Two New Resveratrol Tetramers from *Upuna borneensis*

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Phytochemical investigation of an acetone extract of *Upuna borneensis* (Dipterocarpaceae) resulted in the isolation of two new resveratrol tetramers, upunaphenols O (1) and P (2). The structures were elucidated by spectroscopic analysis including NMR experiments.

Key words *Upuna borneensis*; Dipterocarpaceae; resveratrol oligomer; upunaphenol

During the course of our research on the isolation and identification of bioactive polyphenols in Dipterocarpaceaeous plants, we have previously reported the structural variety of resveratrol oligomers in *Upuna borneensis*.^{1–7} Further detailed examination of the acetone extract of stem yielded two new resveratrol tetramers, upunaphenols O (1) and P (2). The structure of isolates 1 and 2 were elucidated by means of 2D NMR techniques such as ¹H–¹H shift correlation spectroscopy (COSY), ¹³C–¹H COSY, and ¹H–¹³C heteronuclear multiple-bond correlation (HMBC), and the stereostructures were proposed by analysis of the nuclear Overhauser spectroscopy (NOESY) spectra.

Results and Discussion

Upunaphenols O (1) ([α]_D²⁵ +84°), and P (2) ([α]_D²⁵ +180°) were purified from an acetone-soluble stem segment of *U. borneensis* by column chromatography over silica gel, Sephadex LH-20, octadecyl silica (ODS), and PTLC (preparative TLC).

Upunaphenol O (1), obtained as a pale yellow solid, showed positive reaction to the Gibbs reagent. The molecular formula of C₅₆H₄₂O₁₂ was established by an [M+H]⁺ ion peak at *m/z* 907.2732 in the electron spray ionization (ESI)-

MS together with the NMR spectral data, which suggested that 1 is a resveratrol tetramer and bears 36 degrees of unsaturation. Acetylation of 1 yielded a nona-acetate (ESI-MS: *m/z* 1307.3484 [M+Na]⁺), suggesting that 1 bears nine phenolic hydroxyl groups. The ¹H- and ¹³C-NMR spectral data together with ¹H–¹H COSY, ¹³C–¹H COSY and HMBC spectra (Fig. 1, Table 1) showed the presence of *ortho*-coupled aromatic protons assignable to three 4-hydroxyphenyl groups (rings A₁, B₁ and D₁), a 4-oxygenated phenyl group (ring C₁), three sets of *meta*-coupled aromatic protons on a 1,2,3,5-tetrasubstituted benzene ring (rings A₂, B₂ and C₂), and a 3,5-dihydroxyphenyl group (D₂). The NMR spectral data also disclosed the presence of two sets of aliphatic signals characteristic for 2,3-diaryldihydrobenzofuran moieties (H-7a/H-8a; H-7d/H-8d),⁸⁾ two coupled aliphatic methine protons (H-7b/H-8b), *cis*-coupled olefinic protons (H-7c/H-8c), and nine phenolic hydroxyl groups (δ_{H} 7.97–8.64). Considering the molecular formula, the remaining three oxygens can be allocated to ether linkage. In the HMBC spectrum (Fig. 1), significant ³*J* correlations were observed between H-7a/C-2a(6a), H-14a/C-8a, H-7b/C-2b(6b), H-14b/C-8b, H-7c/C-2c(6c), H-8c/C-14c, H-7d/C-2d(6d), and H-8d/C-10d(14d), indicating that rings A₁, A₂, B₁, B₂, C₁, C₂, D₁, and D₂ are attached at C-7a, C-8a, C-7b, C-8b, C-7c, C-8c, C-7d, and C-8d, respectively. Further correlations observed between H-8a/C-11b, H-7b/C-11a, and H-8d/C-11c supported

Fig. 1. Selected Correlations in 2D NMR of 1

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Table 1. ^1H - and ^{13}C -NMR Spectral Data of **1** and **2**

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1a		133.5		133.8
2a(6a)	7.42 (d, 8.4)	130.4	7.24 (d, 8.4)	128.2
3a(5a)	6.94 (d, 8.4)	116.5	6.78 (d, 8.4)	116.1 ⁱ⁾
4a		158.49 ⁱ⁾		158.1 ^{g)}
7a	5.78 (d, 9.5)	93.5	6.10 (d, 4.2)	90.0
8a	5.32 (d, 9.5)	52.1	4.78 (d, 4.2)	52.0
9a		141.4		142.1
10a		117.9		121.1
11a		158.1 ^{c)}	7.93 (br s)	158.5
12a	6.27 (br s) ^{e)}	102.1	6.06 (d, 2.2)	102.4
13a		156.8		156.4 ^{b)}
14a	6.27 (br s) ^{e)}	106.6	6.55 (br s)	105.2
1b		134.0		137.6
2b(6b)	6.82 (d, 8.6)	130.7 ^{d)}	6.89 (d, 8.4)	131.2
3b(5b)	6.52 (d, 8.6)	115.1	6.67 (d, 8.4)	115.4
4b		155.9		156.4 ^{b)}
7b	5.33 (d, 5.8)	47.1	4.04 (d, 12.8)	45.9
8b	5.41 (d, 5.8)	83.0	3.54 (d, 12.8)	46.4
9b		137.4		132.8
10b		118.2		124.4
11b		160.9		156.9
12b	6.03 (d, 2.2)	96.9	6.32 (s)	97.2
13b		158.54 ⁱ⁾		152.7
14b	5.91 (d, 2.2)	109.5		115.5
1c		130.6 ^{e)}		127.2
2c(6c)	7.02 (d, 8.6)	130.6 ^{e)}	6.56 (d, 8.6)	131.1
3c(5c)	6.79 (d, 8.6)	116.8	6.39 (d, 8.6)	114.9
4c		158.0		161.3
7c	6.21 (d, 13.2)	130.7 ^{d)}		195.0
8c	6.03 (d, 13.2)	126.5	4.48 (s)	46.3
9c		136.8		133.9
10c		120.0		122.2
11c		162.5		160.6
12c	6.31 (d, 2.2)	96.7	6.54 (s)	98.9
13c		159.2		155.2
14c	6.25 (d, 2.2)	108.4		116.8
1d		133.7		133.7
2d(6d)	7.11 (d, 8.4)	127.9	7.32 (d, 8.6)	127.9
3d(5d)	6.84 (d, 8.4)	116.1	6.93 (d, 8.6)	116.1 ⁱ⁾
4d (OH)	8.47 (br s)	158.1 ^{c)}		158.1 ^{g)}
7d	5.32 (d, 5.3)	93.8	5.32 (d, 6.2)	94.3
8d	4.04 (d, 5.3)	57.1	4.54 (d, 6.2)	58.4
9d		146.8		145.0
10d(14d)	6.05 (d, 2.2)	106.8	5.75 (d, 2.0)	107.0
11d(13d)		159.5		159.1
12d	6.18 (t, 2.2)	101.9	5.52 (t, 2.0)	101.5
OH groups	8.64 (br s, OH-4a)			
	8.03 (br s, OH-11a)			
	8.10 (br s, OH-13a) ^{b)}			
	7.97 (br s, OH-4b)			
	8.10 (br s, OH-13b) ^{b)}			
	8.37 (br s, OH-13c)			
	8.13 (br s, OH-11d(13d))			

Measured in acetone- d_6 at 300 MHz (^1H -NMR) and 75 MHz (^{13}C -NMR). a—h) Overlapping. i) Interchangeable.

the links between C-8a/C-10b, C-7b/C-10a, and C-8d/C-10c, respectively. Additional cross peaks observed between H-7a/C-11b, H-8b/C-4c, and H-7d/C-11c supported the presence of ether linkage (C-7a/O/C-11b, C-8b/O/C-4c, C-7d/O/C-11c). The planar structure of upunaphenol O, that included two dihydrobenzofuran rings, has been concluded to be **1**. The stereostructure of **1** was determined by analysis of the NOESY spectrum with the assistance of computer-aided

Fig. 2. Selected NOEs Observed in the NOESY Experiment with **1** Model of **1** Drawn by Pcmol 9.1 Molecular Modeling Program

molecular modeling (Fig. 2). A 3D structure of **1** was generated with the Pcmol 9.1 molecular modeling program software, using the MMFF94 force field (MM2 type) for energy minimization.⁹⁾ The *trans* orientations of H-7a/H-8a and H-7d/H-8d on the dihydrobenzofuran rings were confirmed by the distinctive NOEs between H-7a/H-14a, H-14a/H-2a(6a), H-8a/H-2a(6a), H-7d/H-10d(14d), H-2d(6d)/H-10d(14d) and H-8d/H-2d(6d). The strong NOE (H-14a/H-2a(6a)) and the large coupling constant (H-7a/H-8a: $J=9.5$ Hz) supported the *trans* diequatorial orientation of two aromatic rings (rings A₁ and A₂) and *trans* diaxial orientation of H-7a/H-8a. Significant NOE between H-8a/H-2b(6b) indicates that ring B₁ and H-8a are of *syn* orientation. The *syn*-orientation of H-8b and ring B₁ was deduced based on the cross peak for H-8b/H-2b(6b) in the spectrum. From these results, the relative configuration of six asymmetric carbons in **1**, C-7a, C-8a, C-7b, C-8b, C-7d, and C-8d, were determined as *rel-R*, *R*, *R*, *S*, *R*, and *R*, respectively. The structure can be regarded as a complex product composed of two resveratrol dimer units **1A** and **1B** [unit **1A**: resveratrols A—B (resveratrol A: ring A₁-C-7a-C-8a-ring A₂); unit **1B**: resveratrols C—D]. The structures of **1A** and **1B** are identical with resveratrol dimers, ampelopsin A and (*Z*)- ϵ -viniferin, respectively. Both compounds have also been isolated from the same material.³⁾ The both dimers are then presumed to be precursors of **1**.

Upunaphenol P (**2**) was obtained as a pale yellow amorphous solid. The composition was deduced to be C₅₆H₄₀O₁₃ from the pseudo-molecular ion peak of $[\text{M}+\text{H}]^+$ at m/z 921.2527 in the ESI-MS spectrum and the ^{13}C -NMR spectrum which showed 56 carbon signals. Acetylation of **2** yielded a deca-acetate (ESI-MS: m/z 1365 $[\text{M}+\text{Na}]^+$), suggesting the presence of 10 phenolic hydroxyl groups. A signal in the ^{13}C -NMR spectrum (δ_{C} 195.0) showed the presence of a carbonyl group (C-7c) in the molecule. Analysis of the ^1H - and ^{13}C -NMR spectral data (Table 1), ^1H - ^1H , and ^{13}C - ^1H COSY and HMBC spectral data revealed four resveratrol units (resveratrols A—D) consisting of four 4-hydroxyphenyl groups (rings A₁—D₁), a 3,5-dioxygenated-1,2-tetrasubstituted benzene ring (ring A₂), two 3,5-dioxygenated-1,2,6-pentasubstituted benzene rings (rings B₂ and C₂), a 3,5-dihydroxy benzene ring (ring D₂), two set of mutually coupled aliphatic protons characterized 2,3-diaryldihydrobenzofuran moieties (H-7a/H-8a; H-7d/H-8d), and a sequence of three aliphatic protons (H-7b/H-8b/H-8c). Due to 3J cross peaks in the HMBC spectrum (Fig. 3), these partial

Fig. 3. Main Connectivities from the HMBC and ^1H - ^1H COSY Experiments with **2**

Fig. 4. Selected NOEs Observed in the NOESY Experiment with **2** Model of **2** Drawn by Pmodel 9.1 Molecular Modeling program

structures could be connected except for a bond for C-14b and C-14c. The established partial structure satisfied all the oxygen's function and accounted for 36 of the 37 required degrees of unsaturation, which suggested that the ring formation was required. The C-14b-C-14c bond was assumed after consideration of the molecular skeleton. The planar structure of **2** was thus characterized. The planar structure of **2** is similar to upunaphenol I except for a presence of a carbonyl group (C-7c) instead of an absence of a C-C bond (C-7c/C-10d) in upunaphenol I.⁵⁾ The relative configuration of **2** was determined by NOESY experiments (Fig. 4). All *trans* orientations of two 2,3-diaryldihydrobenzofuran rings were determined by the NOEs observed between H-7a/H-14a, H-14a/H-2a(6a), H-8a/H-2b(6b), H-7d/H-10d(14d), and H-8d/H-2d(6d). The relative *cis* disposition for H-8a, ring B₁, H-8b, ring D₂, and a 4-hydroxybenzoyl group (ring C₁) were inferred from NOEs for H-8a/H-2a(6a), H-8a/H-8b, H-8b/H-2c(6c), and H-2c(6c)/H-10d(14d). The results explain well the stereostructure of **2**, where all the asymmetric carbons are situated in the same manner as upunaphenol I.⁵⁾ Previously, we have reported the co-presence of resveratrol tetramers, upunaphenols B and H—J, (–)-hopeaphenol and stenophyllol A, in the same material, that could be derived from the main component, (–)-hopeaphenol.³⁾ Compound **2** is also presumed to have biogenetic relation with hopeaphenol.

Experimental

The following instruments were used: optical rotations, JASCO P-1020 polarimeter; UV spectra, Shimadzu UV-2200 spectrophotometer (in MeOH solution); ^1H - and ^{13}C -NMR spectra, JEOL JNM LA-300 (chemical shift

values are presented as δ values with tetramethylsilane (TMS) as internal standard); ESI-MS, Thermo Fisher Scientific LTQ Orbitrap instrument.

The following adsorbents were used for purification: analytical TLC, Merck Kieselgel 60 F₂₅₄ (0.25 mm); preparative TLC, Merck Kieselgel 60 F₂₅₄ (0.5 mm); column chromatography, Merck Kieselgel 60, Pharmacia Fine Chemicals AB Sephadex LH-20 and Fuji Silysia Chemical Chromatex.

Upuna borneensis SYM. was cultivated in Bogor Botanical Garden, Bogor, Indonesia, and its stems were collected in May 2000 and identified by one of co-authors (D.D.). A voucher specimen (number DP-012) has been deposited in Gifu Pharmaceutical University.

Extraction and Isolation of Compounds (1 and 2): The extraction procedure was the same as that on our previous reports.^{1–7)} Fr. 7 (CHCl_3 -MeOH, 9:1) was further subjected to Sephadex LH-20 CC (MeOH) to give eight fractions (Fr. 7a—Fr. 7h). Compound **2** (17 mg) was purified from the fraction of Fr. 7e after purification by Sephadex LH-20 CC (MeOH) and PTLC (EtOAc- CHCl_3 -MeOH- H_2O , 15:8:4:1). Fr. 8 (CHCl_3 -MeOH, 8:1) was divided into seven parts (Fr. 8a—Fr. 8g) in the same way as that of Fr. 7. Sub fraction of Fr. 8f gave **1** (12 mg) after CC over ODS (MeOH- H_2O , 6:4).

Compound 1 (Upunaphenol O): A pale yellow solid; $[\alpha]_{\text{D}}^{25} + 84^\circ$ ($c=0.1$, MeOH); UV (MeOH) λ_{max} (log ϵ): 207 (4.91), 225sh (4.75), 285 (4.07) nm; positive ion ESI-MS m/z : 907.2732 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{56}\text{H}_{43}\text{O}_{12}$: 907.2749); ^1H - and ^{13}C -NMR spectral data [^1H (300 MHz), ^{13}C (75 MHz), acetone- d_6], see Table 1; HMBC correlations, see Fig. 1 (selected) and H-2a(6a)/C-4a, C-7a; H-3a(5a)/C-1a, C-4a; OH-4a/C-3a(5a), C-4a; H-7a/C-9a; H-8a/C-1a, C-9a, C-10b; OH-11a/C-10a, C-11a, C-12a; H-12a/C-10a, C-11a, C-13a; OH-13a/C-12a, C-13a, C-14a; H-14a/C-10a, C-12a, C-13a; H-2b(6b)/C-4b, C-7b; H-3b(5b)/C-1b, C-4b; OH-4b/C-3b(5b), C-4b; H-7b/C-9a, C-10a, C-1b, C-9b; H-8b/C-10a, C-1b, C-9b, C-10b, C-14b; H-12b/C-10b, C-11b, C-13b, C-14b; OH-13b/C-12b, C-13b, C-14b; H-14b/C-10b, C-12b, C-13b; H-2c/C-4c, C-7c; H-3c(5c)/C-1c, C-4c; H-7c/C-9c; H-8c/C-1c, C-9c, C-10c; H-12c/C-10c, C-11c, C-13c, C-14c; OH-13c/C-12c, C-13c, C-14c; H-14c/C-10c, C-12c; H-2d(6d)/C-4d, C-7d; H-3d(5d)/C-1d, C-4d; OH-4d/C-3d(5d), C-4d; H-7d/C-9d; H-8d/C-9c, C-10c, C-1d; H-10d(14d)/C-11d(13d), C-12d; OH-11d(13d)/C-10d(14d), C-11d(13d), C-12d; H-12d/C-11d(13d); NOESY correlations: see Fig. 2 (selected) and H-3a(5a)/OH-4a; H-2a(6a)/H-7a; OH-11a/H-12a, H-7b; H-12a/OH-13a; OH-13a/H-14a; H-2b(6b)/H-7b; H-3b(5b)/OH-4b; H-8b/H-3c(5c); H-12b/OH-13b; OH-13b/H-14b, H-3c(5c); H-2c(6c)/H-10d(14d), OH-13c; H-8c/H-8d; H-12c/OH-13c; OH-13c/H-14c; H-2d(6d)/H-7d; H-3d(5d)/OH-4d; H-8d/H-10d(14d); H-10d(14d)/H-2c(6c), OH-11d(13d); OH-11d(13d)/H-12d.

Acetylation of 1 A solution of **1** (1 mg) in pyridine (0.5 ml) containing Ac_2O (0.1 ml) was kept at rt for 24 h. Workup in the usual manner and the purification of the resulting crude product (1 mg) by prep. TLC (*n*-hexane/EtOAc 1:1) afforded a nona-acetate (1 mg). A pale yellow solid; positive ion ESI-MS m/z : 1307.3484 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{74}\text{H}_{60}\text{O}_{21}\text{Na}$: 1307.3519).

Compound 2 (Upunaphenol P): A pale yellow amorphous solid; $[\alpha]_{\text{D}}^{25} + 180^\circ$ ($c=0.3$, MeOH); UV (MeOH) λ_{max} (log ϵ): 225sh (4.50), 279 (3.96), 323 (3.52) nm; positive ion ESI-MS m/z : 921.2527 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{56}\text{H}_{41}\text{O}_{13}$: 921.2542); ^1H - and ^{13}C -NMR spectral data [^1H (300 MHz), ^{13}C (75 MHz), acetone- d_6], see Table 1; HMBC correlations: see Fig. 3 (selected) and H-2a(6a)/C-4a, C-7a; H-3a(5a)/C-1a, C-4a; H-7a/C-1a, C-9a; H-8a/C-10a, C-10b; OH-11a/C-10a, C-11a; H-12a/C-10a, C-13a, C-14a; H-14a/C-10a, C-13a; H-2b(6b)/C-4b, C-7b; H-3b(5b)/C-1b, C-4b; H-7b/C-9a, C-10a, C-1b; H-8b/C-1b, C-9b; H-12b/C-10b, C-11b, C-13b, C-14b; H-2c(6c)/C-4c; H-3c(5c)/C-1c, C-4c; H-8c/C-9c; H-12c/C-10c, C-11c, C-13c, C-14c; H-2d(6d)/C-4d, C-7d; H-3d(5d)/C-1d, C-4d; H-7d/C-1d, C-9d; H-8d/C-1d; H-10d(14d)/C-11d(13d), C-12d; NOESY correlations: see Fig. 4 (selected) and H-2a(6a)/H-7a; OH-11a/H-12a, H-7b; H-2b(6b)/H-7b; H-3c(5c)/H-10d(14d); H-2d(6d)/H-7d; H-8d/H-10d(14d).

Acetylation of 2 Compound **2** (2 mg) was treated in the same way as **1** to afford a deca-acetate (2 mg). A pale yellow solid; positive ion ESI-MS m/z : 1363.3398 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{76}\text{H}_{60}\text{O}_{23}\text{Na}$: 1363.3418).

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