ Hz)	128.0	*H6*, H7	H7	C6, C4a, C1
8a		133.4			
1′	a: 2.14 (1H, dt, $J = 14.0, 7.1$ Hz)	30.8	H3, H1′b, H2′, *H5′*	H1′b, H4′b	C2, C3, C4, C2′,C3′
	b: 2.52 (1H, dt, $J = 14.0, 8.0$ Hz)		H3, H1′a, H2′	H3, H4, H1′a, H4′b	C2, C3, C4, C2′, C3′
2′	5.61 (1H, t br, $J = 7.7$ Hz)	126.8	H1′a, H1′b, H5′	H3, H1′a, H1′b, H5′	*C3*, *C1′*, C4′, C5′
3′		143.3			
4′	a: 4.09 (1H, d, $J = 12.0$ Hz)	59.6	H4′b		C3′, C2′, C5′
	b: 4.19 (1H, d, $J = 12.0$ Hz)		H4′a	H1′a, H1′b	C3′, C2′, C5′
5′	4.10 (2H, s br)	66.8	*H1′a*, H2′	H2′	C3′, C2′, C4′

[a] Low interactions are noted in italics.

(α, β-conjugated C=O) and 1588 cm^{-1} (C=C). The ^{1}H NMR, ^{13}C NMR, gCOSY, NOESY, gHSQCAD, and gHMBCAD data are described in Table 1. These data are reported in parts per million downfield from tetramethylsilane. The coupling constants are in hertz, and s stands for singlet, d for doublet, t for triplet, m for multiplet, and br for broad. Hydrogen connectivity (C, CH, CH_2, and CH_3) information was obtained from the edited HSQC experiments.

Based on these spectral data, it was ascertained that P1 is 4′,5′-dihydroxy-epiisocatalponol (Fig. 1A) as follows: ^{1}H NMR exhibits four aromatic proton signals for H-5 (δ 7.51), H-6 (δ 7.59), H-7 (δ 7.41), and H-8 (δ 7.86), which is characteristic of a 1,2-disubstituted aromatic ring. $^{1}H-^{13}C$ long distance correlations with these four aromatic protons allow us to unambiguously assign the two quaternary carbon atoms, C-4a (δ 147.1) and C-8a (δ 133.4). The ^{3}J coupling constants between the two H-2 protons and the neighbouring H-3 proton indicate that the H-2 at δ 2.49 ($^{3}J = 3.8$ Hz) is in the equatorial position, while both the H-2 at δ 2.77 ($^{3}J = 11.4$ Hz) and H-3 are in axial positions. Because H-3 is axial, its coupling constant with H-4 (δ 4.83) indicates that the latter is equatorial, while the related hydroxyl group is axial. Long-distance correlations between the H-2 protons and C-1 (δ 198.9) permitted us to link the two rings and place the ketone moiety. The structure was further ascertained by the observation of long-range $^{1}H-^{13}C$ correlations of H-2a to C-8a, H-4 to C-5, and H-8 to C-1. The COSY correlation between H-1′ and H-3 indicated that the lateral chain was attached to the rings at C-3. The same experiment showed that the H-1′ protons are also coupled to the vinyl proton H-2′, which appeared as a broad triplet with quasi equivalent coupling constants for both H-1′ protons. This vinyl proton did not couple any other vinyl proton, which indicates the double bond was trisubstituted. The quaternary carbon C-3′, at δ 143.3, was identified via its long-range correlation to the H-1′ protons. Finally, it was discovered that C-3′ bore two CH_2OH groups, one with two equivalent protons at δ 4.10 and the other with two separate protons at δ 4.09 and 4.19. NOESY correlations between H-4′ and H-1′ as well as H-5′ and H-2′ indicated that C-4′ and C-5′ were *cis* relative to C-1′ and H-2′, respectively (Fig. 1B).

Further evidence was sought to ascertain the cis relationship between H-3 and H-4. Using molecular mechanics (Chem3D® Ultra v10.0), the H3–C3–C4–H4 dihedral angle was estimated to be 59° for 4′,5′-dihydroxy-epiisocatalponol and 172° for 4′,5′-dihydroxy-isocatalponol (Fig. 2). In our case, the coupling constant between H-3 and H-4 was 2.8 Hz, which does not correspond to a dihedral angle near either 0 or 180°, according to the Karplus equation, and 59° is, therefore, more likely than 172°. In addition, the H-3–H-4 coupling constant ($J = 2.8$ Hz) more closely corresponds to that reported for epiisocatalponol ($J = 2.4$ Hz) and its 4′-hydroxylated analogue (Kizu et al., 1994) than for isocatalponol ($J = 7.0$ Hz) (Inoue et al., 1980; Park and Lim, 1996). Finally, we observed an NOE interaction between H-3 and H-4 that further indicated these two protons were in the cis configuration (Fig. 1B), which implies the hydroxyl and 4′,5′-dihydroxy-prenyl substituents were cis as well.

The absolute configuration of this molecule was established by VCD analysis, and the original report is given in the supplementary material. The theoretical conformational study has shown that four conformations are significantly populated. These four conformations were used to calculate the mean IR and VCD spectra. Although intermolecular hydrogen bonds between the solute and solvent were neglected for the spectral calculations, comparing them to the measured spectra established a satisfactory correlation for 15 bands, therefore allowing us to determine that the absolute configuration of compound P1 is 3R, 4R. It is important to note, however, that this result could be vitiated by the increased risk from the presence of intermolecular hydrogen bonds that may significantly affect the VCD spectrum and partially distort the interpretation.

3.2. Relationship between P1 concentration and the decay resistance of teak wood

The compound P1, identified as 4′,5′-dihydroxy-epiisocatalponol, represents one of the primary compounds that accumulates in teak heartwood (Niamké et al., 2011), although its concentration varies strongly between trees ($U = 18.89$; $\rho < 0.0001$). The highest content (44 μmol g^{-1} dw) was found in tree 1, which provided the heartwood with the highest decay resistance (0.1 relative mass loss, class 1 = very durable) as estimated by the relative mass losses of wood samples caused by Antrodia sp. (brown rot) according to the European standards EN-350-1 (Afnor, 1994) and EN 113 (Afnor, 1996). The choice of a brown rot is due to the fact that teak heartwood is less resistant against brown rot than white rot, so it is easier to highlight the role of the extractives in wood-decay resistance (Kokutse, 2002). Conversely, the lowest decay resistance, found for trees 5 and 6 (0.5 and 0.6 relative mass loss, respectively, class 3 = moderately durable), was associated with low P1 concentrations (1.4 μmol g^{-1} dw; Fig. 3). Therefore, it was clearly shown that teak wood decay resistance was correlated to the P1 content of heartwood ($r2 = 0.8$). A high Spearman negative correlation was found between the P1 content and relative mass loss of the wood samples ($R = -0.9$; $\rho < 0.0001$). The P1 content decreased with increasing relative mass losses after fungal decay. Similar results were observed for roburine D for the decay resistance of oak against *Coriolus versicolor* (Guilley et al., 2004) and for acetone extract, which explain the resistance of *Larix* sp. against *Poria placenta* (Gierlinger et al., 2004). The high correlation between the decay

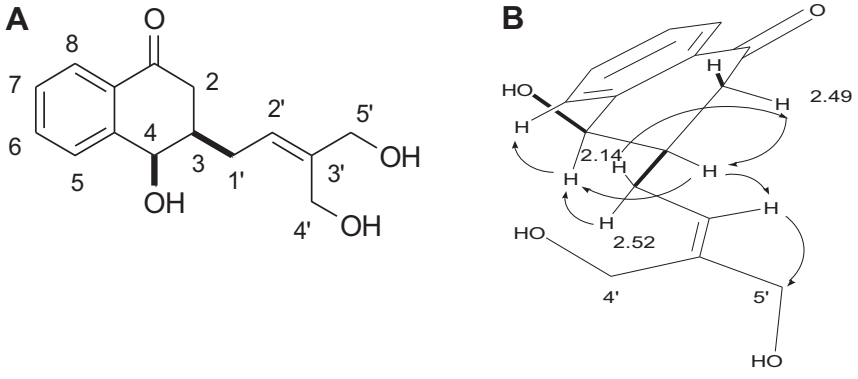

Fig. 1. Compound P1 (4′,5′-dihydroxy-epiisocatalponol (A) and relevant NOE correlations in P1 (B).

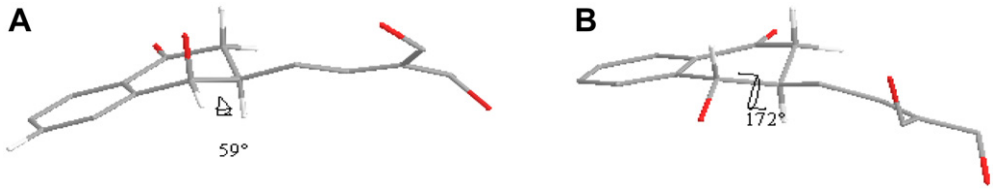

Fig. 2. Mechanical molecular assessment of the H4–C4–C3–H3 dihedral angle for the two epimers 4′,5′-dihydroxy-epiisocatalponol (P1, A) and 4′,5′-dihydroxyisocatalponol (B).

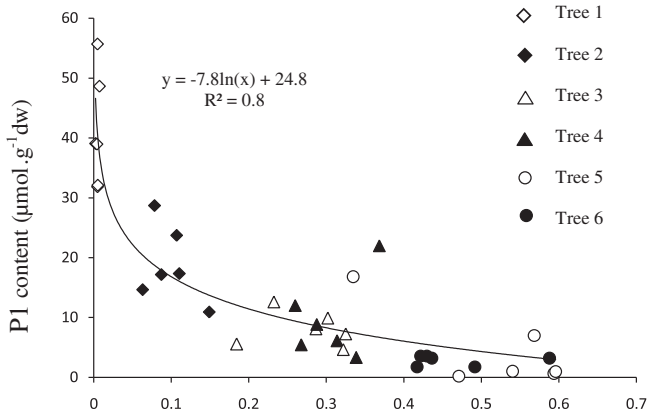

Fig. 3. The relationship between P1 (4′,5′-dihydroxy-epiisocatalponol) content and the relative mass loss of the heartwood samples in six Malaysian teak (*Tectona grandis* L. f.) trees. $n = 6$.

resistance and P1 concentration indicates that this compound is involved in the decay resistances of teak as previously postulated (Niamké et al., 2011).

3.3. Fungicidal activity of P1

4′,5′-Dihydroxy-epiisocatalponol (P1) was assessed for its fungicidal activity against the white rot *T. versicolor* using a broth stock solution method. Sumthong (2007) showed a fungicidal activity of teak extractives against white and brown rot, although the degradation mechanisms are not the same. Brown rot can degrade cellulose and hemicelluloses while white rot produces peroxidases and laccases, which degrade cellulose, hemicelluloses, lignin, and extractives. So, in a first step, we decided to test the bioactivity of P1 against *T. versicolor*. The total test duration was 14 days. Under these conditions, P1 inhibited the growth of *T. versicolor* (minimum inhibitory concentration, MIC = 0.22 mM; IC_{50} = 0.11 mM; Fig. 4), and its fungicidal activity threshold was found to be between 0.11 and 0.22 mM. The fungicidal activity of P1 at the MIC was significantly different ($U = 3.5$, $\rho < 0.003$) than at lower concentrations (0.11–0.03 mM) and for the negative control ($U = 10.5$, $\rho < 0.025$). These results confirm that P1 was toxic, as was observed for catalponol derivatives from the genus *Catalpa* in the Bignoniaceae family (Park et al., 2010). Further tests will be carried out in order to confirm the bioactivity against brown rot.

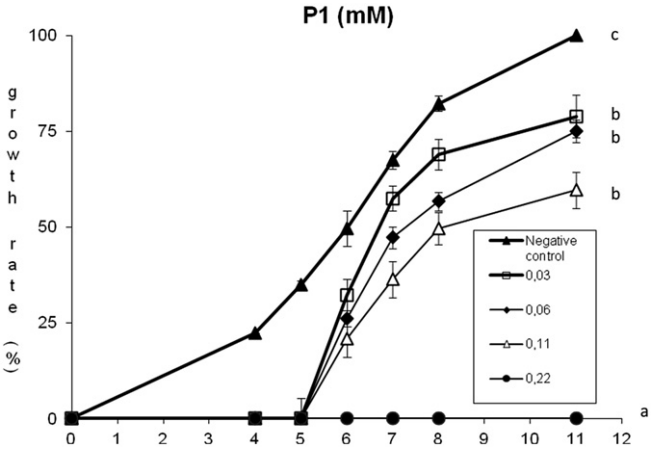

Fig. 4. The growth rate of *Trametes versicolor* inhibited by P1 (4′,5′-dihydroxy-epiisocatalponol), which had been isolated from teak heartwood and tested in agar petri dishes at different concentrations (expressed in mM) during a period of 11 days. The positive concentration is 0.14 µM. Concentrations with different letters are significantly different at $p < 0.5$.

4. Conclusion

4′,5′-Dihydroxy-epiisocatalponol (P1) is a new naphthoquinone derivative isolated from teak heartwood that was characterised and tested for fungicidal activity. The concentration of P1 in heartwood was correlated to the decay resistance of the teak wood ($R = 0.9$; $\rho < 0.0001$) with higher resistance for wood with higher P1 content. In-vitro experiments showed that this compound displayed antifungal properties, with an MIC of 0.22 mM and half minimal inhibitory concentration, IC_{50}, of 0.11 mM. Therefore, our study demonstrated that measuring the P1 content could be used for the rapid pre-evaluation of teak wood's natural durability, and high-P1-content trees should be selected to improve the teak wood on plantations.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ibiod.2012.03.010.

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