HETEROCYCLES, Vol. 63, No. 1, 2004, PP. 129 - 136
Received, 17th September, 2003, Accepted, 31st October, 2003, Published online, 10th November, 2003

A NEW RESVERATROL HEXAMER FROM *UPUNA BORNEENSIS*

Tetsuro Ito,[a,*] Toshiyuki Tanaka,[a] Zulfiqar Ali,[a] Yukihiro Akao,[b] Yoshinori Nozawa,[b] Yoshikazu Takahashi,[c] Ryuichi Sawa,[c] Ken-ichi Nakaya,[a] Jin Murata,[d] Dedy Darnaedi,[e] and Munekazu Iinuma[f]

a) Gifu Prefectural Institute of Health and Environmental Sciences, 1-1 Naka-fudogaoka, Kakamigahara 504-0838, Japan

b) Gifu International Institute of Biotechnology, 1-1 Naka-fudogaoka, Kakamigahara 504-0838, Japan

c) Microbial Chemistry Research Center, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

d) Botanical Gardens, Koishikawa, Graduate School of Science, University of Tokyo, 3-7-1 Hakusan, Bunkyo-Ku, Tokyo, 112-0001, Japan

e) Herbarium Bogoriense, The Indonesian Institute of Science, Research and Development Center for Biology, J1 Ir. Juanda 18, Bogor 16122, Indonesia

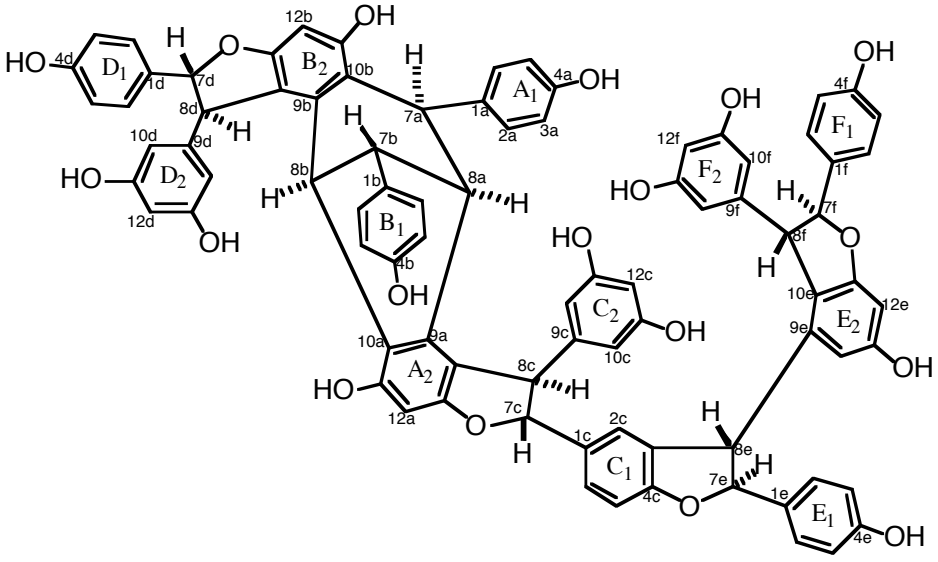
f) Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan

Abstract - A new resveratrol hexamer, upunaphenol A, was isolated from an acetone soluble part of stem of *Upuna borneensis* (Dipterocarpaceae). The structure, which has twelve asymmetric carbon atoms on the partial structures of a dibenzobicyclo[3.2.1]octadiene ring and four dihydrobenzofuran rings, was determined by spectral analysis including 1D and 2D NMR spectral experiments. Resveratrol and four known resveratrol oligomers, ampelopsin F, isoampelopsin F, vaticanols C and B, were also isolated. Upunaphenol A was found to suppress cell growth in HL60 cells through induction of apoptosis with IC_{50} at 9.2 □M.

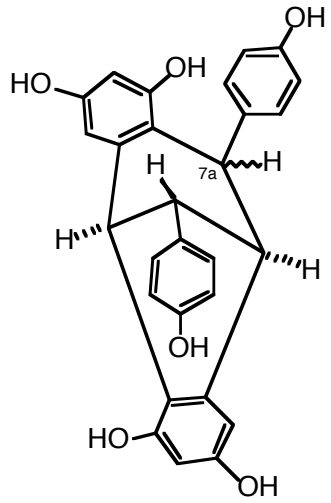
INTRODUCTION

Much attention has been paid to biologically active polyphenols of phytochemicals in the last decade. Among various studies of polyphenols, stilbenoids are the most behind-hand phytochemicals. As the various biological activities of resveratrol (3,5,4'-trihydroxystilbene) oligomers such as cytotoxicity,[1]

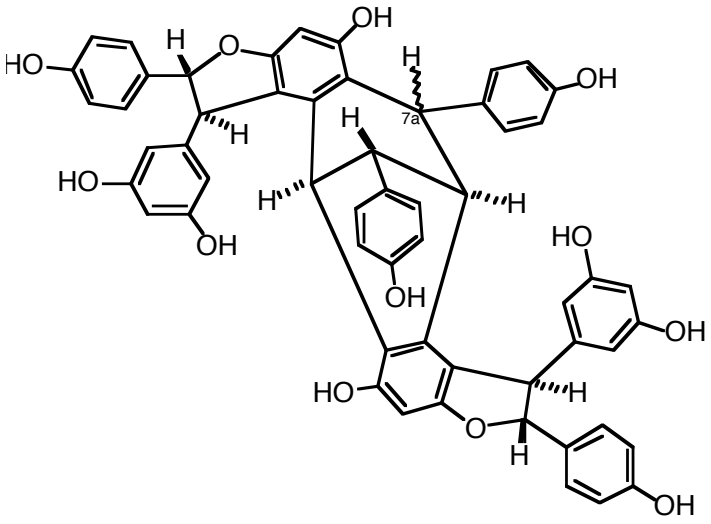
anti-virus[2] and anti-inflammatory[3] have been recently revealed, more detailed and accumulated information on their chemistry is desired for the development of new drug candidates. Dipterocarpaceaeous plants are known to be abundant natural resources of resveratrol oligomers.[4] *Upuna borneensis*, a monotypic genus belongs to the largest subfamily Dipterocarpoideae in Dipterocarpaceae.[5] Although some phytochemicals in the subfamily were investigated, no examination of the genus *Upuna* has been reported yet. In relation to our continuous phytochemical studies of the family,[6–10] the chemical constituents in stem of *U. borneensis* were examined. We report in this paper the isolation and the structure of a new resveratrol hexamer, upunaphenol A (**1**). Inhibitory effects of **1** and the related compounds on growth of HL60 cells are also described.



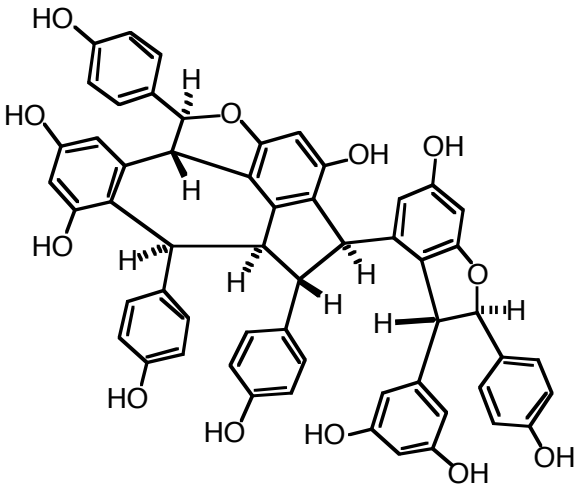
1



3 H-7a = β
4 H-7a = α



5 H-7a = β
7 H-7a = α



6

Figure 1

RESULTS AND DISCUSSION

Upunaphenol A (**1**) ([□]$^{25}_{D}$ □120°) was isolated as a pale yellow amorphous powder from an acetone soluble part of stem of *Upuna borneensis* by column chromatography on silica gel, Sephadex LH-20 and PTLC. Resveratrol (**2**) and four known resveratrol oligomers were also isolated from the acetone soluble part and identified as ampelopsin F (**3**), isoampelopsin F (**4**), vaticanols C (**5**) and B (**6**) (Figure 1),[6] respectively, by spectral comparison with the authentic samples.

Upunaphenol A (**1**) showed positive reaction to the Gibbs reagent. The UV spectrum displayed absorption maxima at 285 nm, which is consistent with the presence of one or more nonconjugated phenyl rings. The ESIMS exhibited an $[M+Na]^+$ ion peak at *m/z* 1381. The molecular formula of $C_{84}H_{62}O_{18}$ was deduced by HR-ESIMS at *m/z* 1381.3858 $[M+Na]^+$ and the ^{13}C NMR spectrum which showed 84 carbon signals. The ^1H and ^{13}C NMR spectral data (Table) coupled with ^1H□^1H COSY, ^{13}C□^1H COSY and HMBC spectra indicated the presence of 12 aromatic rings which formed five 1,4-disubstituted phenyl groups (rings A_1, B_1, D_1□F_1), a 1,3,4-trisubstituted benzene ring (ring C_1), two 1,2,3,5,6-pentasubstituted benzene rings (rings A_2, B_2), three 1,3,5-trisubstituted benzene rings (rings C_2, D_2, F_2) and a 1,2,3,5-tetrasubstituted benzene ring (ring E_2). The chemical shift pattern of the carbons on the rings A_1-F_1 was characteristic of a 4-oxygenated phenyl group, and that of the rings A_2-F_2 was of a 3,5-dioxygenated phenyl groups.[6□10] The spectrum also exhibited four sets of mutually coupled aliphatic protons (H-7c/H-8c, H-7d/H-8d, H-7e/H-8e and H-7f/H-8f). Four aliphatic protons (H-7a, H-8a, H-7b, H-8b) were further observed in the spectrum, and were coupled in this order in the ^1H□^1H long range

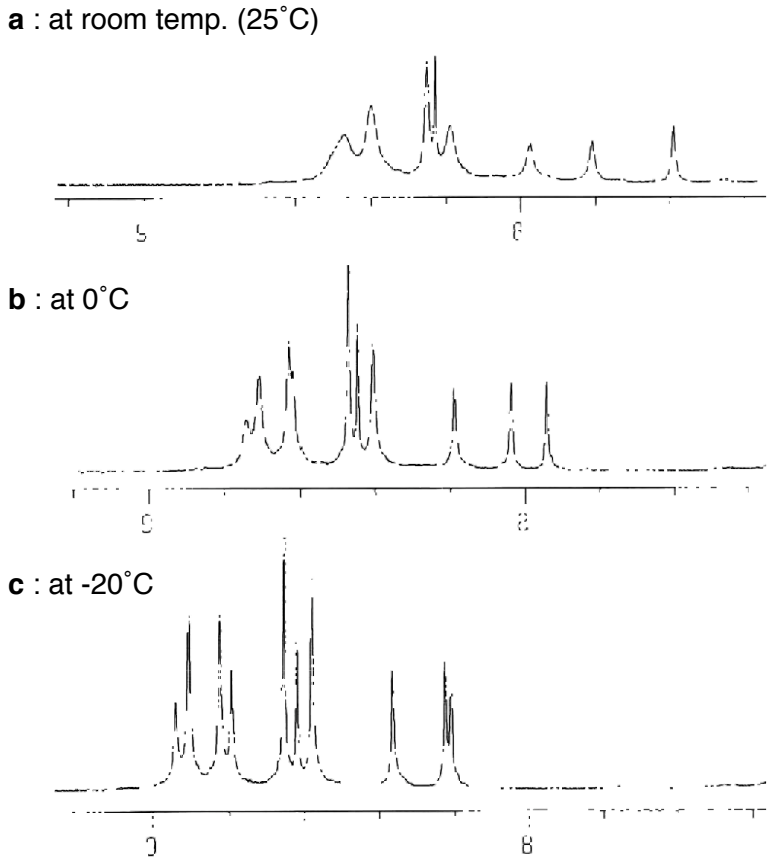


Figure 2 ^1H-NMR spectra at variable temperatures of **1**

)SY spectrum. The spectral pattern of e carbons binding to the former ethine protons (C(7c): □$_c$ 94.04; 8c): □$_c$ 56.75; C(7d): □$_c$ 94.14; C(8d): 56.75 C(7e): □$_c$ 94.40; C(8e): □$_c$ .00; C(7f): □$_c$ 93.91; C(8f): □$_c$ 55.88) is characteristic of a dihydrobenzo-ran ring, and the pattern of the latter (7a): □$_c$ 45.44; C(8a): □$_c$ 50.94; 7b): □$_c$ 49.55; C(8b): □$_c$ 47.40) was nilar to that of a dibenzobicyclo-.2.1]octadiene system observed in npelopsin F (**3**) and vaticanol C (**5**).[6] ne number of phenolic hydroxyl oups (14) was ascertained as follows. e phenolic hydroxyl group appears as oad signal in the ^1H NMR spectrum **1** (Figure 2-a), which causes fficulty for counting the exact number. the ^1H NMR spectral measurement

of **1**, the signals gradually became sharp at lower temperature (Figures 2-b and c), and it made possible to count the number of hydroxyl groups based on the integration value. Thus existence of 14 phenolic hydroxyl groups in **1** was confirmed. These results showed that **1** was a resveratrol hexamer with four dihydrobenzofuran units and a dibenzobicyclo[3.2.1]octadiene system. The connection of these partial structures was decided as follows. In the HMBC spectrum (Figure 3), the significant correlations *via* ^{3}J were observed between H-7a/C-2a(6a), H-8a/C-10a, H-7b/C-2b(6b), H-8b/C-10b, H-7c/C-2c, H-8c/C-10c(14c), H-7d/C-2d(6d), H-8d/C-10d(14d), H-7e/C-2e(6e), H-8e/C-14e, H-7f/C-2f(6f) and H-8f/C-10f(14f), indicating that the rings A_1, A_2, B_1, B_2, C_1, C_2, D_1, D_2, E_1, E_2, F_1 and F_2 were attached at C-7a, C-8a, C-7b, C-8b, C-7c, C-8c, C-7d, C-8d, C-7e, C-8e, C-7f and C-8f, respectively. Thus six resveratrol units (resveratrols A-F) in **1** were confirmed [(resveratrol A: ring A_1-7a-8a-ring A_2)]. The ^{3}J correlations were observed between H-7a/C-7b and H-8a/C-8b, which confirmed the methine sequences of H-7a/H-8a/H-7b/H-8b. Two C-C bonds of C-7a/C-10b and C-8b/C10a were deduced by the correlations of H-7a/C-11b and H-8b/C-11a, respectively. These results established the skeleton of dibenzobicyclo[3.2.1] octadiene system. Among the 3,5-dioxygenated phenyl groups (rings A_2-F_2), the rings A_2 and B_2 were pentasubstituted and condensed to the octadiene ring. The long range correlations between the aliphatic methine protons and the quaternary carbons on the rings (A_2 and B_2) were H-7c/C-13a, H-8c/C-13a, H-7d/C-13b and H-8d/C-13b, which indicated that both rings formed a dihydrobenzofuran ring (rings G and H) with resveratrols C and D, respectively. Formation of a dihydrobenzofuran ring (ring J) and C-C bond formation between C-3c and C-8e were deduced by the correlations of H-7f/C-11e, H-8f/C-11e and H-2c/C-8e. Although no long-range correlation between H-7e/C-4c was observed, the presence of a dihydrobenzofuran ring (ring I) was evident from the molecular formula ($C_{84}H_{62}O_{18}$). The planar structure of upunaphenol A was then determined to be **1** as shown in Figure 3. The partial planar structure in **1** composed of resveratrols A-D is identical with vaticanols C (**5**), F and isovaticanol C (**7**), all of which are stereoisomer each other.[6, 10]

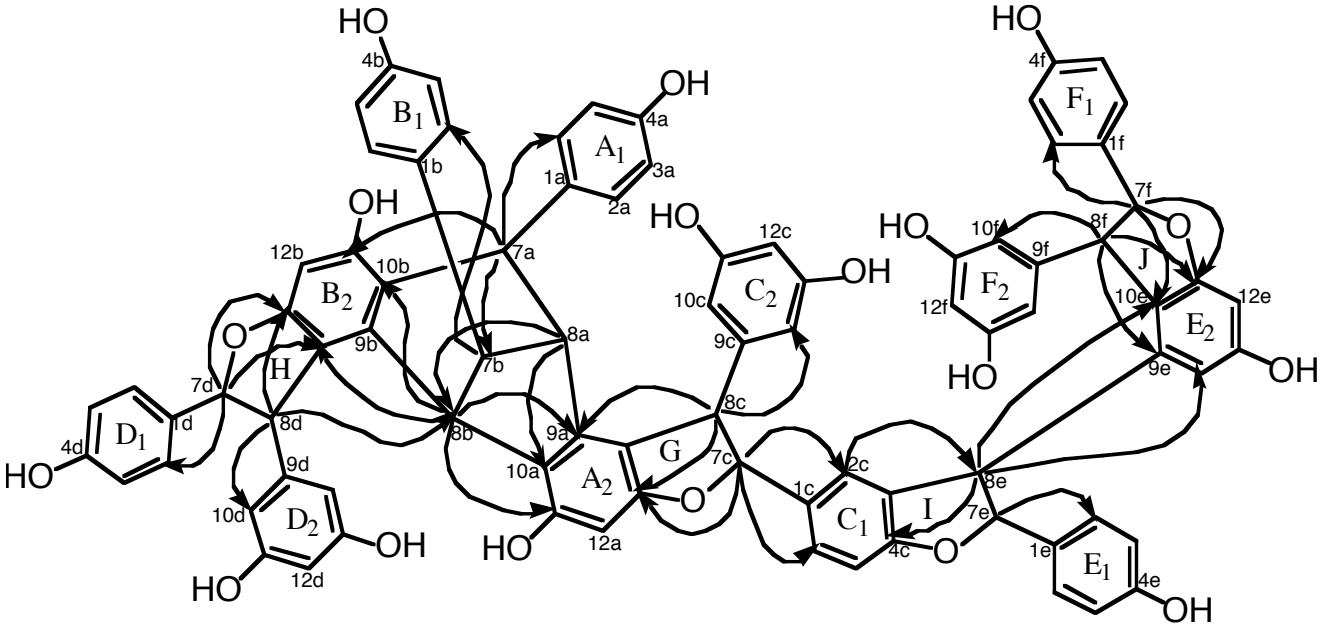


Figure 3 Key correlations in the HMBC spectrum of **1**

To confirm the relative stereochemistry, NOESY experiment was conducted. In this experiment, the NOEs were observed between H-7c/H-10c(14c), H-8c/H-2c, H-8c/H-6c, H-7d/H-10d(14d), H-8d/H-2d(6d), H-7e/H-14e, H-8e/H-2e(6e), H-7f/H-10f(14f), H-8f/H-2f(6f). The results indicated that the orientations of protons on the four dihydrobenzofuran rings were all *trans*. The spectral features of the protons (H-7a, H-8a, H-7b and H-8b), which displayed singlet and/or broad singlet, well similar to those of ampelopsin F (**3**) and vaticanols C (**5**) and F, indicating that the relative stereochemistry of the ring system of **1** was the same as those compounds. Considering the framework of dibenzobicyclo-[3.2.1]octadiene system in **1** (Figure 4), H-8a and H-8b should be located in the same orientation (□-configuration). The orientation of the ring A_1 and H-7b was confirmed to be same (□-configuration) by the NOE between H-2a(6a)/H-7b. Therefore the relative stereochemistry of **1** is as shown in Figure 1.

Table ^{1}H and ^{13}C NMR spectral data of Upunaphenol A(**1**)

No.	□H	□C	No.	□H	□C
1a		138.15	1d		134.14 b
2a, 6a	7.02 (d, 8.6)	129.94	2d, 6d	7.22 (d, 8.6)	128.45
3a, 5a	6.67 (d, 8.6)	115.54	3d, 5d	6.83 (d, 8.6)	116.10
4a		158.08	4d		158.17 c
7a	4.17 (br s)	45.44	7d	5.43 (d, 6.4)	94.14
8a	3.08 (s)	50.94	8d	4.93 (d, 6.4)	56.75
9a		142.88	9d		148.30
10a		128.14 a	10d, 14d	6.40 (d, 2.2)	107.38
11a		153.30	11d, 13d		159.75
12a	6.08 (s)	96.13	12d	6.36 (t, 2.2)	101.85
13a		160.78	1e		131.13
14a		118.51	2e, 6e	7.09 (d, 8.7)	128.69
1b		134.14 b	3e, 5e	6.76 (d, 8.7)	116.16
2b, 6b	6.37 (d, 8.3)	129.35	4e		158.48 c
3b, 5b	6.46 (d, 8.3)	115.40	7e	5.25 (d, 9.8)	94.40
4b		155.73	8e	4.42 (d, 9.8)	55.00
7b	3.37 (s)	49.55	9e		140.17
8b	3.99 (s)	47.40	10e		121.27
9b		144.01	11e		162.23
10b		114.55	12e	6.28 (d, 2.1)	96.62
11b		157.22	13e		159.75
12b	6.19 (s)	95.79	14e	6.25 (d, 2.1)	107.76
13b		160.02	1f		133.40
14b		117.85	2f, 6f	6.99 (d, 8.5)	128.14 a
1c		134.97	3f, 5f	6.83 (d, 8.5)	116.02
2c	7.03 (d, 1.6)	123.98	4f		155.97
3c		131.13	7f	5.17 (d, 4.9)	93.91
4c		160..89	8f	3.65 (d, 4.9)	55.88
5c	6.77 (d, 8.3)	109.72	9f		147.04
6c	7.16 (dd, 8.3, 1.6)	127.01	10f, 14f	5.95 (d, 2.1)	106.59
7c	5.57 (d, 8.4)	94.04	11f, 13f		159.75 c
8c	4.53 (d, 8.4)	56.75	12f	6.19 (t, 2.1)	102.22
9c		145.48	OH groups	7.59, 7.81, 7.98,	
10c, 14c	6.57 (d, 2.0)	108.28		8.19, 8.23, 8.25,	
11c, 13c		159.75		8.40, 8.47 (each br s)	
12c	6.34 (t, 2.0)	102.47			

Measured in CD_3COCD_3. 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). a, b : overlapping. c : interchangeable.

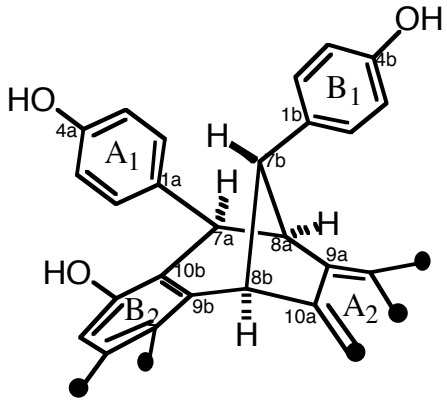


Figure 4 Dibenzobicyclo[3.2.1]octadiene system in **1**

reviously, three hexamers (vaticanols D, H and I) n stem bark of *Vatica rassak* (Dipterocarpaceae) ave been disclosed in varieties of highly ondensed resveratrol cognates.[9] Their epresentative skeleton is a dibenzobicyclo-5.3.0]decadiene system in vaticanols D and I, and tribenzobicyclo[3.3.2]octatriene system in aticanol H. The present hexamer, upunaphenol A **1**), has a dibenzobicyclo[3.2.1]octadiene system, hich is the first instance as resveratrol hexamer. n our previous paper, the antitumor effect of **5** was reported.[11] Further investigation of the effect demonstrated that **5** caused two cell lines (SW480 and HL60) to induce cell death through apoptosis by affecting mitochondrial membrane potential and activation of caspases-9 and 3.[12] In this context, we examined the effects of the related compounds on cell growth. Compounds **1**, **3**, **4** and isovaticanol C (**7**) [6], all of which commonly bear a dibenzobicyclo[3.2.1]octadiene system, were examined for the cell growth assay in HL60 cells. The hexamer (**1**) and the tetramers (**5** and **7**) showed the growth suppression with IC_{50} values at 12.4, 5.9 and 9.2 □M, respectively, while the dimers (**3** and **4**) did not show the suppressive effect. The results implied that two dihydrobenzofuran rings condensed to the octadiene system are necessary for the cell growth suppression. The potent effect of **1** and **7** was found to be due to apoptosis, which was characterized by the morphological aspect of chromatin condensation and fragmentation observed by Hoechst 33342 nuclear staining. We will continue to isolate additional resveratrol oligomers with a dibenzobicyclo[3.2.1]octadiene together with their derivertization and to evaluate the activity in order to define the structure-activity relationship.

EXPERIMENTAL

General method

The following instruments were used: ESIMS spectra, JEOL JMS-T100LC mass spectrometer; ^{1}H and ^{13}C NMR spectra, JEOL JNM LA-300 (TMS as internal standard); UV spectra, Shimadzu UV-2200 spectrophotometer (in methanol solution); optical rotations, JASCO P-1020 polarimeter (in methanol solution). The following adsorbents were used for purification: analytical and preparative TLC, Merck Kieselgel 60 F_{254} (0.25 mm); column chromatography, Merck Kieselgel 60, Pharmacia Fine Chemicals AB Sephadex LH-20 and Fuji Silysia Chemical Chromatorex.

Plant material

Upuna borneensis that was identified by one of the co-authors (D.D.) was cultivated at Bogor Botanical Garden, Bogor, Indonesia, where several stems were collected in May 2000. A voucher specimen has been deposited in Gifu Prefectural Institute of Health and Environmental Sciences, Gifu, Japan.

Extraction and isolation

The dried and ground stem (820 g) of *U. borneensis* was extracted successively with acetone (2 L x 24 h x 3), MeOH (2 L x 24 h x 3) and 70% MeOH (2 L x 24 h x 2) at rt. Concentrated extracts gave respective residues [175 g (acetone), 17 g (MeOH) and 18 g (70% MeOH)]. A part (172 g) of the acetone extract was subjected to chromatography on silica gel column eluted with a mixture of $CHCl_3$-MeOH increasing polarity to give 12 fractions (Fr. 1–12). Fr. 2 ($CHCl_3$/MeOH 15:1, 820 mg) was further purified by Sephadex LH-20 CC (acetone) to give **2** (120 mg). Fr. 6 ($CHCl_3$/MeOH 9:1, 800 mg) was subjected to Sephadex LH-20 CC (MeOH) to give 7 fractions (Fr. 6a–Fr. 6g). Compound (**4**) (24 mg) was obtained from Fr. 6b after purification by PTLC (EtOAc/$CHCl_3$/MeOH/H_2O 15:8:4:1). An acetone soluble part (910 mg) of Fr. 8 ($CHCl_3$/MeOH 8:1, 960 mg) was purified by Sephadex LH-20 CC (MeOH) to give **3** (45 mg). Compounds (**5**) (55 mg) and (**6**) (410 mg) were obtained from a part (2 g) of Fr. 9 ($CHCl_3$/MeOH 7:1, 50 g) after purification by Sephadex LH-20 CC (MeOH). Fr. 10 ($CHCl_3$/MeOH 6:1, 42 g) was further subjected to Sephadex LH-20 CC (MeOH) to give 6 fractions (Fr. 10a–Fr. 10f). Repeated purification of Fr. 10f by PTLC (EtOAc/ $CHCl_3$/MeOH/H_2O 15:8:4:1) achieved the isolation of **1** (30 mg).

Upunaphenol A (1) : A pale yellow amorphous powder. Positive ion HR-ESIMS: $[M+Na]^+$ *m/z* 1381.3858 (Calcd 1381.3834 for $C_{84}H_{62}O_{18}Na$); UV λmax (MeOH) nm: 285, 232; $[\alpha]_D^{25}$ −120° (*c*= 0.1, MeOH); The ^1H and ^{13}C NMR spectral data are listed in Table 1; ^1H–^{13}C HMBC: H-2a(6a)/C-4a,C-7a, H-3a(5a)/C-1a,C-4a, H-7a/C-1a,C-2a(6a),C-8a,C-9a,C-7b, C-9b,C-10b,C-11b, H-8a/C-1a,C-7a,C-9a,C-10a,C-1b,C-8b, H-12a/C-10b,C-11b,C-13b,C-14b, H-2b(6b)/C-4b,C-7b, H-3b(5b)/C-1b,C-4b, H-7b/C-7a,C-8a,C-9a,C-14a,C-1b,C-2b(6b),C-9b, H-8b/C-8a,C-9a,C-13a.C-14a,C-1b,C-9b,C-10b,C-14b, H-12b/C-10b,C-11b,C-13b,C-14b, H-2c/C-3c,C-7c,C-8e, H-5c/C-3c,C-6c, H-6c/C-2c,C-7c, H-7c/C-13a,C-1c,C-2c,C-6c,C-8c,C-9c, H-8c/C-9a,C-13a,C-14a,C-1c,C-7c,C-9c,C-10c(14c), H-10c(14c)/C-8c,C-11c(13c),C-12c, H-12c/C-10c(14c),C-11c(13c), H-2d(6d)/C-4d,C-7d, H-3d(5d)/C-1d,C-4d, H-7d/C-13b,C-14b,C-1d,C-2d(6d),C-8d,C-9d, H-8d/C-8b,C-13b,C-14b,C-1d,C-7d,C-9d,C-10d(14d), H-10d(14d)/C-8d,C-11d(13d),C-12d, H-12d/C-10d(14d),C-11d(13d), H-2e(6e)/C-4e,C-7e, H-3e(5e)/C-1e,C-4e, H-7e/C-1e,C-2e(6e),C-8e,C-9e, H-8e/C-3c,C-4c,C-7e,C-9e,C-10e,C-14e, H-12e/C-10e,C-11e,C-13e,C-14e, H-14e/C-10e,C-12e,C-13e, H-2f(6f)/C-4f,C-7f, H-3f(5f)/C-1f,C-4f, H-7f/C-10e,C-11e,C-1f,C-2f(6f),C-8f,C-9f, H-8f/C-9e,C-10e,C-11e,C-1f,C-9f,C-10f(14f), H-10f(14f)/C-8f,C-11f(13f),C-12f, H-12f/C-10f(14f),C-11f(13f); ^1H–^1H NOESY: H-2a(6a)/H-7a,H-8a,H-7b, H-7a/H-8a, H-8c,H-8e, H-8a/H-2b(6b),H-7b,H-10c(14c), H-2b(6b)/H-7b,H-8b, H-7b/H-8b,H-8e, H-8b/H-8d,H-10d(14d), H-2c/H-7c,H-8c,H-10c(14c),H-14e, H-6c/H-7c,H-8c, H-7c/H-10c(14c), H-8c/H-10c(14c), H-10c(14c)/H-12f, H-2d(6d)/H-7d,H-8d, H-7d/H-10d(14d),H-7e, H-8d/H-10d(14d), H-2e(6e)/H-7e,H-8e,H-14e,H-10f(14f), H-7e/H-14e,H-7f, H-8e/H-14e,H-10f(14f), H-2f(6f)/H-7f,H-8f, H-7f/H-10f(14f), H-8f/H-10f(14f).

Assay for growth inhibitory effect

A stock solution of **1**, **3**–**6** was prepared in 1 mM DMSO, and was further diluted to the working

concentration before use. A human cancer cell, HL60 (leukemia) was grown in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Sigma) and 2 mM L-glutamine under an atmosphere of 95% air and 5% CO_2 at 37˚C. For evaluating IC_{50}, the starting cell number was 1 x 10^5/mL. The number and viability of cells were determined after 72 h exposure of each sample by the trypan blue dye-exclusion assay. For morphological examination of apoptotic changes, cells were stained with Hoechst 33342 (5 mg/mL) at 37°C for 30 min, washed twice with phosphate-buffered saline (PBS), pipetted dropwise onto a glass slide, and examined by fluorescence microscopy using an Olympus microscope (Tokyo, Japan) equipped with an epi-illuminator and appropriate filters.

ACKNOWLEDGEMENT

The authors are indebted to Mr. Y. Doke of Gifu Prefectural Institute of Industrial Product Technology for his technical support during the course of these studies for NMR spectra.

REFERENCES

1. M. Ohyama, T. Tanaka, T. Ito, M. Iinuma, K. F. Bastow, and K.-H. Lee, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 3057.
2. J.-R. Dai, Y. F. Hallock, J. H. Cardellina II, and M. R. Boyd, *J. Nat. Prod.,* 1998, **61**, 351.
3. K.-S. Huang, M. Lin, and G.-F. Cheng, *Phytochemistry,* 2001, **58**, 357.
4. S. Sotheeswaran and V. Pasupathy, *Phytochemistry*, 1993, **32**, 1083.
5. P. S. Ashton and A. Arboretum, "Flora Malesiana", 1982, Vol. 9-2, p. 337, Martinus Nijhoff Publishers, Dordrecht, the Netherlands.
6. T. Ito, T. Tanaka, M. Iinuma, I. Iliya, K. Nakaya, Z. Ali, Y. Takahashi, R. Sawa, Y. Shirataki, J. Murata, and D. Darnaedi, *Tetrahedron*, 2003, **59**, 5347.
7. T. Ito, T. Tanaka, M. Iinuma, K. Nakaya, Y. Takahashi, H. Nakamura, H. Naganawa, and S. Riswan, *Helv. Chim. Acta.*, 2003, in press.
8. T. Ito, T. Tanaka, M. Iinuma, K. Nakaya, Y. Takahashi, R. Sawa, H. Naganawa, and V. Chelladurai, *Tetrahedron,* 2003, **59**, 1255.
9. T. Ito, T. Tanaka, K. Nakaya, M. Iinuma, Y, Takahashi, H. Naganawa, M. Ohyama, Y. Nakanishi, K. F. Bastow, and K.-H. Lee, *Tetrahedron*, 2001, **57**, 7309.
10. T. Tanaka, T. Ito, K. Nakaya, M. Iinuma, and S. Riswan, *Phytochemistry*, 2000, **54**, 63.
11. T. Ito, Y. Akao, T. Tanaka, M. Iinuma, and Y. Nozawa, *Biol. Pharm. Bull.*, 2002, **25**, 147.
12. T. Ito, Y. Akao, H. Yi, K. Ohguchi, K. Matsumoto, T. Tanaka, M. Iinuma, and Y. Nozawa, *Carcinogenesis*, 2003, **24**, 1.