Resveratrol Derivatives from Stem Bark of *Hopea* and Their Biological Activity Test

Sri Atun[1*], Nurfina Aznam[1], Retno Arianingrum[1], Y. Takaya [2] and Niwa Masatake[2]

[1]Department Chemistry Education, Universitas Negeri Yogyakarta, Karangmalang, Depok, Sleman, Yogyakarta, 55281, Indonesia
[2]Faculty of Pharmacy, Meijo University, Tempaku, Nagoya, Japan

*Corresponding author: Atun_1210@yahoo.com

Abstract: *From the stem bark of* Hopea odorata, H. mengarawan and H. nigra, *seven known resveratrol derivatives, named balanocarpol (1), heimiol A (2), vaticanol G (3), vaticanol B (4), hopeaphenol (5), ampelopsin H (6), and hemlesyanol C (7) were isolated. The structure was elucidated by NMR spectroscopy, including 1D and 2D NMR. Some compounds showed antioxidant activity and cytotoxicity againt HeLa-S3 and Raji cell.*

Keywords: resveratrol derivatives, *Hopea odorata, H. mengarawan, H. nigra,* antioxidant, cytotoxicity

1. INTRODUCTION

Hopea is one of 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 oligomers.[3–18] These structures are very interesting and showed interesting biological activity, such as antibacterial, anticancer, antihepatotoxic and anti-HIV.[3–18] Thus Dipterocarpaceae plants are very promising 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 odorata, H. mengarawan* and *H. nigra. Hopea* is widely distributed in tropical rain forest of Sumatra, Malaysia and up to the Andaman islands, and it is locally known as *merawan hitam* or *pengarawan*[3] This paper reports first investigation of seven resveratrol derivatives from the stem bark of these species. The structures of these compounds were derived based on the analysis of the UV, IR, MS and NMR, including 1D and 2D NMR (^{1}H- ^{1}H COSY, HMQC, HMBC and NOESY) spectra.

2. EXPERIMENTAL

2.1 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 spectrometers, 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 Si-gel Merck 60 GF_{254} (230–400 mesh), column chromatography using Si-gel Merck 60 (200–400 mesh) and TLC analysis on precoated Si gel plates Si-gel Merck Kieselgel 60 F_{254} 0.25 mm, 20 x 20 cm.

2.2 Plant Material

Samples of the stem bark of *H. mengarawan, H. odorata* and *H. nigra* were collected in December 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.

2.3 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 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[3–18] 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 **(3)** (200 mg) (Fig. 1).

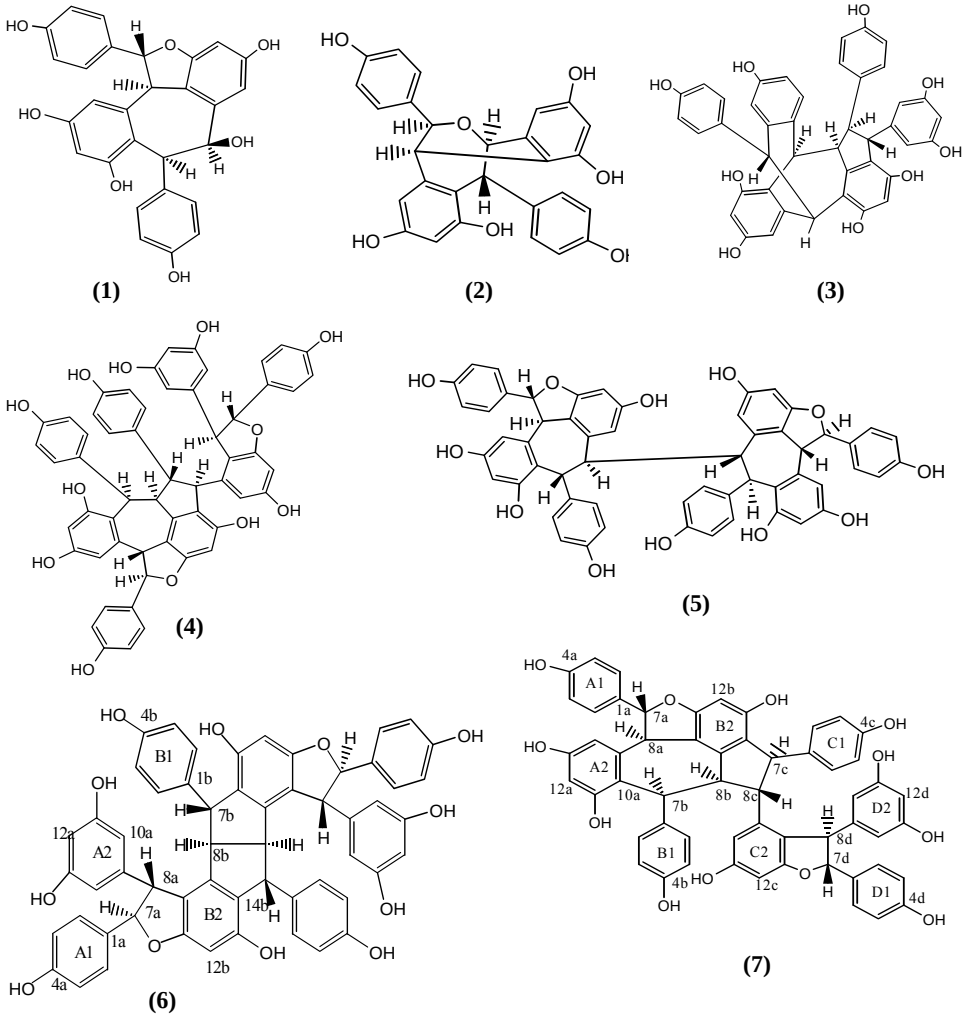

Figure 1: Structure some compounds isolated from *Hopea.*

3. RESULTS AND DISCUSSION

Balanocarpol **(1)** was obtained as a pale yellow powder, m.p. 230°C, UV (MeOH) λ_{max} (log ε) : 227 (5.6); 283 (3.76) nm, IR (KBr) υ_{max}: 3384; 1608; 1405; 1350; 1240; 1132; 1037; 995; 833 cm^{-1}, ^{1}H 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) λ_{max} (log ε) : 225 (6.01); 230 (sh 4.83); 282 (3.65) nm, IR (KBr) υ_{max}: 3352; 1606; 1512; 1450; 1234; 1141; 1068; 954; 835 cm^{-1}, ^{1}H 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) λ_{max} (log ε) : 208 (5.95); 234 (sh) (5.72); 280 (5.16)nm, IR (KBr) υ_{max} : 3296; 1609; 1510; 1445; 1243; 1142; 1012; 833 cm^{-1}, ^{1}H 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) λ_{max} (log ε) : 225 (6.01); 230 (sh 4.83); 282 (3.65) nm, IR (KBr) υ_{max} : 3352; 1606; 1512; 1450; 1234; 1141; 1068; 954; 835 cm^{-1}, ^{1}H and ^{13}C NMR (Me_2CO-d_6, 600.0 and 150 MHz) see Table 2. FABMS *m/z* 906 $[M+H]^+$ ($C_{56}H_{42}O_{12}$).

Hemlesyanol C **(7)** was obtained as white brown powder, UV (MeOH) λ_{max} (log ε): 203 (5.31); 283 (4.33)nm, IR (KBr) υ_{max}: 3200, 1612–1454, and 833 cm,$^{-1}$ ^{1}H 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)** were identified with UV, IR and TLC compared with authentic sample.

Table 1: ^{1}H and ^{13}C NMR data of compounds **(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

(continue on next page)

Table 1: *(continued)*

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
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 OH	5.39 (*br s*) 4.32 (*d*, 4.4)	73.2	4.97 (*d*, 3.3)	81.4	6.02 (*d*, 8.4)	134.9
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					5.92 (*br s*)	127.7
3c					5.98 (*br s*)	114.6
4c (OH)					7.85 (*br s*)	156.4
5c					6.67 (*br s*)	116.2
6c					7.13 (*br s*)	130.3
7c					3.51 (*d*, 8.9)	62.9
8c					4.11	56.9
9c					-	147.5
10c, 4c					5.96 (*d*, 2.6)	106.3
11c, 3c (OH)					7.96 (*br s*)	158.9
12c					6.12 (*t*, 2.6; 2.6)	100.9

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

Table 2: ^{1}H and ^{13}C NMR data of compounds **(6 and 7)*** in acetone-d_6.

No	Ampelopsin H **(6)**		Hemlesyanol C **(7)**	
	δH (*m*, *J* in Hz)	δC	δH (*m*, *J* in Hz)	δC
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	-	134.8	-	136.3
2c,6c	7.11 (*d*, 8.4)	127.3	5.77 (*d*, 8.8)	129.5
3c,5c	6.74 (*d*, 8.4)	116.1	6.20 (*d*, 8.8)	115.1
4c	-	157.9	-	156.1
7c	5.31 (*d*, 2.0)	93.8	3.88 (*d*, 5.8)	61.2
8c	4.33 (*d*, 2.0)	57.1	3.19 (*d*, 5.8)	56.7
9c	-	148.6	-	147.5
10c	6.29 (*br s*)	106.6	-	119.1
11c	-	160.0	-	162.8
12c	6.32 (*t*, 2.1; 2.1)	102.2	6.29 (*d*, 2.7)	95.4

(continue on next page)

Table 2: *(continued)*

No	Ampelopsin H **(6)**		Hemlesyanol C **(7)**	
	δH (*m*, *J* in Hz)	δC	δH (*m*, *J* in Hz)	δC
13c	-	160.0	-	160.2
14c	6.29 *(br s)*	106.6	5.91 (*d*, 2.7)	106.4
1d	-	138.8	-	134.6
2d,6d	6.73 (*d*, 8.4)	129.2	7.07 (*d*, 8.4)	127.6
3d,5d	6.56 (*d*, 8.4)	115.5	6.85 (*d*, 8.4)	116.0
4d	-	155.9	-	157.8
7d	4.29 (*s*)	50.2	5.08 (*d*, 3.3)	93.9
8d	3.85 (*s*)	60.5	3.65 (*d*, 3.3)	56.2
9d	-	144.6	-	148.4
10d	-	126.4	5.91 (*d*, 2.5)	106.6
11d	-	155.5	-	160.1
12d	6.21 (*s*)	96.7	6.11 (*d*, 2.5)	106.6
13d	-	163.2	-	160.1
14d	-	116.2	5.91 (*d*, 2.5)	106.6

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

Balanocarpol **(1)** was obtained as a pale yellow powder, m.p. 230°C. Its UV spectrum showed absorption maximum 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 monosubtituted benzene (833 cm^{-1}), these spectral characteristic absorptions 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 this suggestion was 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), 156.9 (C-13a), 155.8 (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 was attached to a hydroxyl functional group. The ^{1}H 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 ^{1}H 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 ^{1}H NMR spectrum exhibited signals for a set of aliphatics 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 A **(2)** was obtained as a pale yellow powder, with of absorption maxima 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 monosubstituted benzene (835 cm^{-1}). Its molecular formula of $C_{28}H_{22}O_7$ was established by FABMS, showing a $[M+H]^+$ ion at *m/z* 471, together with its NMR spectral data, were evidence 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 coupled 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) 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 (Fig. 2). The HMBC spectrum of **(2)** showed long-range correlations between H-2a with C-7a (δ 81.5 ppm), confirming that a 4-hydroxyphenyl group was attached to an oxygen bearing carbon. Long-range correlations were also observed for the methine proton between H-8b/C-7b H-7b/C-10b, and H-8a/C-10b, pointing to a fused benzopyran-benzo-oxepane structure, in the same pattern as those of heimiol A.[18] The relative configuration of **(2)** was established on the basis of the NOESY spectrum (Fig. 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 which did not show any correlations. Therefore, it may be concluded that **(2)** is heimiol A, a resveratrol dimer.

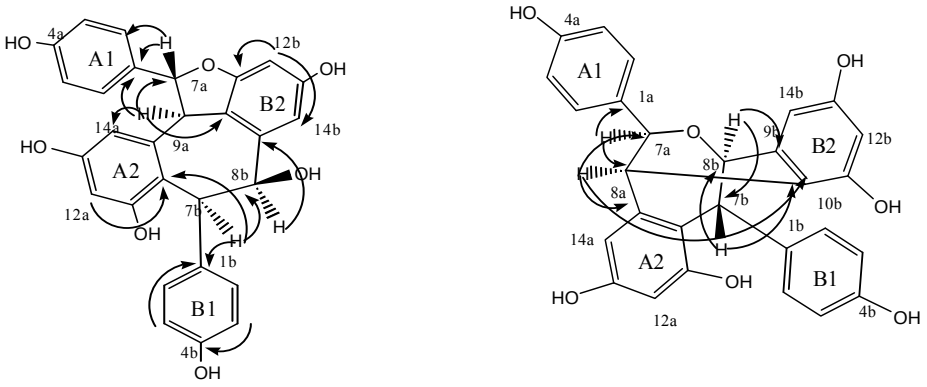

Figure 2: Significant HMBC of (a) balanocarpol **(1)** and (b) heimiol A **(2)**.

Vaticanol G **(3)** was obtained as a brown powder, m.p. 240°C. Its UV spectrum showed absorption maximum 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 monosubstituted benzene (833 cm^{-1}). These were characteristic spectral data for supporting **(3)** to be an oligostilbene. The positive ion FABMS exhibited an $[M]^+$ ion at *m/z* 680, which together with NMR data, were consistent with a molecular formula $C_{42}H_{32}O_9$, for a resveratrol trimer. The ^{1}H 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 (rings of A1 and C1), and one unit of a 1,2,4-trisubstituted benzene at δ 6.07 (1H, *d, J* = 2.6 Hz); 6.02 (1H, *d, J* = 8.4 Hz). Additionally, the ^{1}H 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 of a 1,3,5-trisubstituted 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 of a 1,2,3,5-tetrasubstituted benzene at δ 6.20 (1H, *d, J* = 2.8 Hz) and 5.67 (1H, *d, J* = 2.8 Hz) (ring A2), and one unit of a 1,2,6-trisubstituted-3,5-dihydroxibenzene δ 6.46 (*s*), (ring B2). The six substituted benzene rings suggested 24 DBE (double bond equivalents). Beside that, the ^{1}H NMR spectrum exhibited two aliphatic proton signals which correlated at ^{1}H-^{1}H COSY spectrum, characteristic of a unit -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 characteristic 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 aliphatic proton signal due to a *trans*-2,3-diaryl-dihydrobenzofuran moiety, was not observed, suggesting that **(3)** was a trimeric resveratrol with an aliphatic tricyclic skeleton similar to that of vaticanol G isolated from *Vatica rassak.*[8] 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-peaks in HMBC measurement (Fig. 3).

Ampelopsin H **(6)** was obtained as a pale yellow powder, with absorption maxima observed at 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 cm^{-1}), and monosubstituted benzene (835 cm^{-1}). Its molecular formula of $C_{56}H_{42}O_{12}$ was established by FABMS, showing a $[M+H]^+$ ion at *m/z* 906, which together with the NMR spectral data, suggested that **(5)** was a resveratrol tetramer. The NMR data (^1H and ^{13}C), however showed number of signal corresponding to half the molecular formula, so was suggested that compound **(5)** composed of two symmetrical structural units, and each unit was a resveratrol dimmer (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 indicating the presence of a 3,5-hydroxyphenyl group. Furthermore, the aromatic proton signal at 6.21 (1H, *s*) ppm showed existence of a penta-substituted benzena ring. Two proton signals at δ 5.31 (1H, *d, J* = 2.0 Hz) ppm and δ 4.33 (1H, *d, J* = 2.0 Hz) ppm showed existence of a *trans*-dihydrobenzofuran ring. Two proton signals at δ 4.29 (*s*) ppm and δ 3.85 (*s*) ppm indicated that both protons were at different locations.

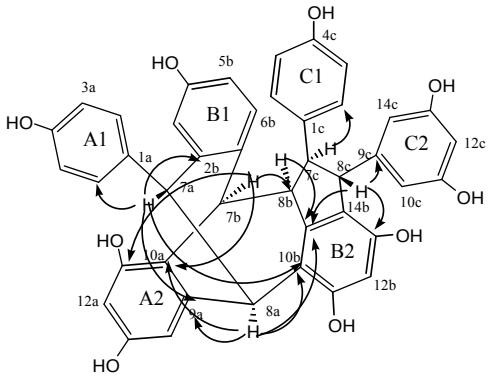

Figure 3: Significant HMBC (H→C) correlations of vaticanol G **(3)**.

Hemlesyanol C **(7)**, was a brown amorphous powder, with absorption band (283 nm) in the UV spectrum showing the presence of aromatic rings. The IR spectrum exhibited hydroxyl group (3200 cm^{-1}), C=C aromatic (1612–1454 cm^{-1}) and monosubstituted benzene (833 cm^{-1}). The [M^+] ion peak at *m/z* 906, corresponded to the molecular formula $C_{56}H_{42}O_{12}$. The ^{1}H-NMR spectrum (Table 2), showed the signals assignable to four 4-hydroxyphenyl groups at δ 7.58 (2H, *d*, *J* = 8.4), 6.91 (2H, *d*, *J* = 8.4 Hz), 6.11 (2H, *d*, *J* = 8.4 Hz), 6.40 (2H, *d*, *J* = 8.4 Hz), δ 5.77 (2H, *d*, *J* = 8.8 Hz), 6.20 (2H, *d*, *J* = 8.8 Hz), 7.07 (2H, *d*, *J* = 8.4 Hz) and 6.85 (2H, *d*, *J* = 8.4 Hz). The presence of a 3,5-dihydroxyphenyl group at δ 5.91 (2H, *d*, *J* = 2.5 Hz) H-10d and 14-d, δ 6.11 (*d*, *J* = 2.5 Hz) H-12d, and two sets of meta-coupled aromatic protons on 1,2,3,5-tetrasubstituted benzene rings at δ 6.23 (*d, J* = 2.2 Hz), H-12a; δ 6.05 (*d*, *J* = 2.2 Hz), H-14a; δ 6.29 (*d*, *J* = 2.7 Hz), H-12c and δ 5.91 (*d*, *J* = 2.7 Hz), H-14c were also exhibited. The spectrum further showed the signals due to an aromatic proton on a pentasubstituted benzene ring at δ 6.00 *(s*), H-12b, a sequence of four aliphatic methine protons coupled successively in the COSY spectrum in the order δ 4.40 (*d*, *J* = 3.3 Hz), H-7b; δ 4.16 (*t*, *J* = 3.3; 3.3 Hz), H-8b; δ 3.88 (*d*, *J* = 5.8 Hz), H-7c and δ 3.19 (*d*, *J* = 5.8 Hz), H-8c, and two sets of mutually coupled aliphatic protons δ 5.68 (*d*, *J* = 10.6 Hz), H-7a and δ 5.35 (*d*, *J* = 10.6 Hz), H-8a; δ 5.08 (*d*, *J* = 3.3 Hz), H-7d and δ 3.65 (*d*, *J* = 3.3 Hz), H-8d, in addition to ten phenolic hydroxyl groups (δ 6.46–8.57) ppm. These results suggested that compound was a stilbene composed of four resveratrol units. Analysis of the HMQC and HMBC spectra enabled the complete assignments of all protonated carbons and quarternary carbons corresponding to respective resveratrol units (A–D). The HMBC spectrum (Fig. 4) showed cross peaks indicating long range correlations between 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, isolated from *Shorea hemsleyana* for the first time.[5]

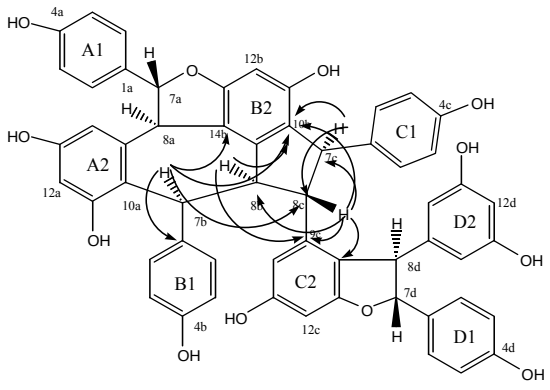

Figure 4; Significant HMBC (H→C) correlations of hemlesyanol C **(7)**.

Activity test as antioxidants based on radical scavenger activity using the Halliwel method,[19] is shown at Table 3. The data IC_{50} showed that the activity as radical hydroxyl scavenger from hopeaphenol **(5)** was more active than ascorbic acid, and the IC_{50} of oligoresveratrol, balanocarpol **(1)**, heimiol A **(2)**, vaticanol B **(4)** and ampelopsin H **(6)** showed them to be less active. For oligoresveratrol, that activity as hydroxyl radical scavenger was due to the existence of phenol ring, stability of molecular structure and existence of double bonds of olefinic unit. Phenol ring can trap hydroxyl radical by releasing hydrogen radical, by condensation with hydroxyl radical and form water molecules, whereas radical phenol will be stabilized by resonance. That is, resveratrol compound is referred for development as antioxidant. An antioxidant is substance that can prevent or slow down the reactions of radical oxidation. The role antioxidant in body is to reduce the amount free radicals, like ROS (reactive oxygen species) that can be formed in course of metabolism in organism. Antioxidant also can function to protect low density lipoprotein (LDL) from oxidation reaction, thus preventing the occurrence of arteriosclerosis.

The *in vitro* cytotoxicity test was investigated using plate with 96 wells, with cell density 2 x 10^4 cells per ml. Into each well was added 100 µl cells in culture medium (87.5% RPMI 10.4 g l^{-1}; 2% penstrep; and 10% FBS) which was then incubated in CO_2 incubator for 12–24 h at 37°C. Each sample was dissolved in culture medium containing 0.05% DMSO, and 100 µl of each sample in different concentrations was added into each well in triplicate and was then incubated in CO_2 incubator for 12–24 h 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 h at 37°C in CO_2 incubator. As much as 100 µl formazon (10% SDS and 0.01 M hydrochloric acid) was added into each well and mixed on a shaker for

Table 3: Data of activity test as radical scavengers.

Sample	IC_{50} (µg ml^{-1})	Observation
Balanocarpol (1)	1802.3	Less active
Heimiol A (2)	4575.3	Less active
Vaticanol G (3)	683.96	Active
Vaticanol B (4)	2146.6	Less active
Hopheaphenol (5)	61.8	High active
Ampelopsin H (6)	4840.0	Less active
Hemlesyanol C (7)	425.5	Active
Ascorbic acid	83.9	High active
Butylated Hydroxy Toluene (BHT)	1328.1	Less active

Note: IC_{50} < 100 µg ml^{-1}: highly active; 100–1000 µg ml^{-1}: active; and 1000–5000 µg ml^{-1}: less active; > 5000 µg ml^{-1}: not active[17]

5 min. The wells were incubated in the dark room for 12–24 h at room temperature. The absorbance was measured using multiwell scanning spectrophotometers (ELISA reader) at wavelength 595 nm. The absorbance is directly proportional to the number of living cells. So the dead cell could be calculated to determine LC_{50}. Doxorubicin, a medicine for lymphoma, leukaemia and acute tumor, 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 lived as adherent cell, is a cell derivate of ephythell cell of human cervix cancer. Further investigation of cytotoxic activity of the samples was held against Raji cell (Table 4), the cell that resembles lymphoblast cell found by R.J.V. Pulvertaft (1963) from *Burkitt's lymphoma* at the left of the upper jaw of an 11 year old negro boy. Table 4 shows that the highest cytotoxic activity against HeLa-S3 and Raji cell is ampelopsin H **(6)**. This compound is more active than doxorubicin. In the other hand, heimiol A **(2)** and vaticanol G **(3)** showed the lowest cytotoxic activity against HeLa-S3 and Raji cell. It is necessary to carry out further investigation about the relationship between the structure and the 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-diketone 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 NAD(P)H quinone reductase (QR).

Table 4: LC_{50} of some compounds from stem bark of *Hopea* against HeLa-S3 and Raji cell.

No	Sample	HeLa S3		Raji	
		LC_{50} ($\mu g\ ml^{-1}$)	Observation	LC_{50} ($\mu g\ ml^{-1}$)	Observation
1	Balanocarpol **(1)**	682.16	Less active	235.29	Active
2	Heimiol A **(2)**	Very high	Not active	Very high	Not active
3	Vaticanol G **(3)**	Very high	Not active	Very high	Not active
4	Vaticanol B **(4)**	92.81	Very active	34.45	Very active
5	Hopeaphenol **(5)**	1931.52	Less active	781.49	Less active
6	Ampelopsin H **(6)**	129.72	Active	34.69	Very active
7	Hemsleyanol C **(7)**	557.44	Less active	292.15	Less active
8	Doxorubisin (positive control)	96.82	Very active	94.38	Very active

4. CONCLUSION

In this paper, we concluded that resveratrol derivatives 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 cell lines. Hopeaphenol **(5)** showed the highest activity as antioxidant, whereas ampelopsin H **(6)** and vaticanol B **(4)** gave the highest cytotoxic effect against HeLa-S3 and Raji cell.

5. ACKNOWLEDGMENT

This work has been supported by competitive grant (Insentif Riset Dasar, Ristek-2008), Ministry Research and Technology, Republic of Indonesia and Fundamental Research from DIKTI (2007; 2008). The authors are grateful to the experimental Garden in Carita, Pandeglang, Banten, Indonesia and Herbarium Bogoriensis for the sample gift and the identification of the plant specimen.

6. REFERENCES

1. Cronquist , A. (1981). *An integrated system of classification of flowering plants*. New York: Columbia In Press.
2. Newman, M.F. (1999). *Pedoman identifikasi pohon-pohon* Dipterocarpaceae: Sumatera. Bogor: Prosea Indonesia.
3. Heyne, K. (1987). *Tumbuhan berguna Indonesia,* Vol. III. Jakarta: Badan Litbang Kehutanan, 1390–1443.
4. Dai, J.R., Hallock, Y.F., Cardellina, J.H. & Boyd, M.R. (1998). HIV-inhihibitory and cytotoxic oligostilbenoids isolated from the leaves of *Hopea malibato. J. Nat. Prod.,* 61, 351–353.
5. Ito, T., Tanaka, T., Ido, Y., Nakaya, K., linuma, M. & Riswan, S. (2000). Stilbenoids isolated from stem bark of *Shorea hemsleyana. Chem. Pharm. Bull*., 48(7), 1001–1005.
6. Ito, T., Tanaka, T., Ido, Y., Nakaya, K., linuma, M. & Riswan, S. (2000*).* Four new stilbene C-glycosides isolated from the stem bark of *Shorea hemsleyana*. *Chem. Pharm. Bull*., 48(12), 1959–1963.
7. Ito, T., Tanaka, T., Nakaya, K., linuma M., Takahashi, Y., Naganawa, H., Ohyama, M., Nakanishi, Y., Bastow, K.F. & Lee, K.-H. (2001). A new resveratrol octamer, vateriaphenol A, in *Vateria indica. Tetrahedron Letters*, 42, 5909–5912.

8. Ito, T., Tanaka, T., Ido, Y., Nakaya, K., linuma, M., Takahashi, Y., Naganawa, H., Ohyama, M., Nakanishi, Y., Bastow, K.F. & Lee, K.-H. (2001*)*. A novel bridged stilbenoid trimer and four highly condensed stilbenoid oligomers in *Vatica rassak*. *Tetrahedron,* 57, 7309–7314.
9. Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W.W., Fong, H.H.S., Farnsworth, N.R., Kinghorn, A.D., Mehta, R.G., Moon, R.C. & Pezzuto, J.M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, 275, 218–220
10. Pryce, R.J. & Langcake, P. (1977). (-)-α-Viniferin: An antifungal resveratrol trimer from grapevines. *Phytochemistry*, 16, 1452–1454.
11. Tanaka, T., Ito, T., Ido, Y., Son, T.K., Nakaya, K., linuma, M., Ohyama, M. & Chelladurai, V.M. (2000). Stilbenoids in the stem bark of *Hopea parviflora*. *Phytochemistry*, 53(8), 1015–1019.
12. Tanaka, T., Ito, T., Nakaya, K., linuma, M. & Riswan, S. (2000). Oligostilbenoids in the stem bark of *Vatica rassak*. *Phytochemistry,* 54, 63–69.
13. Tanaka, T., Ito, T., Nakaya, K., linuma, M., Takahashi, Y., Naganawa, H., Matsuura, N. & Ubukata, M. (2000). Vaticanol D, a novel resveratrol hexamer isolated from *Vatica rassak. Tetrahedron Letters*, 41, 7929–7932.
14. Tanaka, T., Ito, T., Nakaya, K. linuma, M., Takahashi, Y., Naganawa, H. & Riswan, S. (2001). Six new heterocyclic stilbene oligomers from stem bark of *Shorea hemsleyana*. *Heterocycles,* 55, 729–741.
15. Sri Atun**,** Nurfina, A., Retno, A. & Niwa, M. (2005). A trimer stilbenoids compound from stem bark *Hopea nigra* (Dipterocarpaceae). *Indo. J. Chem.*, 5(3), 211–214.
16. Sri Atun**,** Nurfina, A., Retno, A. & Niwa, M. (2006). Balanocarpol and heimiol A, two resveratrol dimers from stem bark *Hopea mengarawan* (Dipterocarpaceae). *Indo. J. Chem.*, 6(1), 75–78.
17. Sri Atun**,** Sjamsul, A.A., Niwa, M., Retno, A. & Nurfina, A. (2006). Oligostilbenoids from *Hopea mengarawan* (Dipterocarpaceae). *Biochem. System. and Ecol.,* 34, 642–644.
18. Weber, J.F., Wahab, I.A., Marzuki, A., Thomas, N.F., Kadir, A.A., Hamid, A., Hadi, A., Awang, K., Latif, A.A., Richomme, P. & Deaunay J. (2001). Heimiol A, a new dimeric stilbenoid from *Neobalanocarpus heimii. Tetrahedron Letters*, 42, 4895–4897.
19. Halliwel, B., Gutteridge, J.M.C. & Aruoma, O.I. (1987). The deoxyribose method: A simple test tub assay for determination of rate constans for reaction of hydroxyl radicals. *Anal. Biochem.,* 165, 215–219.