

Hopeafuran and a C-Glucosyl Resveratrol Isolated from Stem Wood of *Hopea utilis*

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A new resveratrol dimer and a new C-glucosyl resveratrol were isolated from stem wood of *Hopea utilis* along with nine stilbenoid derivatives comprising bergenin and (+)-lyoniresinol. The structures have been elucidated on the basis of the spectroscopic evidence.

Key words *Hopea utilis*; Dipterocarpaceae; stilbenoid; hopeafuran; resveratrol-10-C-glucopyranoside; structure elucidation

Some of stilbene derivatives have been found to show various bioactivities, e.g. chemopreventive,¹⁾ anti-inflammatory activity,²⁾ inhibitory activity of histamine release³⁾ and gastric ATPase.⁴⁾ Dipterocarpaceous plants generally contain stilbene derivatives, which consist of a resveratrol (*E*-3,5,4'-trihydroxystilbene) unit and are sometimes substituted with a glycosyl moiety.^{5,6)} We previously reported the isolation and structure elucidation of resveratrol oligomers in this family (*Hopea*,⁷⁾ *Vatica*,^{8,9)} and *Shorea*^{10,11)} and their distinctive cytotoxicity was disclosed.¹²⁾ The phytochemical constituents of *Hopea utilis* were examined in our continuing phytochemical studies of the Dipterocarpaceae oriented toward searching for leads with bioactivity. We report here the isolation and characterization of two new stilbenoids and 11 known compounds in *H. utilis* (BEDD.) BOLE.

Compound **1** (hopeafuran), [α]_D –46 °C, a yellow solid, showed positive reaction to the Gibbs reagent. The negative ion FAB-MS exhibited an [M–H][–] ion peak at *m/z* 465 indicating the molecular weight to be 466. The high-resolution (HR) negative FAB-MS at *m/z* 465.0974 showed that the molecular formula is corresponding to C₂₈H₁₈O₇. An absorption band in the IR spectrum (1653 cm^{–1}) and a signal in the ¹³C-NMR spectrum (δ 196.4) showed the presence of a carbonyl group in the molecule. Usual methylation of **1** afforded a pentamethyl ether (**1a**), suggesting that **1** has five phenolic hydroxyl groups. Thus the remaining oxygen is attributable to an ether linkage in **1**. The analysis of ¹H-NMR spectrum (Table 1) exhibited the presence of two sets of *ortho*-coupled protons assignable to two 4-hydroxyphenyl groups [δ 7.70

(2H, d, *J*=8.8 Hz, H-2a, 6a), 6.98 (2H, d, *J*=8.8 Hz, H-3a, 5a); δ 6.85 (2H, dd, *J*=8.3, 1.3 Hz, H-2b, 6b), 6.55 (2H, d, *J*=8.3 Hz, H-3b, 5b)] and two sets of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring [δ 6.57 (1H, d, *J*=2.6 Hz, H-12a), 6.70 (1H, d, *J*=2.6 Hz, H-14a); δ 7.04 (1H, d, *J*=2.1 Hz, H-12b), 7.34 (1H, d, *J*=2.1 Hz, H-14b)]. The presence of five phenolic hydroxyl groups [δ 8.00, 8.36, 8.80 (2×OH), 8.92] was also supported by the spectrum. A benzylic methine proton was observed as a signal in a high shifted-field [δ 6.12 (1H, brs, H-7b)] and showed a cross peak with the aromatic protons [H-2b(6b)] in the ¹H–¹H shift correlation spectroscopy spectrum. In the ¹³C-NMR spectrum (Table 2), 24 aromatic carbons and a methine carbon were assigned by the ¹³C–¹H shift correlation spectroscopy and correlation spectroscopy involving long-range coupling (COLOC) spectrum (*J*=8, 10 Hz) (Fig. 2). The remaining carbon signals [δ 153.3 (C-7a), δ 116.4 (C-8a)] were observed as quaternary olefinic carbons, and the behavior of chemical shift was closely similar to those of a benzofuran moiety of malibatol A¹³⁾ (δ 150.6, δ 117.4, Table 2). In the COLOC spectrum (Fig. 2), significant ³*J* long-range correlations were observed between C-7a/H-2a(6a), C-8a/H-14a, C-9a/H-7b, C-11a/H-7b and C-2b(6b)/H-7b, showing that a partial structure of **1** (ring-A₁–C-7a–C-8a–ring-A₂–C-7b–ring B₁) was the same as that of malibatol A. On the other hand, the carbonyl carbon signal (C-8b) displayed cross peaks with the aromatic proton (H-14b) and the aliphatic methine proton (H-7b), indicating that the carbonyl group was attached to C-9b and C-7b. Considering the mole-

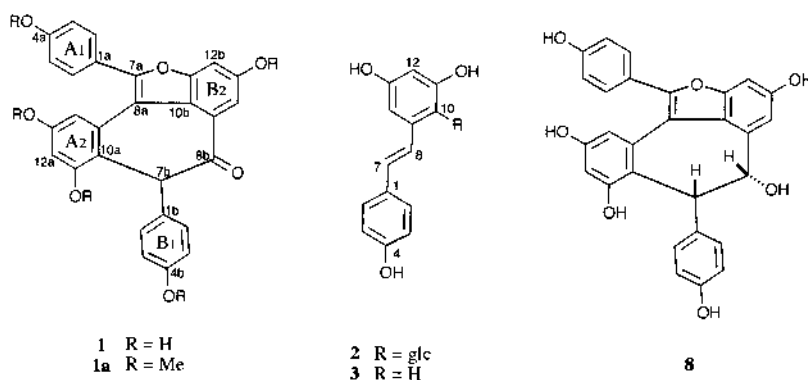


Fig. 1

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Table 1. $^1\text{H-NMR}$ Spectral Data of (300 MHz) **1**, **1a**, **8** and **8a**

No.	δ_{H}			
	1 ^{a)}	1a ^{b)}	8 ^{a)}	8a ^{b)}
2a, 6a	7.70 (2H, d, 8.8)	7.75 (2H, d, 8.8)	7.54 (2H, d, 8.8)	7.61 (2H, d, 8.8)
3a, 5a	6.98 (2H, d, 8.8)	6.97 (2H, d, 8.8)	6.92 (2H, d, 8.8)	6.93 (2H, d, 8.8)
12a	6.57 (1H, d, 2.6)	6.50 (1H, d, 2.6)	6.49 (1H, d, 2.4)	6.41 (1H, d, 2.4)
14a	6.70 (1H, d, 2.6)	6.75 (1H, d, 2.6)	6.66 (1H, d, 2.4)	6.71 (1H, d, 2.4)
2b, 6b	6.85 (2H, dd, 8.3, 1.3)	6.87 (2H, dd, 8.3, 1.3)	7.20 (2H, d, 8.6)	7.11 (2H, d, 8.6)
3b, 5b	6.55 (2H, d, 8.3)	6.55 (2H, d, 8.3)	6.45 (2H, d, 8.6)	6.51 (2H, d, 8.6)
7b	6.12 (1H, br s)	6.20 (1H, br s)	5.59 (1H, br s)	5.57 (1H, br d)
8b			5.43 (1H, br s)	5.40 (1H, br d)
12b	7.04 (1H, d, 2.1)	7.05 (1H, d, 2.1)	6.67 (1H, d, 2.0)	6.78 (1H, d, 2.0)
14b	7.34 (1H, d, 2.1)	7.44 (1H, d, 2.1)	7.22 (1H, d, 2.0)	7.12 (1H, d, 2.0)
OH-8b			5.35 (1H, br d)	4.89 (1H, br s)
Ar-OH(OMe)	8.92 (1H, br s, OH-4a)	3.89 (3H, s, OMe-4a)	7.81–8.46 (5H, br s)	3.84 (3H, s, OMe-4a)
	8.80 (1H, br s, OH-11a)	3.85 (3H, s, OMe-11a)		3.81 (3H, s, OMe-11a)
	8.36 (1H, br s, OH-13a)	3.52 (3H, s, OMe-13a)		3.46 (3H, s, OMe-13a)
	8.00 (1H, br s, OH-4b)	3.60 (3H, s, OMe-4b)		3.60 (3H, s, OMe-4b)
	8.80 (1H, br s, OH-13b)	3.86 (3H, s, OMe-13b)		3.82 (3H, s, OMe-13b)

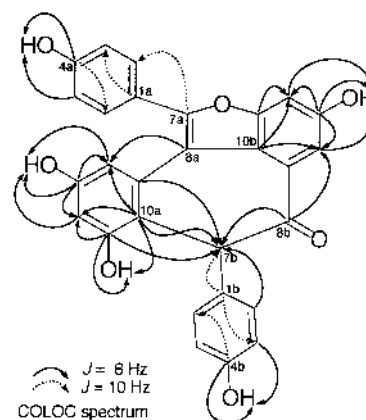
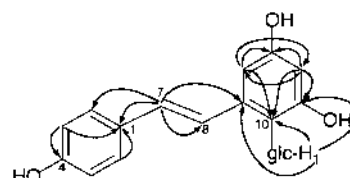
a) Measured in CD_3COCD_3 . b) Measured in CDCl_3 . All protons were assigned by $^1\text{H-NMR}$, $^1\text{H-NMR}$ long range, $^{13}\text{C-NMR}$ COSY, COLOC and HMBC spectrum.

Table 2. $^{13}\text{C-NMR}$ Spectral Data (75 MHz) of **1**, **1a**, **8** and **8a**

No.	δ_{C}			
	1 ^{a)}	1a ^{b)}	8 ^{a)}	8a ^{b)}
1a	123.1	123.2	124.4	124.3
2a, 6a	131.0	130.2	130.8	130.2
3a, 5a	116.7	114.2	116.4	113.9
4a	159.5	160.6	158.6	160.0
7a	153.3	152.9	150.8	150.8
8a	116.4	116.2	117.1	116.3
9a	135.3	134.1	135.6	134.1
10a	114.1	115.8	120.5	121.9
11a	158.3	159.7	157.0	158.5
12a	103.1	98.6	102.4	98.0
13a	157.7	159.0	156.7	158.2
14a	109.0	105.5	109.7	106.0
1b	131.1	130.5	133.0	131.7
2b, 6b	128.5	127.6	130.4	129.5
3b, 5b	115.7	113.3	114.8	113.0
4b	156.4	157.7	155.5	157.4
7b	56.1	56.2	48.4	47.6
8b	196.4	196.0	74.4	73.5
9b	129.9	129.9	139.7	137.2
10b	122.5	122.4	118.5	117.9
11b	154.9	154.0	154.7	153.9
12b	102.5	101.2	95.9	93.8
13b	156.1	157.6	156.1	158.1
14b	112.0	109.7	110.2	108.2
OMe		55.0 (3×C)		55.1 (C-4a)
		55.4		55.6 (C-11a)
		56.1		54.9 (C-13a) ^{c)}
				54.9 (C-4b) ^{c)}
				54.9 (C-13b) ^{c)}

a) Measured in CD_3COCD_3 . b) Measured in CDCl_3 . c) Overlapping. All carbons were assigned by $^{13}\text{C-NMR}$ COSY, COLOC and HMBC spectrum.

cular weight, the structure of **1** was characterized as shown in Fig. 2, where the benzofuran ring was fused to ring B₂. The benzyl methine proton (H-7b) appears in a lower field than that of **8**, which could be explained by the deshielding effect which is caused by the carbonyl group. Stilbenoids having benzofuran moiety such as hopeafuran have been isolated from *Vitis vinifera*,¹⁴ *Hopea malbato*¹³ and *Shorea* sp.

Fig. 2. Correlations Observed in the COLOC Spectrum of **1**Fig. 3. HMBC Spectrum of **2**

(Dipterocarpaceae).¹⁵⁾

Compound **2** was positive to the Gibbs reagent and had the molecular formula of $\text{C}_{20}\text{H}_{22}\text{O}_8$, supported by the HR-FAB-MS (m/z 389.1244). The $^1\text{H-NMR}$ spectrum showed the signals due to a 4-hydroxyphenyl group [δ 7.36 (2H, d, $J=8.5$ Hz, H-2, 6), 6.79 (2H, d, $J=8.5$ Hz, H-3, 5)] and a set of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring [δ 6.22 (1H, d, $J=2.4$ Hz, H-12), 6.56 (1H, d, $J=2.4$ Hz, H-14)]. *Trans* geometry of two olefinic protons [δ 6.76 (1H, d, $J=16.3$ Hz, H-7), 7.48 (1H, br d, $J=16.3$ Hz, H-8)] were also exhibited in the spectrum. The $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectral data showed the presence of a *C*-glucopyranosyl moiety [δ_{H} 4.92 (1H, d, $J=9.6$ Hz); δ_{C} 81.7, 79.5, 78.5, 74.4, 70.9, 61.9].^{10,11)} In the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 3), distinct correlations

were observed between the olefinic proton (H-7) and aromatic carbons [C-2(6), C-9], suggesting that the aglycone moiety was resveratrol. The location of the C-glucosyl moiety was determined to be at C-10 by the HMBC spectrum which displayed the cross peak between the anomeric proton (δ 4.92) and the three aromatic carbons at C-9 (δ 140.7), C-10 (δ 114.7) and C-11 (δ 158.5). Therefore, the structure of **2** was concluded to be 10-C- β -glucopyranosylresveratrol.

Eight known stilbenoids, resveratrol (**3**), dihydroresveratrol (**4**),¹⁶ (-)- ϵ -viniferin (**5**),¹³ (+)-ampelopsin A (**6**),¹⁷ (-)-balanocarpol (**7**),¹⁸ malibatol A (**8**),¹³ vaticanol B (**11**),⁸ (-)-hopeaphenol (**12**)¹⁹ and (+)-isohopeaphenol (**13**)¹⁹ were identified in addition to bergenin (**9**)²⁰ and (+)-lyoniresinol (**10**)²¹ by the analysis of spectral data and by comparison with authentic samples. Isolation and structure elucidation of other stilbenoids in acetone and MeOH extracts is now in progress.

Experimental

¹H- and ¹³C-NMR spectra were recorded on JNM EX-400 and LA-300 (JEOL) spectrometers. Chemical shifts are shown as δ values with tetramethylsilane (TMS) as an internal reference. Peak multiplicities are quoted in Hz. Negative ion FAB-MS was measured on a JMS-DX-300 spectrometer equipped with a JMA 3500 data analysis system (JEOL). UV spectra were recorded on a UV-2200 spectrometer (Shimadzu), IR spectra on a FT-IR-8000 spectrometer (JASCO), and optical rotations on a P-1020 (JASCO) polarimeter. Silica gel 60 (70–230 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography; silica gel 60H (Merck) was used for vacuum liquid chromatography (VLC); Kiesel-gel 60F₂₅₄ (Merck) was used as analytical and preparative TLC.

Extraction and Isolation of Compounds (1–13) Dried stem wood of *Hopea utilis* (1.0 kg) collected in India in October, 1999 was powdered and extracted successively with acetone (31 \times 3), MeOH (31 \times 3) and 70% MeOH (31 \times 3) at room temperature. A part (160 g) of the acetone extract (180 g) was chromatographed on Si gel (1200 g) eluted with CHCl₃–MeOH mixtures to divide into 17 fractions (Fr. 1–17). Compounds **3** (12 mg), **4** (6 mg) and **10** (21 mg) were obtained from Fr. 7 [CHCl₃–MeOH (10:1) fraction] after purification by Sephadex LH-20 (acetone) and preparative TLC (EtOAc–CHCl₃–MeOH–H₂O=80:40:11:2). Fraction 9 [CHCl₃–MeOH (10:1) fraction] was further chromatographed by Sephadex LH-20 using MeOH to give 10 fractions. Compound **9** (1.8 g) was obtained in a pure form after recrystallization (acetone) from the third fraction. The sixth fraction afforded **1** (65 mg) and **5** (250 mg) after purification by Sephadex LH-20 column [acetone–MeOH (5:1)]. Fraction 13 [CHCl₃–MeOH (6:1) fraction] was repeatedly chromatographed by Sephadex LH-20 [MeOH and acetone–MeOH (5:1)] to isolate **8** (80 mg), **6** (85 mg) and **7** (1.3 g). Compounds **2** (12 mg), **11** (2.3 g), **12** (3.6 g) and **13** (9 mg) were obtained from Fr. 16 [CHCl₃–MeOH (6:1) fraction] after purification by Sephadex LH-20 (MeOH) and preparative TLC (EtOAc–CHCl₃–MeOH–H₂O=15:8:4:1).

Compound **1** (Hopeafuran): A yellow solid. Negative ion HR-FAB-MS: ([M–H][–]) *m/z* 465.0961 (Calcd 465.0974 for C₂₈H₁₇O₇); Negative ion FAB-MS: *m/z* 465 ([M–H][–]). UV λ_{\max} (MeOH) nm: 212, 255 sh, 297, 396. IR λ (KBr) cm^{–1}: 3384, 1653, 1613, 1512. [α]_D²⁴ –46° (*c*=0.1, MeOH). The ¹H- and ¹³C-NMR spectral data are listed in Tables 1 and 2, respectively.

Methylation of 1 Compound **1** (20 mg) was allowed to react with K₂CO₃ (2 g) and MeI (0.5 g) in dry acetone under reflux for 6 h. The crude product (22 mg) was purified by preparative TLC [*n*-hexane–EtOAc (1:1)]

to afford **1a** as an amorphous yellow solid (15 mg). The ¹H- and ¹³C-NMR spectral data are listed in Tables 1 and 2, respectively.

Compound **2** (Resveratrol-10-C- β -glucopyranoside): A white amorphous powder. Negative ion HR-FAB-MS: ([M–H][–]) *m/z* 389.1244 (Calcd 389.1236 for C₂₀H₂₁O₈); Negative ion FAB-MS: *m/z* 389 ([M–H][–]). UV λ_{\max} (MeOH) nm: 212, 293. [α]_D²⁴ +23° (*c*=0.1, MeOH). ¹H-NMR (300 MHz, acetone-*d*₆) δ : 7.36 (2H, d, *J*=8.5 Hz, H-2, 6), 6.79 (2H, d, *J*=8.5 Hz, H-3, 5), 6.76 (1H, d, *J*=16.3 Hz, H-7), 7.48 (1H, br d, H-8), 6.22 (1H, d, *J*=2.4 Hz, H-12), 6.56 (1H, d, *J*=2.4 Hz, H-14), 4.92 (1H, d, *J*=9.6 Hz, H-glc-1), 3.71 (1H, t, *J*=9.6 Hz, H-glc-2), 3.58 (1H, t, *J*=9.6 Hz, H-glc-3), 3.67 (1H, t, *J*=9.6 Hz, H-glc-4), 3.44 (1H, dd, *J*=9.6, 3.5 Hz, H-glc-5), 3.81 (2H, m, H-glc-6); ¹³C-NMR [75 MHz, acetone-*d*₆] δ : 130.4 (C-1), 128.8 (C-2, 6), 116.4 (C-3, 5), 158.1 (C-4), 131.0 (C-7), 126.0 (C-8), 140.7 (C-9), 114.7 (C-10), 158.5 (C-11), 104.0 (C-12), 158.5 (C-13), 105.7 (C-14), 78.5 (C-glc-1), 74.4 (C-glc-2), 79.5 (C-glc-3), 70.9 (C-glc-4), 81.7 (C-glc-5), 61.9 (C-glc-6).

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