Bioactive Oligostilbenoids from the Stem Bark of *Hopea exalata*

Hui Ming Ge, Bo Huang, Shu Hua Tan, Da Hua Shi, Yong Chun Song, and Ren Xiang Tan*

Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

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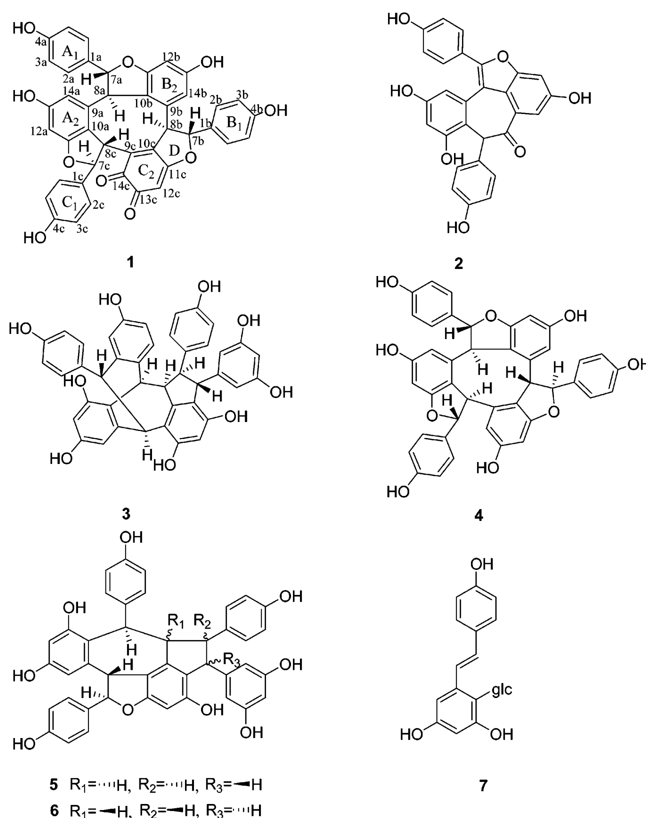
Hopeanolin (**1**), an unusual resveratral trimer with an *ortho*-quinone nucleus, was isolated and characterized from the stem bark of *Hopea exalata*. Also obtained were six known stibenoids, shoreaphenol (**2**), vaticanol G (**3**), α-viniferin (**4**), pauciflorol A (**5**), vaticanol A (**6**), and *trans*-3,5,4′-trihydroxystilbene 2-*C*-glucoside (**7**). The structure of **1** was determined by spectroscopic data interpretation. Compounds **1**−**7** were tested for antifungal activity and inhibitory effects against jack bean urease. Hopeanolin (**1**) demonstrated antifungal activity in the MIC value range 0.1−22.5 μg/mL.

Plants of the family Dipterocapaceae have been shown to be a rich source of resveratrol (3,5,4′-trihydroxystibene) oligomers,[1−4] which are receiving considerable chemical and biological attention owing to their structural complexity as well as their array of bioactivities exhibited such as antifungal,[5,6] anti-HIV,[7] cytotoxic,[8,9] anti-inflammatory,[10] and antibacterial effects.[11] In particular, species in the genus *Hopea* seem to be abundant in oligostilbenoids.[3,7,12] *Hopea exalata* Lin. Yang et Hsue is a woody tree widespread in Hainan Island, People's Republic of China. It is quite competitive in terms of its resistance to the phytopathogens and pests that feed on the other plants growing nearby. The successful ecological adaptation of this species, plus the fact that it has not been studied phytochemically previously, suggested the present investigation. This has led to the characterization of a new antimicrobial resveratrol trimer, hopeanolin (**1**, the first resveratral oligomer with an *ortho*-quinone nucleus), which was found to occur with six known congeners identified as shoreaphenol (**2**),[13] vaticanol G (**3**),[14] α-viniferin (**4**),[15] pauciflorol A (**5**),[16] vaticanol A (**6**),[17] and *trans*-3,5,4′-trihydroxystilbene 2-*C*-glucoside (**7**).[18] Vaticanols A and G have shown antibabesial activity against *Babesia gibsoni*,[19] while α-viniferin has exhibited anti-inflammatory[20] and antifungal effects.[15]



Compound **1** was afforded as a dark yellow, amorphous powder. The polyphenolic nature of **1** was evidenced initially from its UV maxima centered at 233 and 280 nm as well as its IR absorption bands at 3258 (OH), and 1613 and 1517 cm^{-1} (phenyl). Its negative-mode HRESIMS gave a $[M - H]^-$ ion peak at *m/z* 691.1603, indicating that the molecular formula of **1** is $C_{42}H_{28}O_{10}$. These data suggested that compound **1** could be a resveratrol trimer. This assumption was reinforced by the ^1H and ^{13}C NMR data, unambiguously assigned by the interpretation of its $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra (Table 1). Three mutually coupled aliphatic proton pairs at δ_H 5.98/4.75 (H-7a/H-8a), 6.20/3.97 (H-7b/H-8b), and 5.56/4.68 (H-7c/H-8c) were used to assign the six inter-phenyl aliphatic protons of the three resveratrol units. However, only a total of five aromatic rings, with three (rings A_1−A_3) and two (rings B_1−B_2) being 1,4-di- and 1,2,3,5-tetrasubstituted, respectively, could be allocated by the observed chemical shifts and the splitting pattern of the aromatic protons, in conjunction with the five discrete OH singlets between δ_H 8.4 and 8.8. This observation, along with the resonance of an olefinic proton at δ_H 5.80 (H-10c) and two ketone signals at δ_C 180.4 and 177.4, demonstrated that the third 1,2,3,5-tetrasubstituted phenyl group is oxidized in **1** to a 3,4,5-trisubstituted *ortho*-quinone moiety. The planar structure of **1** was generated by the subsequent connection of the aforementioned structural units, mainly by its HMBC and NOESY spectra. The connections proposed for the nonoxidized two and a half resveratrol units were based on the HMBC correlations of H-7a with C-2a/6a, C-9a, and C-11b, of H-8a with C-1a, C-10a, C-14a, and C-11b, of H-7b with C-2b/6b and C-9b, of H-8b with C-1b, C-10b, and C-14b, of H-7c with C-2c/6c, C-10a, and C-11a, and of H-8c with C-1c, C-9a, and C-11a. This was confirmed by the anticipated NOE correlations observed between the proton pairs H-8a/H-2a(6a), H-8b/H-2b(6b), and H-8c/H-2c(6c). Furthermore, a fragment, C_6HO_3, and seven indices of unsaturation were evident by subtracting these two and a half resveratrol units from the molecular formula. The HMBC correlation (Figure S3, Supporting Information) of H-8c (δ_H 4.68) with C-10c (δ_C 148.1) and C-14c (δ_C 180.1) and of H-12c (δ_H 5.80) with C-10c, C-11c (δ_C 171.4), and C-14c suggested again the presence of an *ortho*-quinone moiety (ring C_2, bearing five indices of unsaturation). The two remaining unsaturation indices could only be utilized by proposing two more rings for the molecule of **1**. The observed HMBC correlations of

* Corresponding author. Tel: +86-25-8359 2945. Fax: +86-25-8330 2728. E-mail: rxtan@nju.edu.cn.

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Table 1. NMR Spectroscopic Data of Compound **1**[a]

position	δ_C	δ_H (mult., *J* Hz)	$^1H-^{13}C$ HMBC	$^1H-^1H$ NOESY
1a	130.6			
2a, 6a	128.9	7.32 (d, 8.6)	1a, 4a, 7a	3a(5a), 8a
3a, 5a	116.1	6.75 (d, 8.6)	1a, 4a	OH-4a, 2a(6a)
4a	158.6			
7a	90.0	5.98 (d, 12.0)	2a(6a), 8a, 9a, 11b	14a
8a	52.8	4.75 (d, 12.0)	1a, 7a, 9a, 10a, 14a, 10b, 11b	2a(6a), 8b
9a	137.6			
10a	118.0			
11a	162.8			
12a	97.6	6.29 (d, 1.8)	10a, 13a, 14a,	OH-13a
13a	161.6			
14a	106.2	6.69 (d, 1.8)	8a, 10a, 12a, 13a	OH-13a, 7a
1b	134.6			
2b, 6b	129.2	7.19 (d, 8.5)	1b, 3b(5b), 4b, 7b	3b(5b), 8b
3b, 5b	116.4	6.81 (d, 8.5)	1b, 4b	2b(6b), OH-4b
4b	159.0			
7b	88.9	6.20 (brs)	1b, 2b(6b), 8b, 9b, 10c, 11c,	14b
8b	44.7	3.97 (brs)	1b, 9b, 10b, 14b, 9c, 10c, 11c	8a, 2b(6b)
9b	139.8			
10b	122.1			
11b	161.0			
12b	97.8	6.32 (d, 1.8)	10b, 13b, 14b	OH-13b
13b	159.9			
14b	106.5	6.54 (d, 1.8)	8b, 10b, 12b, 13b	7b, OH-13b
1c	133.8			
2c, 6c	128.0	7.26 (d, 8.5)	4c, 7c	3c(5c), 8c
3c, 5c	115.9	6.75 (d, 8.5)	1c, 4c	2c(6c), OH-4c
4c	158.2			
7c	91.2	5.56 (d, 2.2)	10a, 11a, 1c, 2c(6c), 8c	
8c	50.2	4.68 (d, 2.2)	9a, 10a, 11a, 1c, 7c, 10c, 14c	2c(6c)
9c	129.5			
10c	148.1			
11c	171.4			
12c	101.7	5.80 (s)	10c, 11c, 14c	
13c	177.4			
14c	180.4			
OH-4a		8.52 (brs)	3a(5a), 4a	3a(5a)
OH-13a		8.78 (brs)	12a, 13a, 14a	12a, 14a
OH-4b		8.71 (brs)	3b(5b), 4b	3b(5b)
OH-13b		8.62 (brs)	12b, 13b, 14b	12b, 14b
OH-4c		8.44 (brs)	3c(5c), 4c	3c(5c)

[a] Measured in acetone-d_6 at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR).

Table 2. MIC Values (μg/mL) of Compounds **1**−**7** against a Panel of Pathogenic Fungi

compound	*Alternaria attenata*	*Alternaria solani*	*Colletotrichum lagenarium*	*Fusarium oxysporum* f. sp. *vasinfectum*	*Pyricularia oryzae*	*Valsa mali*
1	22.5	1.56	10.6	6.22	0.10	1.55
2	—[b]	—[b]	—[b]	—[b]	27.3	7.13
3	—[b]	24.6	—[b]	—[b]	0.78	—[b]
4	—[b]	3.12	12.5	25.2	6.25	—[b]
5	1.56	—[b]	—[b]	—[b]	11.5	—[b]
6	—[b]	6.25	—[b]	12.7	3.12	20.1
7	—[b]	—[b]	—[b]	—[b]	22.3	6.25
ketoconazole[a]	1.04	0.83	0.97	2.21	0.03	0.51

[a] Used as a positive control. [b]Value >50 μg/mL.

H-7b with C-10c and C-11 and of H-8b with C-10c and C-11c together with the downfield chemical shifts of a tertiary carbon C-7b (δ_C 88.9) and an olefinic carbon C-11c (δ_C 171.4) were used to deduce the presence of a furan ring (ring D), which also led to the formation of a cyclononane ring.

The relative configuration of **1** was determined from its NOESY spectrum. The *trans* orientations of H-7a/H-8a, H-7b/H-8b, and H-7c/H-8c at the dihydrofuran rings were established by the distinctive NOEs between H-8a/H-2a(6a), H-8b/H-2b(6b), and H-8c/H-2c(6c).[1,2] H-8a and H-8b could be proposed as having a *cis* orientation to one another from the observation of a mutual cross-peak in the NOESY spectrum, whereas H-8c and H-8a and H-8c and H-8b were assigned as *trans* due to the lack of any observed NOE correlations between them.

Biosynthetically, hopeanolin (**1**) may originate from a precursor, miyabenol C, a metabolite of *Dipterocarpus grandiflorus* (Dipterocapaceae),[21] via a set of sequential bioreactions (Scheme S1, Supporting Information).

Compounds **1**−**7** were tested against a small panel of pathogenic fungi (Table 2). Compound **1** showed potent activity against six types of pathogenic fungi with MIC values in the range 0.10−22.5 μg/mL. These compounds were also tested for their inhibitory effects against jack bean urease. Only compound **2** exhibited inhibitory activity with an IC_{50} of 126.8 $\pm$ 2.73 μM, compared with the IC_{50} value of the positive control, acetohydroxamic acid (AHA), of 17.3 $\pm$ 0.64 μM. When the amount of urease was fixed at 4 U in the assay buffer, 2 h of preincubation with the compound was needed for maximum inhibition, which was similar to that of the positive control, AHA (Figure S4, Supporting Information). This indicated that compound **2** might inhibit jack bean urease in a reversible manner.[22]

The known compounds **2**−**6** were isolated from *H. exalata* for

the first time. The antibactial substance, hopeanolin (**1**), is the first resveratrol oligomer possessing an *ortho*-quinone moiety. Its isolation and characterization, along with the isolation of known antibabesial [vaticanols A (**6**) and G (**3**)][19] and antifungal (α-viniferin **4**)[15] constituents of *H. exalata*, may contribute to the natural defense of this tree against attack from microbial pathogens and pests.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 digital polarimeter. UV spectra were recorded on a Hitachi U-3000 spectrophotometer. IR spectra were measured on a Nexus 870 FT-IR spectrometer. 1D NMR data were acquired on a Bruker DPX-300 NMR spectrometer with tetramethylsilane (TMS) and solvent signals as internal references. 2D NMR data were acquired on a Bruker DRX-500 NMR spectrometer. ESIMS were recorded on a Mariner System 5304 mass spectrometer. Silica gel (200−300 mesh) for column chromatography was produced by Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Sephadex LH-20 was purchased from Pharmacia Biotech, Uppsala, Sweden. All other chemicals used in this study were of analytical grade.

Plant Material. The stems and twigs of *H. exlata* were collected in July 2004 from the Botanical Garden at Jianfeng Town, Ledong County, Hainan Province, People's Republic of China. A voucher specimen (no. IFB-20040719) is preserved at the Institute of Functional Biomolecules, Nanjing University. The specimen was identified by Prof. X. J. Tian (Nanjing University, Nanjing, People's Republic of China).

Extraction and Isolation. The air-dried and powdered stems of *H. exalata* (1.8 kg) were extracted with MeOH (3 × 10 L) at room temperature and concentrated in vacuo to give a crude extract (240 g). This was subsequently diluted with H_2O (500 mL) to give an aqueous suspension. After defatting by partitioning with *n*-hexane (3 × 500 mL), the suspension was extracted with EtOAc (3 × 500 mL). The EtOAc extract (110 g) was chromatographed on a silica gel column eluted with mixtures of $CHCl_3$−MeOH increasing in polarity, to give a total of 60 fractions. Fractions of similar compositions, as determined by TLC, were pooled, resulting in ten fractions (A−J). Fraction B was subjected to passage over a second silica gel column, eluted with $CHCl_3$−MeOH (50:1, 25:1, 20:1, 10:1, 5:1, each 1.5 L), with 250 mL fractions collected. Fractions B-2, B-5, B-6, and B-11 were purified using Sephadex LH-20 by elution with MeOH, to afford **1** (31 mg), **6** (210 mg), **3** (1.1 g), and **4** (780 mg). Gel filtration of fraction D over a Sephadex LH-20 column, using MeOH, yielded **5** (780 mg). Fraction G was subjected to reversed-phase column chromatography (H_2O−MeOH gradient system, 10%−60% MeOH) to give **2** (202 mg) and **7** (120 mg).

Hopeanolin (1): dark yellow, amorphous powder; $[\alpha]_D^{20}$ −18.6 (*c* 0.1 MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.95), 233 (sh), 280 (2.2) nm; IR (KBr) ν_{max} 3258, 2955, 2925, 2855, 1685, 1613, 1517, 1448, 1360, 1216, 1166, 1126 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS $[M - H]^-$ *m/z* 691.1603 (calcd for $C_{42}H_{27}O_{10}$, 691.1610).

Antifungal Assay. The MICs were determined by a colorimetric method using the dye MTT.[23] After the MIC values were determined visually on each of the microtiter plates, 25 μL of RPMI 1640 containing 5 mg of MTT/mL was added to each well. Incubation was continued at 37 °C for 3 h. The content of each well was removed, and 100 μL of isopropanol containing 5% mol/L HCl was added to extract the dye. After 30 min of incubation at room temperature and gentle agitation, the optical density (OD) was measured with a microplate reader at 550 nm.

Assay for the Inhibition of Jack Bean Urease. The measurement of urease was carried out using an established method.[22] The assay mixture, containing 25 μL (4 U) of jack bean urease and 25 μL (100 μg) of the test compound, was preincubated for 2 h at room temperature in a 96-well assay plate. After preincubation, 0.2 mL of 100 mM phosphate buffer at pH 6.8, containing 500 mM urea and 0.002% phenol red, was added, and the mixture was incubated at room temperature. The reaction time was measured on a microplate reader (570 nm), which was required for enough ammonium carbonate to form to raise the pH of the phosphate buffer from 6.8 to 7.7.

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Supporting Information Available: ^1H and ^{13}C NMR spectra, selected HMBC correlations, and biogenetic pathway for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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