DOI: 10.1002/ejoc.200600661

Hopeanol: A Potent Cytotoxin with A Novel Skeleton from *Hopea exalata*

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Keywords: Natural products / Hopeanol / Structure elucidation / *Hopea exalata* / Cytotoxic activity

Hopeanol, a rearranged resveratrol dimer ester with an unprecedent carbon skeleton, was isolated from the bark of *Hopea exalata*. Its structure was determined by comprehensive spectroscopic analysis. Hopeanol exhibited potent cytotoxicity against six human cancer cell lines with its IC_{50} = 0.52–19.36 μM.

Introduction

Plants belonging to the family Dipterocarpaceous have been recognized for their biosynthesis of a diversity of resveratrol (3,5,4′-trihydroxystilbene) oligomers,[1] some of which display pronounced biological activities such as cytotoxicity,[2] fungicide,[3] antiinflammation[4] and anti-HIV.[5] In our continuous characterization of biologically active new metabolites from plants,[6] endophytes[7] and marine microbes,[8] we have found that one of the most substantially cytotoxic extracts was derived from the bark of *Hopea exalata* Lin. Yang et Hsue, collected from Hainan Province of China. Surprisingly, few reports could be found to date concerning any biological and/or chemical investigation of this plant. Therefore, a bioassay-guided fractionation was performed to lead to the full characterization and biological evaluation of hopeanol (**1**), a cytotoxic rearranged resveratrol dimer ester with a hitherto-undescribed carbon skeleton.

Results and Discussion

Sturcture Elucidation

Hopeanol (**1**) possessed a molecular formula of $C_{29}H_{20}O_9$ (necessitating 20 unsaturation indices) as evidenced from its positive-ion HR-ESIMS (*m*/*z* $[M + H]^+$ 513.1160, calcd. for $C_{29}H_{21}O_9$: 513.1180, Δ3.90 ppm). Its IR absorption bands at 3377, 1724, 1679, and 1661 cm^{-1} indicated the existence of hydroxyls and three carbonyls, including presumably an ester and two conjugated ketones. Co-edited with DEPT and HMQC experiments, the 29 resonance lines in the ^{13}C-NMR spectrum of **1** were attributable to a methoxy, 12 methine and 16 quaternary carbons (see Table 1), while the presence of 5 hydroxyl groups was evidenced from non-carbonated proton singlets at δ = 7.89, 8.42, 8.71, 8.92, and 9.35 in the ^1H-NMR spectrum. Subsequent scrutiny of the ^1H-^1H COSY, NOESY, and HMBC spectra (Table 1 and Figure 1) revealed the presence of the following four substructures as detailed below. A 4-hy-

Table 1. ^1H,^{13}C NMR assignments and HMBC correlations of **1**.[a]

no.	δ_H (mult., *J*, Hz)	δ_C	$HMBC_{(C\rightarrow H)}$
1		124.5 (s)	2(6), 3(5)
2, 6	7.02 (d, 8.7)	132.0 (d)	2(6), 3(5), 4-OH
3, 5	6.64 (d, 8.7)	114.1 (d)	3(5), 4-OH
4		157.3 (s)	2(6), 3(5), 4-OH
7		71.2 (s)	2(6), 3(5), 14, 4′, 10′, 11′
8		189.8 (s)	14
9		132.7 (s)	11-OH
10		120.9 (s)	12, 14, 11-OH
11		154.0 (s)	12, 11-OH, 13-OH
12	6.55 (d, 2.2)	108.8 (d)	14, 11-OH, 13-OH
13		158.5 (s)	12, 13-OH
14	7.05 (d, 2.2)	107.3 (d)	12, 13-OH
1′		152.4 (s)	6′, 3′-OH, 5′-OH
2′		112.6 (s)	4′, 6′, 3′-OH
3′		157.2 (s)	4′, 3′-OH
4′	6.23(d, 2.1)	103.2 (d)	6′, 3′-OH, 5′-OH
5′		160.4 (s)	4′, 6′, 5′-OH
6′	7.03(d, 2.1)	107.8 (d)	4′, 5′-OH
7′		63.5 (s)	12, 6′, 10′,-OCH_3
8′		170.2 (s)	–OCH_3
9′		66.6 (s)	2(6), 10′, 11′, 13′,14′
10′	6.93 (dd, 10.4, 3.2)	149.4 (d)	14′
11′	5.81 (dd, 10.4, 1.9)	131.5 (d)	13′
12′		184.6 (s)	10′, 13′, 14′
13′	6.27 (dd, 10.5, 1.9)	133.7 (d)	11′
14′	7.34 (dd, 10.5, 3.2)	147.3 (d)	10′
–OCH_3	3.52 (s)	51.8 (q)	
4-OH	8.42 (br. s)		
11-OH	9.35 (br. s)		
13-OH	8.92 (br. s)		
3′-OH	7.89 (br. s)		
5′-OH	8.71 (br. s)		

[a] Measured in $[D_6]$acetone at 500 MHz.

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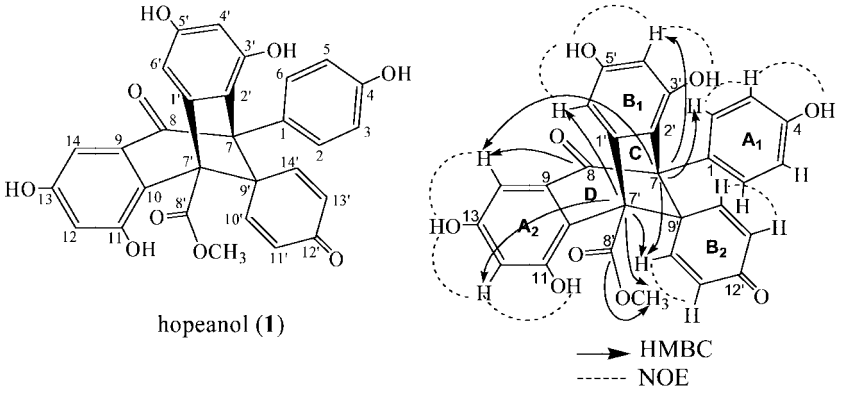
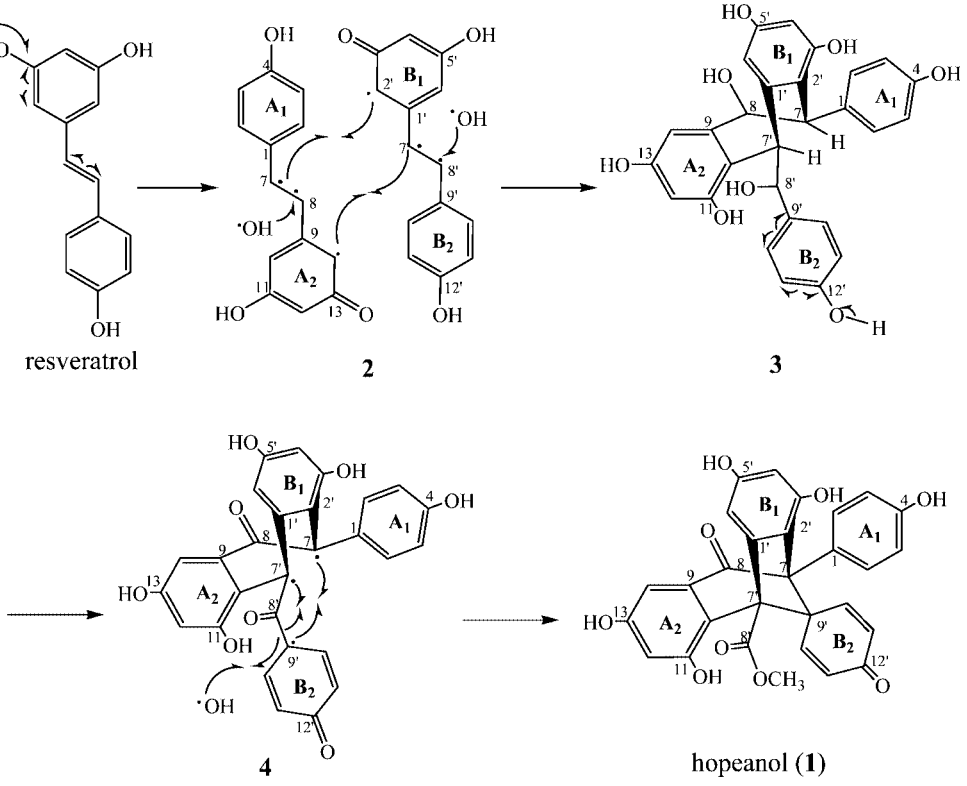


Figure 1. Selected two-dimensional NMR correlations for hopeanol (**1**).

droxypenyl group (ring $\mathbf{A_1}$) was required by a pair of two-proton doublets (J = 8.7 Hz) at δ_H = 7.02 (H-2,6), 6.64 (H-3,5), the latter showing a clear NOE correlation with the broadened 4-OH singlet at δ_H = 8.42. Furthermore, a pair of 4,5-disubstituted 1,3-benzenediol nuclei (rings $\mathbf{A_2}$ and $\mathbf{B_1}$) could be proposed from two sets of *meta*-coupled aromatic proton doublets at δ_H = 7.05 (J = 2.2 Hz, H-14) and 6.55 (J = 2.2 Hz, H-12, exhibiting NOE correlations with the broadened singlets of 11-OH and 13-OH at δ = 9.35 and 8.92 ppm, respectively), as well as at δ_H = 7.03 (J = 2.1 Hz, H-6′) and 6.23 (J = 2.1 Hz, H-4′, displaying NOE cross peaks with 3′-OH and 5′-OH resonating at δ = 7.89 and 8.71 ppm, respectively). Finally, an α,β,α′,β′-unsaturated ketonic system (ring $\mathbf{B_2}$) was evidenced from the four mutually coupled olefinic double doublets at δ_H = 6.93 (J = 10.4, 3.2 Hz, H-10′), 5.81 (J = 10.4, 1.9 Hz, H-11′), 6.27 (J = 10.5, 1.9 Hz, H-13′), and 7.34 (J = 10.5, 3.2 Hz, H-14′). The magnitude (≈ 10.4 Hz) of $J_{10',11'}$ and $J_{13',14'}$ highlighted that this system was actually formulated in a six-membered ring together with a quatenary carbon (C-9′) at δ_C = 66.6, showing HMBC correlations with H-11′ and H-13′ in HMBC correlations. Furthermore, the kentonic carbon (C-12′) at δ_C = 184.6 showed HMBC correlations with H-10′ and H-14′. This ring conjunction gained additional reinforcement from the discerned "W-type" couplings $J_{10',14'}$ (= 3.2 Hz) and $J_{11',13'}$ (= 1.9 Hz).

The above four rings, plus two carbonyl groups, took a total of 18 degrees of unsaturation. Owing to the shortage of any more double bonds, the two remaining unsaturation indices could only be "consumed" by proposing two more



Scheme 1. Plausible biogenetic pathway to hopeanol (**1**) from resveratrol.

rings for the molecule. The subsequent scrutiny of its HMBC spectrum revealed the given connectivity of the aforementioned four rings ($\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{B_1}$ and $\mathbf{B_2}$). Thus, the HMBC correlations of C-7 with H-2(6), H-4′, and H-10′ indicated that this quaternary carbon bonded to C-1 (ring $\mathbf{A_1}$), C-2′ (ring $\mathbf{B_1}$), and C-9′ (ring $\mathbf{B_2}$). This observation, along with those of C-7′ with H-6′ and H-10′, required the formation of a five-membered ring (**C**). In addition to the anticipated HMBC cross peak with ester carbonyl (C-8′), the three-proton methoxy singlet (δ_H 3.52) showed discernible 4-bond correlations with C-7′, demonstrating the linkage of C-7′ with C-10 and the 8′-carboxylic methyl ester. The final one unsaturation index, coupled with the HMBC correlations of H-14 with C-7 and C-8, both covalently connnected to generate a six-membered ring (**D**). The relative configuration of hopeanol (**1**) had to be proposed owing to the geometrical requirement for the fusions of rings $\mathbf{A_2}$, $\mathbf{B_1}$, $\mathbf{B_2}$, **C**, and **D**.

A plausible biogenetic pathway (Scheme 1) for hopeanol (**1**) was supposed on the basis of previous work.[9] Resveratrol may be its biosynthetic precusor through radical intermediates (**2**) that tend to combine into **3**. Oxidation of the 8- and 8′-hydroxyls in **3** may generate radical **4**, in which migration of ring $\mathbf{B_2}$ to C-7 forms a secondary C-8′ active (radical) site prone to anchor on C-7′. This process was accompanied or followed by further oxidation of C-8′ to liberate an acid intermediate which forms hopeanol (**1**) upon methyl esterification.

It was possible that the methyl ester of **1** was a result of a reaction with MeOH in the procedure of extraction or isolation. HPLC and TLC experiments were performed to determine whether hopeanol was a natural product or an artefact. When extracted with acetone, isolated with a petroleum ether–acetone mixture, and both detected by HPLC with a CH_3CN–H_2O system and TLC with a petroleum ether–acetone system, the dried stem bark of *H. exalata* was confirmed to contain hopeanol (**1**) as a natual product.

Biological Activity

Hopeanol (**1**) exhibited significant cytotoxic activity against KB, AGS, Hela, BEL-7402, SW1116 and BGC-803 cell lines. The inhibitions of hopeanol on the tumor cells were demonstrated to be much stronger than 5-fluorouracil, a clinically prescribed antitumor drug (Table 2).

Table 2. The in vitro cytotoxicity (IC_{50}, μM) of **1** against six cell lines.

Cell lines	Hopeanol (**1**)	5-Fluorouracil[a]
KB	0.52 ± 0.04	17.82 ± 1.29
AGS	4.28 ± 0.15	105.83 ± 9.12
Hela	3.21 ± 0.09	182.49 ± 8.14
BEL-7402	13.95 ± 0.39	146.77 ± 10.25
SW1116	19.36 ± 0.20	54.02 ± 7.41
BGC-803	3.29 ± 0.05	124.80 ± 8.57

[a] a positive control.

Experimental Section

General Procedure: IR Spectra were obtained with a Nexus 870 FT-IR spectrometer. CD spectra were obtained with a JASCO J-725 spectrometer. UV Spectra were recorded with a Hitachi U-3000 spectrophotometer. HR-ESIMS were recorded with a Mariner System 5304 mass spectrometer. All NMR spectra were recorded with a Bruker DRX-500 spectrometer, with chemical shifts δ in ppm relative to $SiMe_4$ as internal standard and coupling constants J in Hz. Silica gel (200–300 mesh) for column chromatography was produced by Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was purchased from Pharmacia Biotech, Sweden. All chemicals used in this study were of analytical grade.

Isolation of Hopeanol: *H. exalata* was collected in July 2004 from the Botanical Garden at Ledong County, Hainan, China with a voucher specimen preserved at the Institute of Functional Biomolecules, Nanjing University. The dried stem bark of *H. exalata* (1.8 kg) was extracted with MeOH (3 × 10 L) at room temperature. In vacuo evaporation of solvent from the extract gave a black syrup (*ca*. 240 g), which was subsequently diluted with H_2O to give an aqueous suspension. After defatting by partitioning with *n*-hexane, the suspension was extracted with EtOAc. The EtOAc extract (110 g) was chromatographed on a silica gel column eluted with a mixture of $CHCl_3$/MeOH (100:0, 100:4, 100:8, 100:16, 100:32, 100:64, 0:100, each 3.5 L) to give a total of 60 fractions. Fractions of similar compositions as determined by TLC were pooled, resulting in 10 fractions, A–J. Fraction B (5.8 g) was subjected to a second silica gel column eluted with $CHCl_3$/MeOH (50:1, 25:1, 20:1, 10:1, 5:1, each 1.5 L). Subfraction B-3 (0.12 g), after being subjected to Sephadex LH-20 with MeOH, yielded hopeanol (**1**) (12.4 mg) as light yellow amorphous powder.

Hopeanol (1): Light yellow armorphous powder. $[\alpha]_D^{20}$ = +40.1 (MeOH, *c* 0.23). UV (MeOH) λ_{max} (log ε): 204 (4.84), 221(4.83), 358 (3.63) nm. CD (MeOH): Δε = 242 (+0.91), 293 (–0.53), 322 (+0.14), 346 (–0.01), 390 (+0.35) nm. IR (KBr): $\tilde{\nu}$ = 3377, 2925, 2881, 2883, 1724, 1679, 1661, 1611, 1517, 1462, 1337, 1280, 1261 cm^{-1}. EIMS: *m*/*z* (rel. int.%) = 480 (51) $[M]^+$, 387 (40), 94 (100), 66 (23), 65 (19), 44 (13). HRESIMS (positive ion mode): *m*/*z* $[M + H]^+$ 513.1160 (calcd. for $C_{29}H_{21}O_9$: 513.1180, Δ3.90 ppm).

Cytotoxicity Assay: The cytotoxicity of **1** was tested on a series of cancer cell lines, listed as follows: human nasopharynyeal epidermoid tumor (KB), human gastric ademocarcinoma (AGS), human cervical carcinoma (Hela), human liver cancer (BEL-7402), human colon cancer (SW1116) and human gastric cancer (BGC-803). The effects of **1** on the viability of these cells were assayed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[10] Briefly, cells at the exponential phase were collected and transferred into 96- well plates. After a 24 h incubation, compound dilutions were dispensed to the established culture plates for 48 h. An MTT solution was then added to each well (0.1 mg/well). After a further incubation for 4 h, the supernatant was removed, the crystals were fully dissolved in 150 μL of DMSO, and the absorbencies of each well were read at 570 nm. The value for IC_{50} was determined at the concentration that inhibited cell growth by 50% using the MTT assay. Data are expressed as means ± standard error (SE).

Supporting Information (see also the footnote on the first page of this article): 1-D, 2-D, and HR-ESIMS spectra of compound **1**.

Acknowledgments

This work was financed by NSFC (Key Project No.: 20432030).

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Received: July 30, 2006
Published Online: October 18, 2006

