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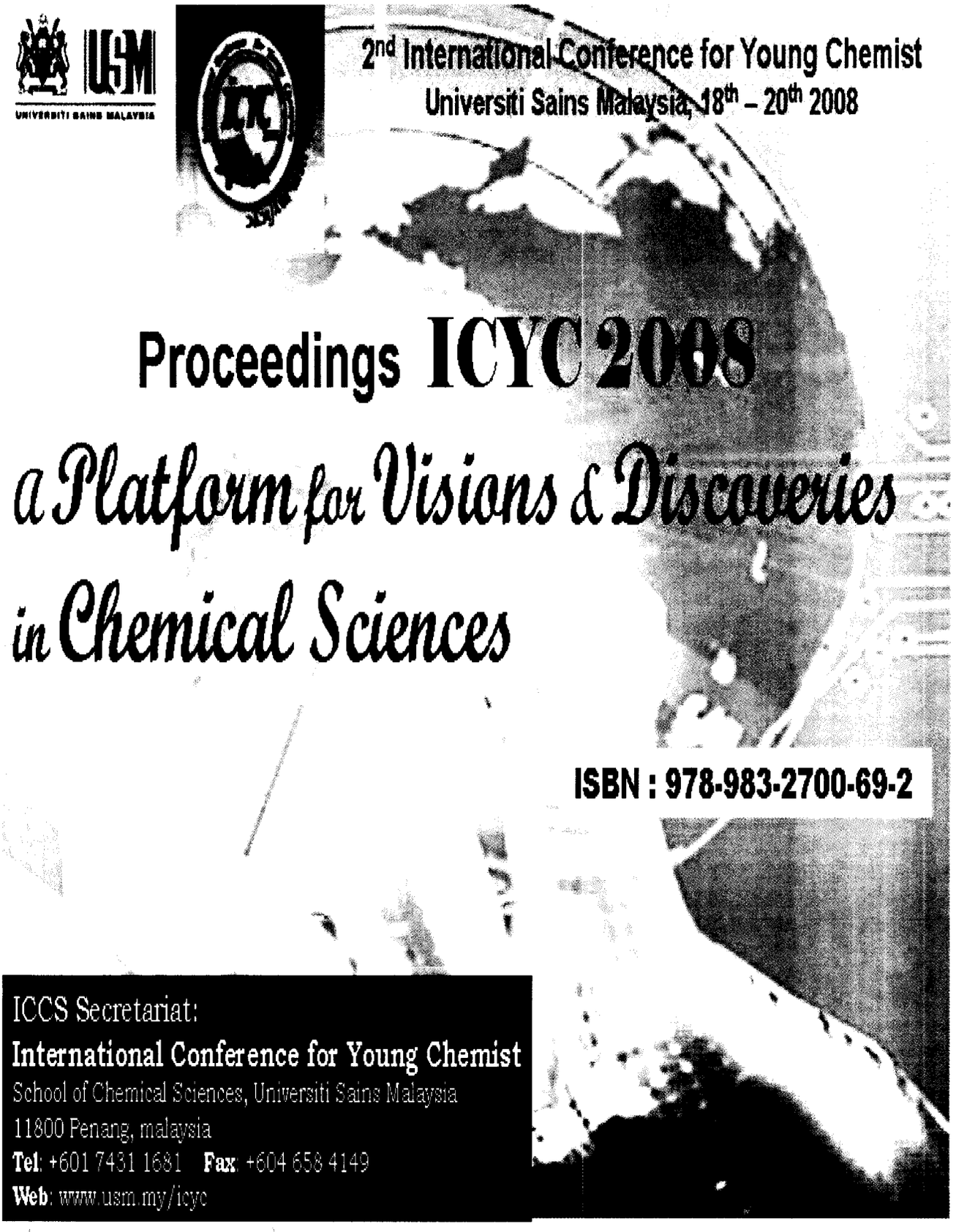

2nd International Conference for Young Chemist
Universiti Sains Malaysia, 18th – 20th 2008

Proceedings ICYC 2008

a Platform for Visions & Discoveries in Chemical Sciences

ISBN : 978-983-2700-69-2

ICCS Secretariat:
International Conference for Young Chemist
School of Chemical Sciences, Universiti Sains Malaysia
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Web: www.usm.my/icyc

2nd International Conference for Young Chemist 2008
Universiti Sains Malaysia, 18 – 20 June 2008

CHEM/I-3

RESVERATROL DERIVATIVE COMPOUNDS FROM STEM BARK OF *HOPEA* AND THEIR BIOLOGICAL ACTIVITY TEST

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ABSTRACT

From the stem bark of Hopea odorata, H. mengarawan, and H. nigra seven known resveratrol derivative, named balanocarpol, heimiol A, vaticanol B, vaticanol G, ampelopsin H, hopeaphenol, and hemlesyanol C were isolated. The structure was elucidated by NMR spectroscopy, including ID and 2D NMR. Some compounds showed activity as antioxidant and cytotoxicity againt cell Hela S3 and Ragi.

***Keywords** : Resveratrol derivative, Hopea odorata, H. mengarawan, H. nigra, antioxidant, cytotoxicity*

INTRODUCTION

Hopea is one the main genus of Dipterocarpaceae, consisting of approximately 100 species and widely distributed in Indonesia specially in Kalimantan [1,2] and until now only few species have been investigated. This family of plant is known to produce a variety of resveratrol oligomer [3-15]. These structures are very interesting and showed interesting biological activity, such as antibacterial, anticancer, antihepatotoxic, and anti-HIV [3-15]. Thus Dipterocarpaceae plants are very potential for chemical research in natural product and pharmaceutical industry. In our continuing phytochemical study of the Dipterocarpaceae family occuring in Indonesia, we have examined resveratrol oligomer constituents from some species of *Hopea*. *H. mengarawan* is widely distributed in tropical rain forest of Sumatra, Malaysia, until Andaman islands, and it is locally known as " merawan hitam " or ' pengarawan" [3]. This paper will report our first investigation of seven resveratrols derivative from stem bark of these species. The structure of this compound based on the analysis spectrum of UV, IR, MS and NMR included ID and 2D NMR (^{1}H- ^{1}H COSY, HMQC, HMBC and NOESY).

EXPERIMENTAL

General Experimental Procedure

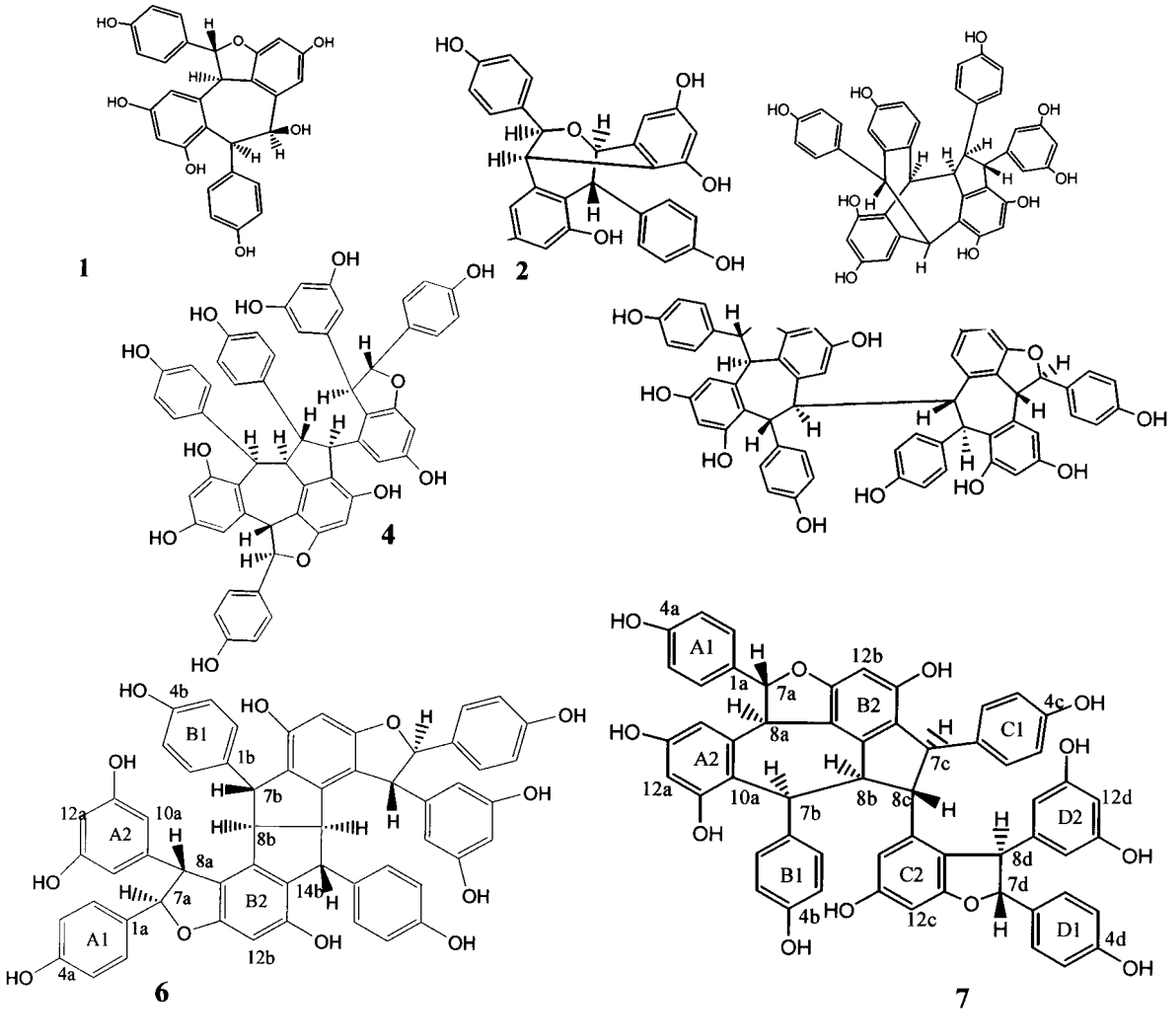
UV and IR spectra were measured with varian cary 100 Conc and Shimadzu 8300 FTIR respectively. ^{1}H and ^{13}C NMR spectra were recorded with Jeol JNM A-5000 spectrophotometers, operating at 600.0 MHZ (^{1}H) and 150.0 MHZ (^{13}C) using residual and deuterated solvent peaks as internal standards. MS spectra were obtained with a JMS-AM 20 spectrometer, using the mode FAB. Vacuum liquid chromatography (VLC) was carried out using Merck Si gel Merk 60 GF_{254} (230-400 mesh), column chromatography using Si-gel Merk 60 (200-400 mesh), and TLC analysis on precoated Si gel plates Si-gel Merk Kieselgel 60 F_{254} 0.25 mm, 20 x 20 cm.

Plant Material

Samples of the stem bark of *H. Mengarawan, H. odorata,* and *H. nigra* were collected in Desember 2003 from the Experimental Garden in Carita, Banten, Indonesia. The plant was identified by the staff at the Herbarium Bogoriense, Kebun Raya Bogor, Bogor, and a voucher specimen had been deposited at the Herbarium.

Extraction and Isolation

The milled dried stem bark of *H. mengarawan* (5 kg) was extracted exhaustively with acetone. The acetone extract on removal of the solvent under reduced pressure gave a brown residue (400 g). A portion (40 g) of the total acetone extract was fractionated by vacuum liquid chromatography (VLC) and purified by repeated column chromatography on silica gel eluted with various solvent systems. From this method we obtained four oligostilbenes, namely balanocarpol (**1**) (300 mg), heimiol A (**2**) (200 mg), vaticanol G (**3**) (70 mg), and vaticanol B (**4**) (200 mg). The structures of these compounds (**1** – **4**) were established on the basis of their spectral data, including UV, IR, and NMR spectra in comparison with the previously reported data and by direct comparison with the authentic samples. From the dried and milled stem bark of *H. odorata* (3.8 kg) was isolated four componds, namely balanocarpol (**1**) 300 mg, hopeaphenol (**5**) 1500 mg, ampelopsin H (**6**) 250 mg, and hemlesyanol C (**7**) 120 mg, whereas from the dried and milled stem bark of *H. nigra* (4,6 kg) to give vaticanol G (**1**) 200 mg.



RESULTS AND DISCUSSION

Balanocarpol (1) was obtained as a pale yellow powder, m.p. 230 °C, UV (MeOH) $\lambda_{max.}$ (log ε) : 227 (5.6); 283 (3.76) nm, IR (KBr) $\upsilon_{max.}$: 3384; 1608; 1405; 1350; 1240; 1132; 1037; 995; 833 cm^{-1}, ^1H and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see Table 1. FABMS *m/z* 470 $[M^+]$ ($C_{28}H_{22}O_7$).

Heimiol A (2) was obtained as a pale yellow powder, m.p. 240 °C, UV (MeOH) $\lambda_{max.}$ (log ε) : 225 (6.01); 230 (sh 4.83); 282 (3.65) nm, IR (KBr) $\upsilon_{max.}$: 3352; 1606; 1512; 1450; 1234; 1141; 1068; 954; 835 cm^{-1}, ^1H and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see Table 1. FABMS *m/z* 471 $[M+H]^+$ ($C_{28}H_{22}O_7$).

Vaticanol G (3) was obtained as a brown powder, m.p. 240 °C, UV (MeOH) $\lambda_{max.}$ (log ε) : 208 (5.95); 234 (sh) (5.72); 280 (5.16), IR (KBr) $\upsilon_{max.}$: 3296; 1609; 1510; 1445; 1243; 1142; 1012; 833 cm^{-1}, ^1H and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see Table 1. FABMS *m/z* 680 $[M^+]$ ($C_{42}H_{32}O_9$).

Ampelopsin H (6) was obtained as a pale yellow powder, m.p. 240 °C, UV (MeOH) $\lambda_{max.}$ (log ε) : 225 (6.01); 230 (sh 4.83); 282 (3.65) nm, IR (KBr) $\upsilon_{max.}$: 3352; 1606; 1512; 1450; 1234; 1141; 1068; 954; 835 cm^{-1}, ^1H and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see Table 2. FABMS *m/z* 906 $[M+H]^+$ ($C_{56}H_{42}O_{14}$).

Hemlesyanol C (7) was obtained as white brown powder, UV (MeOH) $\lambda_{max.}$ (log ε) 203 (5.31); 283 (4.33), IR (KBr) υ_{max} 3200, 612–1454 , and 833 $cm^{-1}$$^1H$ and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see Table 2. FABMS *m/z* 906 $[M^+]$ ($C_{56}H_{42}O_{12}$).

Vaticanol B (4) and hopeaphenol (5) was identified with UV, IR, and TLC compared with authentic sample.

Balanocarpol (**1**) was obtained as a pale yellow powder, m.p. 230 °C. Its UV spectrum showed absorption maxima at 283 nm suggesting the presence of unconjugated phenolic chromophore. The IR spectrum exhibited hydroxyl group (3384 cm^{-1}), C=C aromatic (1608; 1405; 1350 cm^{-1}), and monosubtituen benzene (833 cm^{-1}), these spectra characteristic absorptions for supporting **1** to be an oligoresveratrol. The positive ion FABMS exhibited an $[M]^+$ ion at *m/z* 470 consistent with a molecular formula $C_{28}H_{22}O_7$ for a resveratrol dimer and supported by the NMR data. ^{13}C NMR spectra showed six signals for oxyaryl carbon at δ 159.2 (C-4a), 157.4 (C-11a), 154.6 (C-13a), 157.2 (C-4b), 159.2 (C-11b), and 159.7 (C-13b) ppm, characteristics for resveratrol dimer. Additionally, the ^{13}C NMR also exhibited one oxyalkyl carbon at δ 73.2 (C-8b) indicating that C-8b attached with hydroxyl fungtional group. The ^1H NMR spectrum of **1** in acetone-d_6 exhibited signals for two sets of 4-hydroxybenzene at δ 7.48 (*d*, *J* = 8.8 Hz) and 6.95 (*d*, *J* = 8.8 Hz) ppm, each 2H (ring A1) and at δ 6.75 (*d*, *J* = 9.5 Hz) and 6.42 (*d*, *J* = 9.5 Hz) ppm, each 2H (ring B1). The ^1H NMR spectrum also showed two sets of meta-coupled aromatic protons signals at δ 6.09 (*d*, *J* = 2.2 Hz) and 5.96 (*d*, *J* = 2.2 Hz) ppm, each 1H (ring A2), and at δ 6.20 (*d*, *J* = 2.2 Hz) and 6.25 (*d*, *J* = 2.2 Hz) ppm, each 1H (ring B2). Additionally, the ^1H NMR spectrum exhibited signals for a set of aliphatic proton at δ 5.70 (*d*, *J* = 9.5 Hz) and 5.16 (*d*, *J* = 9.5 Hz), each 1H, characteristic for *trans*-2,3-diaryl-dihydrobenzofuran moiety, and signals assignable two coupled aliphatic protons at δ 4.89 (*br. s*) and 5.39 (*br s*) ppm, each 1H. These spectral data indicated that compound **1** has a dimeric stilbene skeleton as part of its structure.

Heimiol (**2**) was obtained as a light brown powder, maxima of absorption were observed at 225; 230 sh; 282 nm in the UV spectrum attributable to the phenol rings. The IR spectrum exhibited hydroxyl group (3352 cm^{-1}), C=C aromatic (1606; 1512; 1450 cm^{-1}), and monosubtituen benzene (835 cm^{-1}). Its molecular formula of $C_{28}H_{22}O_7$ was establisehed by FABMS, showing a $[M+H]^+$ ion at m/z 471, together with its NMR spectral data, suggesting that **2** was resveratrol dimer. The ^1H NMR (Table 2) and ^1H-^1H COSY spectra showed two sets of AA'BB' system of aromatic protons assignable to two independent 4-hydroxyphenyl groups at δ 6.90 (2H, *d*, *J* = 8.4 Hz) and 6.69 (2H, *d*, *J* = 8.4 Hz) (ring A1), and δ 7.14 (2H, *d*, *J* = 8.4 Hz) and 6.72 (2H, *d*, *J* = 8.4 Hz) (ring B2), two sets of *meta*-coupled aromatic protons at δ 6.41 (1H, *d*, J = 2.6 Hz) and δ 6.16 (1H, *d*, J = 2.6 Hz) (ring A2), 6.48 (1H, *d*, J = 2.2 Hz) and 6.21 (1H, *d*, J = 2.2 Hz) (ring B2) assignable to two units 1,2,3,5-tetrasubstituted benzene group. They also displayed two set of copuled benzyl methine protons at δ 5.57 (1H, *br s*) (7a), 4.24 (1H, *br. s*) (8a), 4.32 (1H, *d*, J = 3.3 Hz) (7b), 4.97 (1H, *d*, J = 3.3 Hz) (8b). The ^{13}C NMR spectrum showed that C-7a (δ 81.5 ppm) and C-8b (δ 81.4 ppm) indicated that they might both be attached to benzylic carbons bearing an oxygen atom. The connection between protons and their corresponding carbons was established by HMQC. Further support for the structure **2** was obtained form HMBC measurement (Figure 2). The HMBC spectrum of **2** showed long-range correlations between H-2a with C-7a (δ 81,5 ppm) confirmed that a 4-hydroxyphenyl group is attached to an oxygen bearing carbon. Long-range correlation were also observed for the methine proton between H-8b/C-7b, H-7b/C-10b, and H-8a/C-10b showed to a fused benzopyran-benzo-oxepane structure, in the same pattern with those of heimiol A[15]. The relatif configuration of **2** was established on the basis of the NOESY spectra (Figure 2). The NOE correlation showed that the H-8a and H-8b are in a *syn* configuration, deduced from the NOE correlations between H-8b/H-7a/H-8a, as well as H-7b does not show any correlations. Therefore, it may be concluded that the **2** is heimiol A, a resveratrol dimer.

Table 1. ^{1}H and ^{13}C NMR data of compound **1**, **2** and **3*** in acetone-d_6

No	Balanocarpol (1)		Heimiol (2)		Vaticanol G (3)	
	δ H (*m.*, J in Hz)	δ C	δ H (m, J in Hz)	δ C	δ H (m, J in Hz)	δ C
1a	-	133.7	-	136.8	-	139.8
2a,6a	7.48 (*d*, 8.8)	131.5	6.90 (*d*, 8.4)	127.9	6.45 (*br s*)	130.1
3a,5a	6.95 (*d*, 8.8)	116.4	6.69 (*d*, 8.4)	115.3	6.46 (*br s*)	114.6
4a	-	159.2	-	157.2	7.89 (*br s*)	155.4
7a	5.70 (*d*, 9.5)	93.5	5.57 (*br s*)	81.5	4.55 (*d*, 4.3)	57.1
8a	5.16 (*d*, 9.5)	52.5	4.24 (*br. s*)	46.9	4.63 (*d*, 4.3)	50.2
9a	-	142.8	-	147.4	-	141.8
10a	-	120.5	6.41 (*d*, 2.6)	107.4	-	125.9
11a	-	157.4	-	157.1	8.01 (*br s*)	153.1
12a	6.09 (*d*, 2.2)	102.0	6.16 (*d*, 2.6)	102.0	6,20 (*d*, 2.8)	101.6
13a	-	156.9	-	154.6	7.59 (*br s*)	155.8
14a	5.96 (*d*, 2.2)	106.8	-	116.0	5.67 (*d*, 2.8)	111.4
1b	-	133.4	-	136.9	-	129.1
2b,6b	6.75 (*d*, 9.5)	132.0	7.14 (*d*, 8.4)	130.0	-	141.6
3b,5b	6.42 (*d*, 9.5)	114.1	6.72 (*d*, 8.4)	115.5	6.07 (*d*, 2.6)	119.7
4b	-	155.8	-	157.2	7.40 (*br s*)	154.8
7b	4.89 (*br. s*)	50.2	4.32 (*d*, 3.3)	50.9	5.77 (*dd*, 8.4; 2.6)	112.7
8b	5.39 (*br s*)	73.2	4.97 (*d*, 3.3)	81.4	6.02 (*d*, 8.4)	134.9
OH	4.32 (*d*, 4.4)					
9b	-	140.8	-	142.6	4.89 (*d*, 3.0)	42.6
10b	-	113.9	6.48 (*d*, 2.2)	104.8	3.85 (*dd*, 8.9; 3.0)	53.8
11b	-	159.2	-	158.1	-	146.9
12b	6.20 (*d*, 2.2)	95.1	6.21 (*d*, 2.2)	102.1	-	117.5
13b	-	159.7	-	156.2	8.48 (*br s*)	154.9
14b	6.25 (*d*, 2.2)	104.5	-	117.0	6.46 (*s*)	101.8
1c					7.59 (*br s*)	152.8
2c					-	121.8
3c					-	136.9
4c (OH)					5.92 (*br s*)	127.7
5c					5.98 (*br s*)	114.6
6c					7.85 (*br s*)	156.4
7c					6.67 (*br s*)	116.2
8c					7.13 (*br s*)	130.3
9c					3.51 (*d*, 8.9)	62.9
10c, 14c					4.11 (*s*)	56.9
11c, 13c (OH)					-	147.5
12c					5.96 (*d*, 2.6)	106.3

* measured with acetone-d_6 600.0 MHz (^{1}H) and 150.0 MHz (^{13}C)

Tabel 2. ^{1}H and ^{13}C NMR data of compound **6** and **7*** in acetone-d_6

No	Ampelopsin H (6)		hemlesyanol C (7)	
	δ_H (*m*, *J* Hz)	δ_C	δ_H (*m*, *J* Hz)	δ_C
1a	-	134.8	-	133.2
2a,6a	7.11 (*d*, 8.4)	127.3	7.58 (*d*, 8.4)	130.8
3a,5a	6.74 (*d*, 8.4)	116.1	6.91 (*d*, 8.4)	115.2
4a	-	157.9	-	158.7
7a	5.31 (*d*, 2.0)	93.8	5.68 (*d*, 10.6)	94.7
8a	4.33 (*d*, 2.0)	57.1	5.35 (*d*, 10.6)	51.8
9a	-	148.6	-	138.9
10a	6.29 (*br s*)	106.6	-	122.8
11a	-	160.0	-	157.8
12a	6.32 (t, 2.1; 2.1)	102.2	6.23 (*d*, 2.2)	102.0
13a	-	160.0	-	156.3
14a	6.29 (br s)	106.6	6.05 (*d*, 2.2)	107.9
1b	-	138.8	-	133.4
2b,6b	6.73 (d, 8.4)	129.2	6.11 (*d*, 8.4)	133.5
3b,5b	6.56 (*d*, 8.4)	115.5	6.40 (*d*, 8.4)	114.8
4b	-	155.9	-	156.3
7b	4.29 (*s*)	50.2	4.40 (*d*, 3.3)	46.2
8b	3.85 (*s*)	60.5	4.16 (*t*, 3.3; 3.3)	55.4
9b	-	144.6	-	144.3
10b	-	126.4	-	115.2
11b	-	155.5	-	160.1
12b	6.21 (s)	96.7	6,00 (*s*)	96.2
13b	-	163.2	-	154.7
14b	-	116.2	-	122.8
1c			-	136.3
2c,6c			5.77 (*d*, 8.8)	129.5
3c,5c			6.20 (*d*, 8.8)	115.1
4c			-	156.1
7c			3.88 (*d*, 5.8)	61.2
8c			3.19 (*d*, 5.8)	56.7
9c			-	147.5
10c			-	119.1
11c			-	162.8
12c			6.29 (*d*, 2.7)	95.4
13c			-	160.2
14c			5.91 (*d*, 2.7)	106.4
1d			-	134.6
2d,6d			7.07 (*d*, 8.4)	127.6
3d,5d			6.85 (*d*, 8.4)	116.0
4d			-	157.8
7d			5.08 (*d*, 3.3)	93.9
8d			3.65 (*d*, 3.3)	56.2
9d			-	148.4
10d			5.91 (*d*, 2.5)	106.6
11d			-	160.1
12d			6.11 (*d*, 2.5)	106.6
13d			-	160.1
14d			5.91 (*d*, 2.5)	106.6

* diukur dalam aseton-d_6 600 MHz (^{1}H) dan 150 MHz (^{13}C)

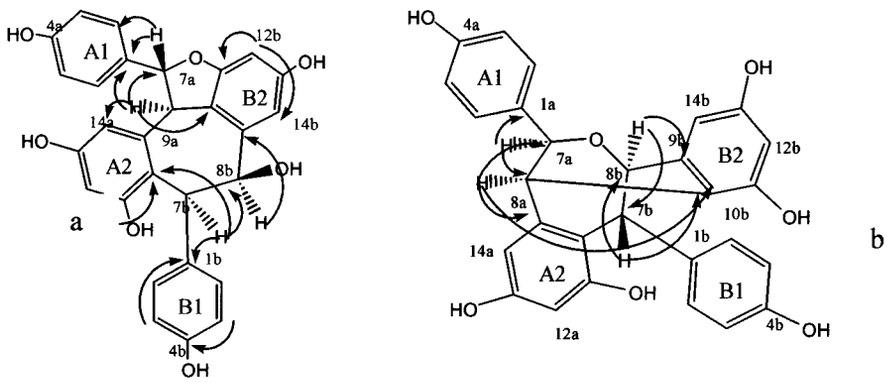


Figure 1. Significant HMBC of balanocarpol (a) and Heimiol A (b)

Vaticanol G (**3**) was obtained as a brown powder, m.p. 240 °C. Its UV spectrum showed absorption maxima at 280 nm, suggesting the presence of unconjugated phenolic chromophore. The IR spectrum exhibited hydroxyl group (3296 cm^{-1}), C=C aromatic (1609; 1510; 1445 cm^{-1}), and monosubtituen benzene (833 cm-1), these spectra characteristic absorptions for supporting **3** to be an oligostilbene. The positive ion FABMS exhibited an $[M]^+$ ion at *m/z* 680, together with NMR data, corresponding to a molecular formula $C_{42}H_{32}O_9$, for a resveratrol trimer. The ^1H NMR spectrum of **3** in acetone-d_6 exhibited signals for two sets of 4-hydroxybenzene at δ 6.45 (*br s*) and 6.46 (*br s*), each 2H, at δ 7.13 (*br s*); 6.67 (*br s*); 5.98 (*br s*); and 5.92 (*br s*), each 1H (ring of A1 and C1), and one unit 1,2,4-trisubstitued benzene at δ 6.07 (1H, *d, J* = 2.6 Hz); 6.02 (1H, *d, J* = 10.6 Hz). Additionally, the ^1H NMR spectrum exhibited signals for a set of aromatic signals at δ 5.77 (1H, *dd, J* = 8.4; 2.6 Hz) (ring B1), one unit 1,3,5-trisubstitued benzene at δ 6.12 (1H, *t, J* = 2.6; 2.6 Hz) and 5.96 (2H, *d, J* = 2.6 Hz) (ring C2), one unit 1,2,3,5-tetrasubstitued benzene at δ 6.20 (1H, *d, J* = 2.8 Hz) and 5.67 (1H, *d, J* = 2.8 Hz) (unit A2), and one unit 1,2,6-trisubstitued-3,5-dihidroksibenzene (δ 6.46 (*s*), (ring B2). The six substitued benzene ring suggesting with 24 DBE (Double Bond Equivalent). Beside that, the ^1H NMR spectrum exhibited two signals aliphatic proton which correlated at ^1H-^1H COSY spectrum, characteristic with unit of -CH-CH- [δ 4.63 (1H, *d, J* = 4.3 Hz) and 4.55 (1H, *d, J* = 4.3 Hz) (unit D), and four signals assignable to two-coupled aliphatic protons charactristic with unit of -CH-CH-CH-CH- [δ 4.89 (1H, *d, J* = 3.0 Hz); 3.85 (1H, *dd, J* = 8.9; 3,0 Hz); 3.51 (1H, *d, J* = 8.9 Hz); and 4.11 (1H, *s*) (unit E)]. The characteristic of aliphatic protons signal was not indicated for trans-2,3-diaryl-dihydrobenzofuran moiety, and support for structure **3** was a trimeric resveratrol with aliphatic trisiclo skeleton, were similar to those of vaticanol G isolated from *Vatica* rassak[13]. Complete assignment of all proton - bearing carbon signals were made possible by analysis of the HMQC spectrum, and support for structure **3** was obtained from significant cross-peak in HMBC measurement.

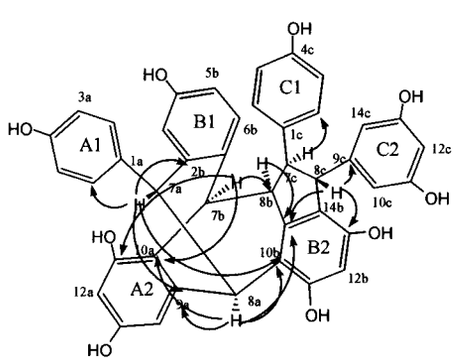


Fig. 2. Significant HMBC (H→C) correlation of vaticanol G **(3)**

Ampelopsin H (**5**) was obtained as a light brown powder, maxima of absorption were observed at 228; 282 nm in the UV spectrum attributable to the phenol rings. The IR spectrum exhibited hydroxyl group (3274 cm^{-1}), C=C aromatic (1608; 1454 cm^{-1}), and monosubtituen benzene (833 cm^{-1}). Its molecular formula of $C_{56}H_{42}O_{12}$ was establisehed by FABMS, showing a $[M+H]^+$ ion at m/z 906, together with its NMR spectral data, suggesting that **5** was tetramer resveratrol, but from NMR data (^1H and ^{13}C) showed amount half from molecular formula, so it's can be suggested compound isolated **5** is are compiled by two symmetrical structure units, that each unit is dimer resveratrol (Table 2). The ^1H NMR spectrum of **5** in acetone-d_6 exhibited signals for two sets of 4-hydroxybenzene at δ 7.11 (2H, *d*, J = 8.4 Hz) and 6.74 (2H, *d*, J = 8.4 Hz) ppm, with δ 6.73 (2H, *d*, J = 8.4 Hz) and 6.56 (2H, *d*, J = 8.4 Hz) ppm. The ^1H NMR spectrum also showed two sets of meta-coupled aromatic protons signals at δ 6.32 (1H, *t, J* = 2.1; 2.1 Hz) ppm and 6.29 (2H, *br s*) ppm indicated of 3,5-hydroxyphenyl group. Furthermore proton signal aromatic at 6,21 (1H, s) ppm showed existence of pentasubstitutedbenzena ring. Two proton signals at δ5.31 (1H, *d, J* = 2.0 Hz) ppm and d 4.33 (1H, *d, J* = 2.0 Hz) ppm showed existence of ring trans-dihydrobenzofuran. Two proton signals at aliphatic area that is at δ 4.29 (s) ppm and 3.85 (s) ppm indicated that both proton is at different position.

Hemlesyanol C (7), is a brown amorphous powder, absorption band (283 nm) in the UV spectra showed the presence of aromatic rings. The IR spectrum exhibited hydroxyl group (3200 cm^{-1}), C=C aromatic (1612–1454 cm^{-1}), and monosubtituen benzene (833 cm^{-1}). The $[M^+]$ ion peak at *m/z* 906, cooresponds to the molecular formula $C_{56}H_{42}O_{12}$. The ^1H-NMR spectrum (Tabel 2), showed the signal assignable to four 4-hydroxyphenyl groups at δ 7.58 (*d*, 8.4); 6.91 (*d*, 8.4); 6.11 (*d*, 8.4); 6.40 (*d*, 8.4); δ 5.77 (*d*, 8.8); 6.20 (*d*, 8.8); 7.07 (*d*, 8.4); 6.85 (*d*, 8.4); each 2H. The presence of a 3,5-dihydroxyphenyl group at δ 5.91 (2H, *d*, 2.5) H-10d and 14d, δ 6.11 (*d*, 2.5) H-12d, two sets of meta coupled aromatic protons on 1,2,3,5-tetrasubstituted benzene rings at δ 6.23 (*d*, 2.2), H-12a; 6.05 (*d*, 2.2), H-14a; 6.29 (*d*, 2.7), H-12c and 5.91 (*d*, 2.7), H-14c was also exhibited. The spectrum further showed the signals due to an aromatic proton on a pentasubstituted benzene ring δ 6.00 *(s)*, H-12b, a sequence of four aliphatic methine protons coupled succesively in the COSY spectrum in this order δ 4.40 (*d*, 3.3), H-7b; 4.16 (*t*, 3.3; 3.3), H-8b; 5.77 (*d*, 8.8), H-7c and 6.20 (*d*, 8.8), H-8c and two sets of mutually coupled aliphatic protons δ 5.68 (*d*, 10.6), H-7a and 5.35 (*d*, 10.6), H-8a; δ 5.08 (*d*, 3.3), H-7d and 3.65 (*d*, 3.3), H-8d, in addition to ten phenolic hydroxyl groups (δ 6.46 -8.57). These results suggested that compound is a stlbene composed of four resveratrol unit, analysis of the HMQC and HMBC spectrum enabled the complete assignmentsof all protonated carbons and quartenary carbons corresponding to respective resveratrol units (A-D). In the HMBC spectrum (Fig. 3) showed cross peaks were observed long range correlation betwen H-7b/ C-14b, C-8c, H-8b/ C-14b, and C-9c, H-7c/ C-14b; H-8c/ C-14b; and C-8b. Therefore, it may be concluded that the **7** is hemlesyanol C, a resveratrol tetramer, that the firs isolated from *Shorea hemsleyana*[12].

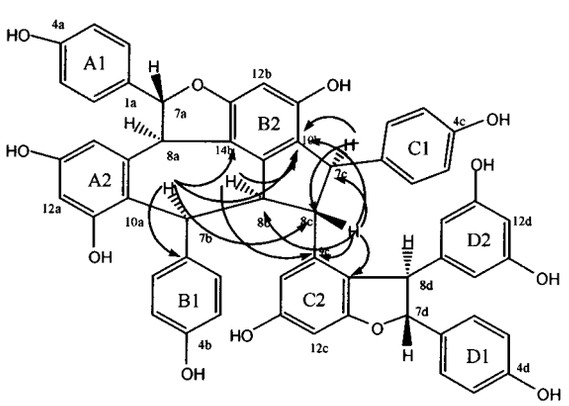


Fig. 3. Significant HMBC (H→C) correlation of hemlesyanol C (7)

Activity test as antioxidant conducted by radical scavenger activity from chloroform and ethyl acetate with Halliwel methode [16], showed at table 3. The data IC_{50} showed that the activity as radical hidroxyl scavenger from hopeaphenol more active than ascorbat acid and the other of oligoresveratrol, balanocarpol, heimiol A, vaticanol B, ampelopsin H showed less active. From the sample oligoresveratrol indicated that activity as hydroxyl radical scavenger caused by existence of phenol ring, stability of molecular structure, and existence of bonding double bond of olefenic unit. Phenol ring can catch hydroxyl radical by release hydrogen radical that of condensation with hydroxyl radical, form of water molecule, whereas radical phenol will be stabilized by resonance. That is, resveratrol compound is referred for developed as antioxidant. Because of antioxidant is substance that can prevent or slow down the happening of free reaction of radical oxidation. Role antioxidant in body is lessen free radical, like SOR (reactive oxygen species) that can be formed in course of metabolism in organism. Antioxidant also can be functioned protect low lipoprotein densitas (Low Density Lypoprotein) from oxidation reaction, so it's can prevent the happening of arteriosklerosis.

The in-vitro cytotoxicity test was investigated using 96 wells plate with cell density $2x10^4$ cells per ml. Into each well was added with 100 µl cells in culture medium (87,5% RPMI 10,4 g/L; 2% penstrep; and 10% FBS) and was then incubated in CO_2 incubator for 12-24 hours at 37^0C. Each sample was dissolved in culture medium containing 0,05% DMSO, and 100 µl of each sample in the different concentrations was added into each well in triplicate and was then incubated in CO_2 incubator for 12-24 hours at 37°C. MTT solution (10 µl per 100 µl medium) was added to all wells of an assay, and plates were incubated for 4 hours at 37°C in CO_2 incubator. As much as 100µl formazon (10% SDS and 0, 01 N chloride acid) was added into each well and mixed on a shaker for 5 minutes. The wells were incubated in the dark room for 12-24 hours at room temperature. The absorbance was measured using multiwell scanning spectrophotometers (ELISA reader) at wavelength 595 nm. The absorbance is directly pro-portional to the number of living cells. So the dead cell could be calculated to determine LC_{50}. Doxorubicin, a medicine for lymphoma, leukaemia and tumor acute, was also measured its cytotoxic activity as standard comparison. The cytotoxic activity of the samples against HeLa-S3 cell measured as LC_{50} were provided in Table 4. HeLa-S3, a *continuous cell line* that living as adherent cell, is a cell derivate of ephythell cell of human cervix cancer. Further investigation of cytotoxic activity of the samples was hold against Raji cell (Table 5). The cell that resembles lymphoblast cell found by R.J.V Pulvertaft (1963) from *Burkitt's lymphoma* at the left of upper jaw of negro boy oldest 11 years. Table 4 and 5 showed that the highest cytotoxic activity against HeLa-S3 and Raji is ampelopsin H. This compound is more active than doxorubicin.. in the other hand heimiol A and vaticanol G showed the lowest cytotoxic activity against HeLa-S3 and Raji. It is necessary to held further investigation about the relationship between the structure and their activities of these compounds. Some studies of curcumin that has been known as anticancer indicated that the existence of hydroxyl group at ortho position and β-diketon gave a big contribution as inducer of enzymes in phase two that their function as protector from carcinogenesis as epoxy hydrolyse, glutathione S-transferase (GST), and NAP(P)H quinone reductase (QR).[18]

Table 3. Data activity test as radical scavenger

Sample	IC_{50} (µg/ml)	Note
Balanocarpol	1802,3	Less active
Heimiol A	4575.3	Less active
Vaticanol G	683.96	active
Vaticanol B	2146.6	Less active
Hopheaphenol	61,8	High active
Ampelopsin H	4840,0	Less active
Hemlesyanol C	425,5	active
Ascorbat acid	83,9	High active
Butylated Hydroxy Toluene (BHT)	1328,1	Less active

Note: IC_{50} < 100 µg/ml: High active; 100 -1000 µg/ml: active; dan 1000-5000 µg/ml: Less active; > 5000 µg/ml: not active[17]

Table. 4. LC_{50} of some compounds from steam bark of *Hopea* against HeLa-S3 cell

No	Sample	LC_{50} µg/ml	Note
1	Balanocarpol	682,16	Less active
2	Heimiol A	Very high	Not active
3	Vaticanol G	Very high	Not active
4	Ampelopsin H	8,12	Very active
5	Vaticanol B	92,04	Very active
6	Hopeaphenol	1931,52	Less active
7	Hemsleyanol C	531,00	Active
8	Doksorubisin (positif control)	96,27	Very active

Table. 5. LC_{50} of some compounds from steam bark of *Hopea* against Raji cell

No	Sample	LC_{50} µg/ml	Note
1	Balanocarpol	277,58	Active
2	Heimiol A	Very high	Not active
3	Vaticanol G	11050,96	Not active
4	Ampelopsin H	91,07	Very active
5	Vaticanol B	107,00	Very active
6	Hopeaphenol	135,64	Active
7	Hemsleyanol C	166,84	Active
8	Doksorubisin (positif control)	156,64	Active

CONCLUSION

In this paper we concluded that resveratrol derivative isolated from the stem bark of *Hopea* consist of dimer, trimer, and tetramer resveratrol. Some compounds have biological activity as antioxidant and cytotoxic effect against Raji and HeLa-S3 lines cell. Hopeaphenol showed the highest activity as antioxidant. Whereas ampelopsin H and vaticanol B gives the highest cytotoxic effect against HeLa-S3 and Raji.

ACKNOWLEDGMENT

This work has been supported by competitive grant (Insentif Riset Dasar, Ristek- 2008), Ministry Reseach and Technology, Republic of Indonesia. The authors are grateful to the experimental Garden in Carita, Pandeglang, Banten, Indonesia and Herbarium Bogoriensis for supported the sample and identification of the plant specimen.

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