A New and Known Cytotoxic Aryltetralin-Type Lignans from Stems of *Bursera graveolens*

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A new 4α-aryltetralin-type lignan called burseranin (1) and a known analogous lignan picropolygamain (2) were isolated along with known triterpenes, lupeol and epi-lupeol from the methanol extract of stems of *Bursera graveolens*, which showed a remarkable inhibitory activity against human HT1080 fibrosarcoma cells. The whole structure of 1 was established based on combined spectral studies and the absolute structure for 2 was first confirmed by CD spectral evidence. In addition, cytotoxic activities of the stem (methanol) extract and its components are evaluated in this paper.

Key words *Bursera graveolens*; 4α-aryltetralin-type lignan; burseranin; picropolygamain; cytotoxic activity; Burseraceae

The genus *Bursera* (in Burseraceae) comprises about 40 species distributed in tropical zones of Latin and South America. Some species in this genus are used as perfumes and folk medicines.[1] *Bursera graveolens* HBK is a wild tree native from Mexico and Yucatan to Peru and Venezuela and has been used as a remedy for stomach ache, sudorific, and a liniment for rheumatism.[2] In the previous paper,[3] we reported the isolation of luteolin 3′-*O*-α-L-rhamnopyranoside and three of its new acylated derivatives along with four known flavonol glycosides from leaves of *B. graveolens*, and the inhibitory activity of the isolated compounds for the Maillard reaction.

In our continuing study on *B. graveolens*, we found that the MeOH extract from the stems showed a potent cytotoxic activity in *in vitro* assay (*vide post*). This finding prompted us to investigate the chemical components in the MeOH extract of the stems and, as a result, a new podophyllotoxin-like 4α-aryltetralin-type lignan termed burseranin (**1**) and a known analogous lignan picropolygamain (**2**) were isolated together with two known triterpenes. In the present report, we describe the isolation, the structural determination, and biological activity of the isolated compounds in detail. The MeOH extract was partitioned with AcOEt and water. The AcOEt soluble part was subjected to repeated separation by silica gel columns, followed by a Sep-Pak octadecyl silica gel (ODS) column to afford **1** and **2** together with known triterpenes, lupeol and epi-lupeol.

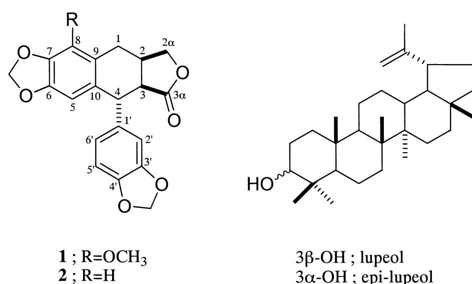

Chart 1

Burseranin **1**, a colorless waxy solid, gave the molecular ion peak at *m/z* 382 (M^+) as the base peak, along with a significant fragment peak at *m/z* 337 [$(M-COOH)^+$, 14%] in the electron impact (EI)-MS; its molecular formula was determined to be $C_{21}H_{18}O_7$ based on the high resolution (HR)-MS data (*m/z* 382.1053) of the M^+ ion. The ^{1}H-NMR spectrum of **1** exhibited signals due to a methoxy (δ 3.99, 3H, s, 8-OCH_3), two methylenedioxy groups (δ 5.88, 5.89, each 1H, both broad s, 6-*O*-CH_2-*O*-7 and δ 5.92, 2H, s, 3′-*O*-CH_2-*O*-4′), a singlet aromatic proton (δ 6.33, H-5), a 1,3,4-trisubstituted aromatic ring (δ 6.59, s, H-2′, δ 6.60, d, J=8.1 Hz, H-6′, and δ 6.72, d, J=8.1 Hz, H-5′), and other aliphatic proton signals [two methylenes (H_2-1 and H_2-2α) and three methines (H-2, H-3, and H-4)] (Table 1). A carbon signal at δ_C 178.3, reasonably assigned to a γ-lactone carbonyl carbon in the ^{13}C-NMR spectrum (Table 1),[4] in conjunction with detailed two dimensional (2D) analysis by the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiquantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) (Fig. 1), gave the podophillotoxin-like planar structure for **1** shown in Fig. 1. Furthermore, nuclear Overhauser enhancement spectroscopy (NOESY) correlations observed between H-4 and H-5 and between H-4 and H-2′ also supported the validity of this established plane structure. The relative structure of **1** was determined based on the ^{1}H coupling constants (J values) and the NOESY data as follows. In 4-aryltetralin-type lignans having a fused 2,3-γ-lactone moiety, the $J_{2,3}$-values of the 2,3-*cis*-γ-lactone derivatives were reported to be about 9.3—9.6 Hz and those of the 2,3-*trans*-γ-lactone series about 14.8—15.5 Hz.[5—9] According to these reported data, the $J_{2,3}$-value (9.5 Hz) of **1** suggested that **1** carries a 2,3-*cis*-γ-lactone moiety in the molecule. Analogously, the $J_{3,4}$-value (3.0 Hz) of **1** was consistent with the reported values for the 3,4-*trans*-configuration in 4-aryltetralin lignans with the 2,3-*cis*-γ-lactone.[6—9] Furthermore, the va-

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

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	2.66 (dd, 5.5, 16.1)	24.2	2.46 (dd, 5.1, 15.3)	32.1
	2.70 (dd, 6.5, 16.1)		2.81 (dd, 6.3, 15.3)	
2	2.97 (m)	32.3	3.01 (m)	32.9
2α	3.94 (dd, 3.0, 9.1)	73.1	3.96 (dd, 3.0, 9.6)	72.8
	4.41 (dd, 7.1, 9.1)		4.43 (dd, 7.5, 9.6)	
3	3.25 (dd, 3.0, 9.5)	46.1	3.30 (dd, 3.0, 9.9)	46.2
3α		178.3		178.3
4	4.33 (d, 3.0)	44.8	4.36 (d, 3.0)	44.8
5	6.33 (s)	104.2	6.59 (s)	109.9
6		147.9$^{a)}$		146.9$^{a)}$
7		135.4		146.8$^{a)}$
8		140.9	6.64 (s)	108.9
9		119.9		128.2
10		131.5		130.1
1′		136.4		136.3
2′	6.59 (s)	108.2	6.60 (br s)	108.2$^{b)}$
3′		148.0$^{a)}$		148.1
4′		146.3		146.3
5′	6.72 (d, 8.1)	108.2	6.73 (d, 8.4)	108.3$^{b)}$
6′	6.60 (d, 8.1)	120.7	6.60 (br d, 8.4)	120.7
6-*O*-CH_2-	5.88 (br s)	100.8	5.91 (d, 1.5)	101.0$^{c)}$
	5.89 (br s)		5.92 (d, 1.5)	
8-*O*-CH_3	3.99 (3H, s)	59.7		
3′-*O*-CH_2-	5.92 (2H, s)	101.1	5.93 (2H, s)	101.1$^{c)}$

a—c) Assignments for values in each compound bearing the same superscript can be reversed.

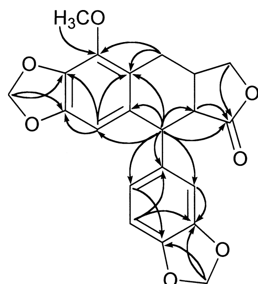

Fig. 1. Key HMBC Correlations in **1**

lidity of the 3,4-*trans* configuration was confirmed by the following NOESY evidence. In addition to the abovementioned two cross peaks between 4-H and 5-H and between 4-H and 2′-H (*vide ante*), another significant and strong cross peak was observed between 3-H and 2′-H. These NOESY proofs showed that 3-H and the 4-aryl ring are in *cis* relation, and thus 3-H and 4-H are in *trans* relation. In conclusion, **1** is composed of 2,3-*cis* and 3,4-*trans* configurations. Finally, the absolute structure of **1** was established based on the following CD spectral evidence. Some 35 years ago Klyne, Swan *et al.* proposed a CD empirical rule that at around 280—290 nm (the first Cotton), all 4α-aryl derivatives in 4-aryltetralin-type lignans give a positive Cotton effect and, contrary to this, all 4β-aryl groups, a negative Cotton curve.[10,11] In the CD spectrum, **1** afforded a positively signed maximum ($\Delta\varepsilon$=1.19) at 291.9 nm and hence, the aryl group at C-4 in **1** is oriented to α (*R* configuration at C-4).[12] On the basis of the above-mentioned accumulated evidence, compound **1** is now defined as structural formula **1** with 2*R*, 3*S*, and 4*R* configurations.

The second lignan **2** gave the molecular formula of $C_{20}H_{16}O_6$, determined based on the HR-EI-MS (*m/z* 352.094 M^+). The molecular formula of **2** differs in composition by the decrement of CH_2O compared with that of **1**. Detailed 2D NMR investigations of **2** were performed in a similar manner to **1** and revealed that **2** coincides in relative structure with the 5-demethoxy derivative of **1**. In addition, the assignments (chemical shifts, multiplicities, and coupling constants) achieved for all protons and carbons of **2** (Table 1) were in agreement with those reported for a known lignan, picropolygamain identified from the resin of *Commiphora incisa* Chiov. (Burseraceae).[13] Picropolygamain has also been isolated from the resin of a Yukatecan plant *Bursera simaruba* (L.) Sarg. (Burseraceae).[8,14] In both reports on the identification of picropolygamain, the absolute structure of picropolygamain was shown but no definite proof (CD spectral, X-ray analytical, synthetic *etc.*) for determination of the absolute structure of picropolygamain was provided.[8,13] Therefore, in the present study, the sign of the CD Cotton curve of **2** at around 280—290 nm was examined and a positive Cotton ($\Delta\varepsilon$ 2.71) at 295.4 nm was observed, suggesting the absolute configuration of C-4 position of **2** to be *R* (*vide ante*). Thus, the absolute structure of **2** is shown in formula **2** with 2*R*, 3*S*, 4*R* configurations, which is the same as the absolute structures previously reported for picropolygamain.[8,13]

Known triterpenes, epi-lupeol and lupeol were also isolated as a major and a predominant component, respectively, in the stems and identified by comparison with the ^{13}C-NMR data reported for the respective triterpenes.[15]

The inhibitory effects of the MeOH extract and its components, **1**, **2**, epi-lupeol, and lupeol, against HT1080 human fibrosarcoma cells, were evaluated according to the bioassay procedure described in Experimental, and the respective ED_{50} values are listed in Table 2. Nowadays, it is well known

Table 2. Cytotoxic Activities of the MeOH Extract and Its Components

Sample	Cell culture [ED_{50} (μg/ml)] HT1080
MeOH extract	60.0
Burseranin (**1**)	5.5
Picropolygamain (**2**)	1.9
Epi-lupeol	>100
Lupeol	16.7
Adriamycin	0.1

that podophyllotoxin and some of its analogs, 4-aryltetralin-type lignans show biological activities such as antineoplastic, antitumor, antiviral *etc.*[16] As expected, both 4α-aryltetralin-type lignans with the 2,3-*cis*-γ-lactone group (**1**, **2**) exhibited potent inhibitory effects in comparison with adriamycin as a positive control as shown in Table 2. Furthermore, a triterpene lupeol showed comparatively strong activity, which is a bit surprising and an interesting result. Although the potent cytotoxicity of picropolygamain (**2**) on three human cell lines, A-549 (lung), MCF-7 (breast), and HT-29 (colon) has previously been reported,[8] that against human HT1080 cells is provided for the first time in this report.

Experimental

General Remarks ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-ECA 600 (^{1}H at 600 and ^{13}C at 150 MHz) or JNM-GX 400 (^{1}H at 400 and ^{13}C at 100 MHz) spectrometers with $CDCl_3$ as a solvent. Chemical shifts were given in δ values (ppm) relative to tetramethylsilane as an internal standard. EI- and HR-EI-MS spectra were obtained with a JEOL JMS-700T spectrometer. UV spectra were recorded on a Shimadzu UV-2200 spectrometer, CD spectra on a JASCO J-820 spectropolarimeter, and optical rotation on a JASCO DIP-140 polarimeter, respectively. For column chromatography, silica gel 60 (70—230 mesh, Merck) was used and for analytical TLC, Kieselgel 60 F_{254} (Merck).

Plant Material Stems of *Bursera graveolens* were collected in July, 1998 at Chiapas in Mexico and the voucher specimen of the plant has been deposited in the Herbarium, Botanical Gardens, the University of Tokyo, Japan.

Extraction and Isolation The dried and cut stems (453 g) of *B. graveolens* were extracted three times with MeOH (1.6 l×5 d and then, 1.6 l×8 d×twice) at room temperature and the solvent was removed under reduced pressure to give a MeOH extract (21.7 g), a part (21.0 g) of which was suspended in water (300 ml) and extracted three times with AcOEt (400 ml×1 and then, 250 ml×2). A large portion (11.6 g) of the resulting AcOEt extract (11.7 g) was subjected to column chromatography on silica gel (550 g) eluted (100 ml per fraction) with $CHCl_3$–MeOH (40 : 1) [giving 31 fractions], (20 : 1) [8 fractions], and (10 : 1) [35 fractions], successively. Fractions 10—17 (2.70 g) eluted with $CHCl_3$–MeOH (40 : 1) were recrystallized from acetone to give epi-lupeol in a pure form. Fraction 22 (770 mg) eluted with the (40 : 1) solvent mixture was further separated on a column of silica gel (15 g) eluted with *n*-hexane–AcOEt (6 : 1) [10 fractions; Nos. 22-1 to 22-10] and (2 : 1) [13 fractions; Nos. 22-11 to 22-23], successively. Fractions 22-2 and 22-3 (540 mg) eluted with the (6 : 1) solvent mixture corresponded to pure lupeol. Fractions 22-11 and 22-12 (51 mg) eluted with the (2 : 1) solvent mixture were further purified by passing through Sep-Pak cartridge ODS C_{18} (Waters Co.) eluted with CH_3CN–H_2O (3 : 1) to give pure **1** (34 mg). Fraction 22-14 (5 mg) eluted with the (2 : 1) solvent mixture was also further purified by Sep-Pak cartridge ODS C_{18} (Waters Co.) eluted with CH_3CN–H_2O (3 : 1) to afford pure **2** (3 mg).

Burseranin (**1**): A colorless waxy solid, $[\alpha]_D$ +32° (c=0.27, $CHCl_3$). EI-MS *m/z* (%): 382 (M^+, 100), 337 (M^+-CO_2H, 14), 310 (27), 297 (17). HR-EI-MS: *m/z* 382.1053 (Calcd for $C_{21}H_{18}O_7$, M^+: 382.1052). UV: λ_{max} (c=1.1×10^{-4} mol/l, MeOH) nm (ε): 287 (5100), 206 (25200). CD (c=1.1×10^{-4} mol/l, MeOH) nm ($\Delta\varepsilon$): 291.9 (+1.19), 259.2 (+0.22), 253.9 (−0.14), 243.1 (+1.63), 208.5 (+13.6). ^{1}H- and ^{13}C-NMR ($CDCl_3$): Given in Table 1.

Picropolygamain (**2**): A colorless oil. EI-MS *m/z* (%): 352 (M^+, 100), 280 (25), 267 (20). HR-EI-MS: *m/z* 352.0941 (Calcd for $C_{20}H_{16}O_6$, M^+: 352.0947). UV: λ_{max} (c=1.4×10^{-4} mol/l, MeOH) nm (ε): 287 (11600), 206 (21500). CD (c=1.4×10^{-4} mol/l, MeOH) nm ($\Delta\varepsilon$): 295.4 (+2.71), 259.1 (+0.23), 249.4 (−1.33), 235.3 (+2.47), 207.6 (+27.5). ^{1}H- and ^{13}C-NMR ($CDCl_3$): Given in Table 1.

Bioassay HT1080 human fibrosarcoma cells were grown in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS, JRH Biosciences, Lenexa, KS, U.S.A.) at 37 °C in a 5% CO_2 atmosphere. The cytotoxic effect of the samples (the MeOH extract, each of the components **1**, **2**, epi-lupeol, and lupeol, and adriamycin as a positive standard) on HT1080 cells was examined by Tetracolor One assay. HT1080 cells (2×10^3 cells/well) were seeded on a 96-well culture plate and incubated in RPMI1640 medium supplemented with 10% FBS (10% FBS/RPMI1640) for 24 h at 37 °C. Then, cells were incubated in the presence of various concentrations of the samples (200 μl) for an additional 48 h at 37 °C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), cells were incubated in RPMI1640 (190 μl) with Tetracolor One (10 μl) for 3 h at 37 °C. Viable cells were determined by the absorbance at 492 nm with reference at 630 nm.

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