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Bioactive constituents from roots of *Bursera tonkinensis*

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Abstract

Bioassay directed-fractionation led to isolation of 12 compounds from the roots of *Bursera tonkinensis* Guillaum (Burseraceae), including burselignan, bursephenylpropane, and burseneolignan. Of the 12 compounds, only 4′-demethyldesoxypodophyllotoxin exhibited significant cytotoxic activities against KB, Col2 and LNCaP cell lines.

Keywords: *Bursera tonkinensis*; Burseraceae; Cytotoxicity; Burselignan; Burseneolignan; Bursephenylpropane

1. Introduction

Bursera tonkinensis Guillaum (Burseraceae) is among the plants that showed cytotoxic potential in our search for anticancer active compounds from plants of Vietnam and Laos, as part of our ICBG (International Cooperative Biodiversity Group) program (Soejarto et al., 1999). Our initial bioassay showed that the CH_2Cl_2 extract from the roots of *B. tonkinensis* collected in Cuc Phuong National Park (Nho Quan District, Ninh Binh Province, Vietnam) exhibited cytotoxicity against KB cells with an IC_{50} value of 4.1 μg/ml. While *B. tonkinensis* has not heretofore been studied phytochemically, several lignans possessing antitumor properties have been reported from other species of the genus *Bursera* (Wickramaratne et al., 1995; McDoniel and Cole, 1972; Bianchi et al., 1968; Cole et al., 1969; Sergio and Luis, 1992; Jolad et al., 1977a,b; Hernández et al., 1983).

A recollected 5.6 kg sample of the roots of this plant was extracted by MeOH, followed by sequential partitioning with petroleum ether and $CHCl_3$. The chloroform extract was subjected to column chromatography and subsequent HPLC separation to give 12 compounds (**1**–**12**), including three new compounds, burselignan (**1**), bursephenylpropane (**6**) and burseneolignan (**7**), along with four known lignans, (+)-isolariciresinol (**2**) (Baderschneider and Winterhalter, 2001), 5-methoxy-(+)-isolariciresinol (**3**) (Zhang et al., 1999), 4′-demethyldesoxypodophyllotoxin (**4**) (Jackson and Dewick, 1984), and 4′-demethyldesoxypodophyllotoxin-4-*O*-β-D-glucoside (**5**) (Broomhead and Dewick, 1990); three known neolignans, 1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-hydroxypropyl)-2″,6″-dimethoxyphenoxy] propane-1,3-diol (**8**) (Hwang et al., 1981), dihydrodehydrodiconiferyl alcohol (**9**) (Seidel et al., 2000), and 5-methoxy-*trans*-dihydrodehydrodiconiferyl alcohol (**10**) (Baderschneider and Winterhalter, 2001); scopoletin (**11**) (Źolek et al., 2003) and the ubiquitous β-daucosterol (**12**)

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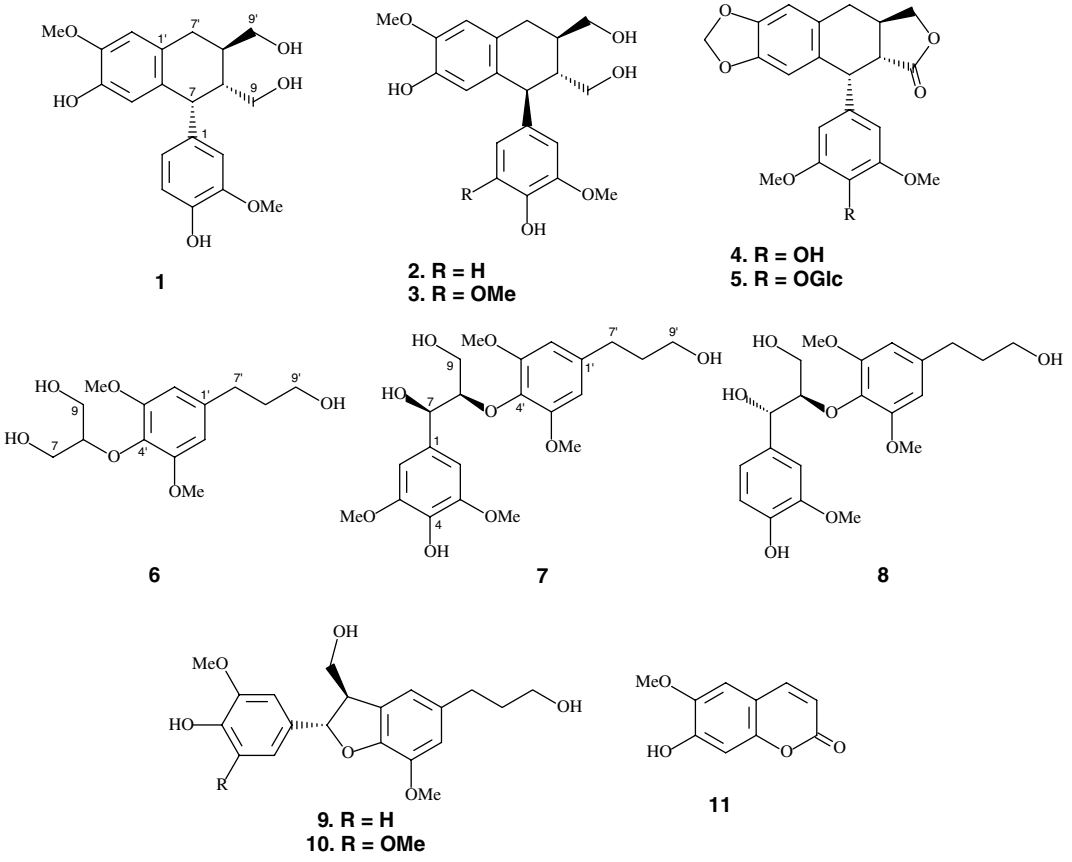
(structure not shown) (Yayli et al., 2003). The current paper describes the isolation, identification and biological evaluation of the cytotoxic compounds from this species.



2. Results and discussion

Burselignan (**1**) was obtained as a white powder with a molecular formula of $C_{20}H_{24}O_6$ based on the positive HRTOFESIMS data ($[M+Na]^+$ m/z 383.1475, calcd. 383.1471). The ^{13}C NMR and DEPT-135 spectroscopic data showed signals for seven aromatic quaternary carbons, four of which are oxy-quaternary; eight methines including five aromatic methines; one methylene; two oxy-methylenes; and two methoxy carbons. (Table 1) The ^1H NMR spectrum showed a 1,3,4-trisubstituted aromatic ring system [δ_H 6.46 (1H, *dd*, $J = 1.7$, 8.1 Hz, H-6), 6.66 (1H, *d*, $J = 8.1$ Hz, H-5), and 6.70 (1H, *d*, $J = 1.7$ Hz, H-2)] and a 1′,3′,4′,6′-tetrasubstituted aromatic ring [δ_H 6.36 (1H, *s*, H-5′) and 6.71 (1H, *s*, H-2′)]. A comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** to those of **2** and **3** indicated that **1** was an aryl-tetralin type lignan. An α-aromatic substituent at C-7 was determined in **1**, as opposed to a β-aromatic substituent in the known (+)-isolariciresinol (**2**), based on the following: The configuration of the hydroxymethyl group at C-8′ in **1** was assigned to be β-oriented due to the presence of the NOE correlation between H-7′β and H_2-9′ (Fig. 1), which in turn, led to the aromatic group at C-7 being assigned as α-oriented based on the presence of the NOE correlation between H-8′α and H-2. Whereas, the configuration of the aromatic group at C-7 in **2** was assigned as β-oriented due to the presence of the NOE correlations between H-7 and H-8′, and between H-2 and H-8. The configuration of the hydroxymethyl group at C-8 in **2** was reported to be α-oriented due to the large coupling constants ($J = 10.7$ Hz) between H-7 and H-8. On the other hand, the small coupling constant ($J = 3.2$ Hz) of H-7 and H-8 observed in the ^1H NMR spectrum of **1**, established that the configuration of the hydroxymethyl group at C-8 in **1** was α-oriented. The change of the aromatic group at C-7 from the β-configuration in **2** and **3** to the α-configuration in **1** led to upfield shifts of the ^{13}C NMR signals at C-1, C-7, C-8, C-6′ and C-8′, and a downfield shift of the H-7 signal. An aryl-tetralin lignan that has the same configurations at C-7, C-8 and C-8′ to **1** has been synthesized from α-conidendrol (Dantzig et al., 2001). This synthetic analog differs from **1** only by the presence of methoxyl groups at C-4 and C-4′, and showed very similar ^1H and ^{13}C NMR spectroscopic data to those of **1**. As a result, **1** was determined to be 8α-(4-hydroxy-3-methoxy-phenyl)-6β,7α-bis-hydroxymethyl-3-methoxy-5,6,7,8-tetrahydro-naphthalen-2-ol, and was given the trivial name of burselignan.

Table 1
NMR spectroscopic data for **1**–**3** and **6**–**8** (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, CD_3OD, *J* in Hz)

Position	**1**		**2**		**3**		**6**		**7**	**8**	
	δ									δ_H	δ_c
1		135.9 *s*		138.6 *s*		137.8 *s*				132.7 *s*	133.7 *s*
2	6.70 *d* (1.7)	115.2 *d*	6.66 *d* (1.8)	113.8 *d*	6.44 *s*	107.7 *d*			6.74 *s*	105.3 *d* 6.97 *brs*	111.4 *d*
3		148.2 *s*		149.0 *s*		149.2 *s*				149.0 *s*	148.7 *s*
4		145.8 *s*		145.9 *s*		135.0 *s*				135.9 *s*	146.8 *s*
5	6.66 *d* (8.1)	115.4 *d*	6.73 *d* (8.0)	115.9 *d*		149.2 *s*				149.0 *s* 6.73 *d* (8.1)	115.7 *d*
6	6.46 *dd* (1.7, 8.1)	124.0 *d*	6.60 *dd* (1.8, 8.0)	123.2 *d*	6.44 *s*	107.7 *d*			6.74 *s*	105.3 *d* 6.77 *brd* (8.1)	120.6 *d*
7	4.21 *d* (3.2)	46.5 *d*	3.79 *d* (10.7)	48.1 *d*	3.83 *d* (8.5)	48.5 *d*	3.75 *d* (5.0)	62.2 *t*	5.00 *d* (7.0)	74.7 *d* 4.90 *d* (4.8)	74.0 *d*
8	2.07 *m*	44.7 *d*	1.75 *tt* (3.9, 10.0)	48.0 *d*	1.80 *m*	47.8 *d*	3.97 *qu*[a] (5.0)	84.8 *d*	4.03 *td* (3.7, 7.0)	89.0 *d* 4.16 *m*	87.5 *d*
9	3.38 *dd* (6.7, 10.6)	63.4 *t*	3.38 *dd* (3.9,11.0)	62.2 *t*	3.42 *dd* (3.9,11.2)	62.1 *t*	3.75 *d* (5.0)	62.2 *t*	3.78 *m*	61.8 *t* 3.52 *dd* (3.4, 12.0)	61.5 *t*
	3.53 *dd* (6.0, 10.4)		3.65 *dd* (4.5,11.0)		3.69 *m*					3.88 *dd* (5.6, 12.0)	
1′		128.4 *s*		129.0 *s*		129.0 *s*		140.0 *s*		140.1 *s*	139.9 *s*
2′	6.71 *s*	112.3 *d*	6.64 *s*	112.4 *d*	6.71 *s*	112.4 *d*	6.56 *s*	106.8 *d*	6.56 *s*	106.8 *d* 6.53 *s*	106.9 *d*
3′		147.8 *s*		147.2 *s*		147.3 *s*		154.4 *s*		154.1 *s*	154.5 *s*
4′		145.5 *s*		145.3 *s*		145.3 *s*		135.0 *s*		135.4 *s*	134.7 *s*
5′	6.36 *s*	117.0 *d*	6.17 *s*	117.3 *d*	6.21 *s*	117.3 *d*		154.4 *s*		154.1 *s*	154.4 *s*
6′		133.0 *s*		134.2 *s*		134.0 *s*	6.56 *s*	106.8 *d*	6.56 *s*	106.8 *d* 6.53 *s*	106.9 *d*
7′	2.65 *dd* (9.3, 16.7)	33.1 *t*	2.77 *d* (7.7)	33.6 *t*	2.80 *d* (7.5)	33.6 *t*	2.66 *t* (7.5)	33.4 *t*	2.65 *t* (7.4)	33.4 *t* 2.63 *t* (7.4)	33.4 *t*
	2.96 *dd* (4.3, 16.7)										
8′	2.07 *m*	35.5 *d*	1.99 *m*	40.0 *d*	2.03 *m*	40.0 *d*	1.84 *tt* (7.5,6.4)	35.5 *t*	1.84 *m*	35.5 *t* 1.82 *m*	35.4 *t*
9′	3.58 *dd* (4.6, 7.0)	65.8 *t*	3.66 *m*	65.9 *t*	3.71 *m*	65.8 *t*	3.58 *t* (6.4)	62.2 *t*	3.58 *t* (6.4)	62.1 *t* 3.56 *t* (6.4)	62.1 *t*
3-OMe	3.76 *s*	56.3 *q*	3.76 *s*	56.3 *q*	3.80 *s*	56.7 *q*			3.86 *s*	56.7 *q* 3.82 *s*	56.3 *q*
5-OMe					3.80 *s*	56.7 *q*			3.86 *s*	56.7 *q*	
3′-OMe	3.84 *s*	56.3 *q*	3.79 *s*	56.4 *q*	3.82 *s*	56.4 *q*	3.85 *s*	56.6 *q*	3.84 *s*	56.6 *q* 3.80 *s*	56.6 *q*
5′-OMe							3.85 *s*	56.6 *q*	3.84 *s*	56.6 *q* 3.80 *s*	56.6 *q*

[a] *qu* represents quintet.

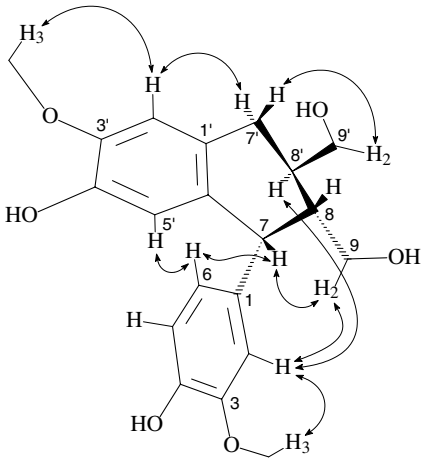


Fig. 1. Selected NOESY correlations for compound **1**.

Bursephenylpropane (**6**), a colorless syrup, was shown to have a molecular formula of $C_{14}H_{22}O_6$ by positive HRTOFESIMS ($[M + Na]^+$ *m/z* 309.1311, calcd. 309.1314). The ^1H NMR spectroscopic data of **6** displayed signals for a tetrasubstituted aromatic ring with two equivalent aromatic protons at δ_H 6.56 (2H, *s*, H-2′ and H-6′); two methoxyl groups at δ_H 3.85 (*s*, 6H); two methylene groups at δ_H 1.84 (2H, *tt*, $J = 6.4$, 7.5 Hz, H_2-8′) and 2.66 (2H, *t*, $J = 7.5$ Hz, H_2-7′), respectively; and a hydroxymethyl group at δ_H 3.58 (2H, *t*, $J = 6.4$ Hz, H_2-9′) (Table 1). On the basis of the ^1H–^1H COSY and HMBC spectra, a dihydrosinapyl alcohol moiety was assigned to the structure of **6**, in good agreement with the reported ^1H NMR data of dihydrosinapyl alcohol (Rustaiyan et al., 1991). In the ^1H–^1H COSY spectra of **6**, a quintuplet signal of the methine proton at δ_H 3.97 (1H, $J = 5.0$ Hz, H-8) was observed to correlate with four equivalent doublet protons at δ_H 3.75 (4H, $J = 5.0$ Hz, H_2-7 and H_2-9), which were assigned to a glycerol moiety. The HMBC experiment displayed a correlation between H-8 and C-4′ (Fig. 2), that linked the glycerol and dihydrosinapyl alcohol moieties bridged by an oxygen atom. Thus, **6** was established as 2-[4-(3-hydroxy-propyl)-2,6-dimethoxy-phenoxy]-propane-1,3-diol, and was given the trivial name of bursephenylpropane.

Burseneolignan (**7**) was obtained as a colorless syrup with a molecular formula of $C_{22}H_{30}O_9$ based on the positive HRTOFESIMS data ($[M + Na]^+$ *m/z* 461.1780, calcd. 461.1788). The signals belonging to a dihydrosinapyl alcohol moiety, which occurred in **6**, were also observed in the NMR spectra of **7**. The ^1H NMR spectrum of **7** showed an additional tetrasubstituted aromatic ring with two equivalent aromatic protons at δ_H 6.74 (2H, *s*, H-2 and H-6), two methoxyl groups at δ_H 3.86 (6H, *s*), two oxymethines at δ_H 4.03 (1H, *td*, $J = 3.7$, 7.0 Hz, H-8) and 5.0 (1H, *d*, $J = 7.0$ Hz, H-7), respectively, and a hydroxymethyl group at δ_H 3.78 (2H, *m*, H_2-9) (Table 1). The long-range correlations of the oxy-methine proton at δ_H 5.0 to C-1, -2, and -6 and the methoxyl protons to C-3 and -5 were observed in the HMBC spectrum (Fig. 3), which led to the tetrasubstituted aromatic ring being determined as a 4-hydroxy-3,5-dimethoxy-phenyl group in **7**. The proton–proton correlations among the oxy-methine groups and the hydroxymethylene group observed in the ^1H–^1H COSY spectrum led to the assignment of a 2-hydroxy-1-hydroxymethyl-ethyl group in **7**. The presence of the HMBC correlations of H-2/H-6 to C-7 connected the 4-hydroxy-3,5-dimethoxy-phenyl group and the 2-hydroxy-l-hydroxymethyl-ethyl group as a 2-hydroxy-2-(4-hydroxy-3,5-dimethoxy-phenyl)-l-hydroxymethyl-ethyl. This was then *O*-linked to the dihydrosinapyl alcohol moiety to form a 8-*O*-4′ neolignan structure (**7**) as indicated through the observed long-range HMBC correlation between C-4′ and H-8. The coupling constant between H-7 and H-8 was measured to be 7.0 Hz in CD_3OD and 8.4 Hz in $CDCl_3$, which resulted in a *threo* configuration at C-7 and C-8 for **7**. Otherwise, a small coupling constant ($J_{7,8} = 4.8$ Hz) would be expected if the stereochemistry of C-7 and C-8 is in an *erythro* form such as the case of compound **8** (Braga et al., 1984) (Table 1). Thus, **7** was determined to be 1*R**-(4-hydroxy-3,5-dimethoxy-phenyl)-2*R**-[4-(3-hydroxy-propyl)-2,6-dimethoxy-phenoxy]-propane-1,3-diol, and was given the trivial name of burseneolignan.

Burselignan (**1**) differs from (+)-isolariciresinol (**2**) (Baderschneider and Winterhalter, 2001) only by the configuration of the aromatic group at C-7. Although a number of natural 7′-deoxy-aryltetralin-type lignans possessing 8α- and 8′β-hydroxymethyl groups and the same configurations at C-7, C-8 and C-8′ of **2** have been reported (Baderschneider and Winterhalter, 2001; Das et al., 1994; Erdemoglu and Sener, 2001; Ferreira Fonseca et al., 1978; Mujumdar et al., 1972; Sicilia et al., 2003; Urones

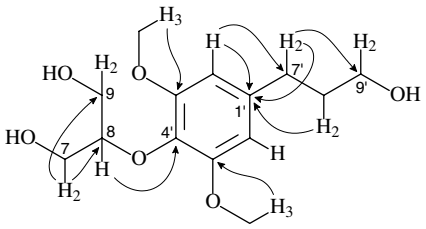


Fig. 2. Selected HMBC correlations for compound **6**.

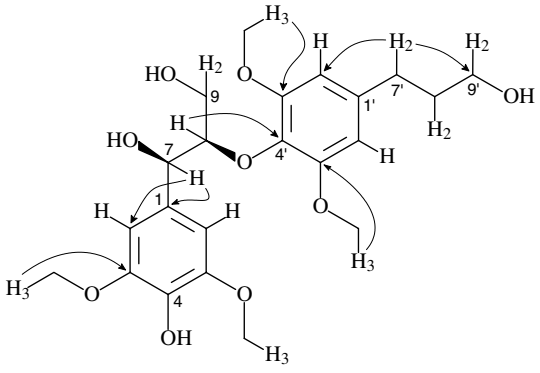


Fig. 3. Selected HMBC correlations for compound **7**.

et al., 1987; Vardamides et al., 2003; Yang et al., 1999; Zhang et al., 1999), none of the 7′-deoxy-aryltetralin-type lignans with 8α- and 8′β-hydroxymethyl groups and the same configurations at C-7, C-8 and C-8′ as **1** has been reported from nature; only a few such compounds have been obtained as synthetic analogs (Dantzig et al., 2001; LaLonde et al., 2003; San Feliciano et al., 1991). Bursephenylpropane (**6**) is probably derived from **7** or **8** by degradation of the aromatic group at C-7 instead of a direct etherization between dihydrosinapyl alcohol and glycerol, considering the structural similarity among compounds **6**–**8**. Burseneolignan (**7**) is a new *p*-(3-hydroxy-propyl)-phenyl neolignan possessing *threo* configurations at C-7 and C-8. Neolignans with both *threo* and *erythro* forms at C-7 and C-8 were reported previously from nature (Matsuda and Kikuchi, 1996; Matsushita et al., 1991).

The in vitro cytotoxicity of isolated compounds (**1**–**8**) was evaluated against the KB, LNCaP, and Col2 cell lines, in comparison to the reference drug, vinblastine. Except for 4′-demethyldesoxypodophyllotoxin (**4**), none of the other compounds (**1**–**3**, **5**–**8**) showed cytotoxicity against these three cancer cells at a concentration of 100 μg/ml. Among the isolates, only 4′-demethyldesoxypodophyllotoxin (**4**) showed cytotoxic activity, as expected based on literature reports (You et al., 2004). Compound **4** exhibited equivalent cytotoxic activity to those of vinblastine in all three cancer cell lines [IC_{50} values (ng/ml) for **4** at 17.7 (KB), 10.0 (LNCaP) and 23.1 (Col2); for vinblastine at 9.7 (KB), 10.5 (LNCaP) and 8.1 (Col2)]. Interestingly, although many C-4 ester derivatives of **4** were reported to possess equivalent or even higher cytotoxic activity against human lung adenocarcinoma (A-549) and human melanoma (SK-MEL-2) cell lines (You et al., 2004), the C-4 glucoside (**5**) of compound **4** was completely devoid of cytotoxicity against the KB, LNCaP, and Col2 cell lines at a concentration of 100 μg/ml.

3. Experimental

3.1. General

Optical rotations were measured with a Perkin–Elmer 241 polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. IR spectra were run on a Jasco FT/IR-410 spectrometer, equipped with a Specac Silver Gate ATR system by applying a film on a Germanium plate. NMR spectra were recorded on a Bruker DPX-300 or a Bruker DPX-400 NMR spectrometer. Chemical shifts (δ) were expressed in ppm with reference to TMS or the solvent signals. All NMR spectroscopic data were obtained by using standard pulse sequences supplied by the vendor. LREIMS were recorded on a Thermo Finnigan LCQ mass spectrometer. HRTOFMS spectra were recorded on a Micromass QTOF-2 spectrometer or a JEOL GCmate II spectrometer. Reversed-phase HPLC was carried out on a Waters 600E Delivery System pump, equipped with a Waters 996 photodiode detector, and a Phenomenex LUNA C18 (2) column (120 Å, 10 μm, 50 × 250 mm) at 20 mL/min. Silica gel 60 (230–400 mesh, Natland International Corporation) and silica gel RP-18 (40–63 μm, EM Science) were used for column chromatography. Thin-layer chromatography was performed on Whatman glass-backed plates coated with 0.25 mm layers of Silica gel 60. Fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 10% H_2SO_4 in MeOH.

3.2. Plant material

The initial collection of root sample (SV2180) of *Bursera tonkinensis* Guillaum (Burseraceae) was made on August 5, 1999 at Cuc Phuong National Park (Vietnam), and was documented by voucher specimens (Nguyen Manh Cuong et al.) 1965. A larger amount of the plant root sample (SVA2180, 5.6 kg) for the current isolation work was subsequently re-collected on March 5, 2003, documented by voucher specimens (Nguyen Manh Cuong et al.) 1753. Duplicate voucher specimens of both the initial collection and the recollected samples have been deposited at the herbaria of Cuc Phuong National Park, Institute of Ecology and Biological Resources of the Vietnamese Academy of Science and Technology in Hanoi, and at the John G. Searle Herbarium of the Field Museum of Natural History (Chicago, IL, USA).

3.3. Evaluation of cytotoxic potential

Cytotoxicity evaluations were conducted as previously reported (Likhitwitayawuid et al., 1993). In brief, human prostate cancer (LNCaP) and human oral epidermoid carcinoma (KB) cell lines were purchased from the American Type Culture Collection and human colon cancer (Col2) cell line was established from Department of Surgical Oncology, University of Illinois College of Medicine at Chicago. Col2 cells were maintained in MEME medium. KB cells were maintained in DMEM medium. LNCaP cells were maintained in RPMI1640 medium with hormone-free 10% heat-activated FBS (fetal bovine serum) supplemented with 0.1 nM testosterone. In each case, PSF (100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, 250 ng/ml amphotericin B) was added. All media were supplemented with 10% heat-inactivated FBS. Serial dilutions of the isolated compounds were prepared using 10% aqueous DMSO as solvent. The 190-μl cell suspension (3×10^4 cells in 1 ml media for KB and 7×10^4 cells in 1 ml media for both LNCaP and Col2) was incubated with 10 μl sample solutions, in triplicate, in 96-well tissue culture plate at 37 °C in a humidified atmosphere of 5% CO_2 in air for 72 h. Ten μl of 10% aqueous DMSO was used as control group. Then the cells were fixed to plastic substratum by the addition of 100 μl cold 20% aqueous trichloroacetic acid and washing with water after incubation at 4 °C for 30 min. After staining cells with 100 μl of 0.4% sulforhodamine B in 1% aqueous AcOH for 30 min, unbound dye was removed by rinsing with 1% aqueous AcOH. The bound

dye was solubilized with 200 μl of 10 mM unbuffered Tris base, pH 10, and the optical density was measured at 515 nm using an ELISA plate reader. The average data were expressed as a percentage, relative to the control. The ED_{50} values, the dose that inhibited cell growth by 50%, were calculated using nonlinear regression analysis (percent survival versus concentration).

3.4. Extraction and isolation

The dry, milled plant material (5.6 kg) was extracted with MeOH to yield 436.8 g of crude extract, which was subsequently defatted with petroleum ether and partitioned with $CHCl_3$. The $CHCl_3$-soluble fraction (32.3 g) was subjected to Si gel column (1 kg) chromatography by elution with increasing polarity of eluents using $CHCl_3$, Me_2CO and MeOH to give 33 fractions [$CHCl_3$ (eluates F1–F12, each 0.5 l); $CHCl_3$–Me_2CO/95:5 (eluates F13–F18, each 1.0 l); $CHCl_3$–Me_2CO/9:1 (eluates F19–F21, each 2.0 l), 7:3 [eluates F22 (1.0 l), F23 (2.0 l) and F24 (8.5 l)]; $CHCl_3$–MeOH/97:3 (eluates F25–F27, each 4.0 l), 95:5 (eluates F28, 8.0 l), 9:1 (eluates F29–F31, each 4.0 l), 7:3 (eluate F32, 5.0 l), 1:1 (eluate F33, 7.6 l), respectively]. Bioactive fraction 11 (1.16 g) was dissolved in MeOH and filtered to yield **4** in crystalline form [366.0 mg, $[\alpha]_D^{20}$ −127.3° (*c* 0.11, $CHCl_3$)]. Scopoletin (167.5 mg) was crystallized from the MeOH solution of fraction 12 (0.44 g) as a major component. Fraction 28 was dissolved in MeOH, which was filtered to afford the precipitate of β-daucosterol (8.49 mg). Compound **5** [27.17 mg, $[\alpha]_D^{20}$ +20.6° (*c* 0.14, $CHCl_3$)] was crystallized from the remaining supernatant of the same fraction. Chromatography of combined fractions 24–26 over a Si gel column (380 g) by gradient elution with $CHCl_3$ and MeOH afforded subfractions F34–F58 [$CHCl_3$ (eluates F34–F37, each 2.0 l); $CHCl_3$–MeOH/99:1 [eluates F38 (4.0 l), F39 (100 ml), F40 (200 ml), F41 (400 ml), F42–F43 (each 200 ml), F44 (540 ml), F45 (400 ml), F46–F47 (each 300 ml), F48 (800 ml), F49 (600 ml), F50 (1.0 l), F51 (600 ml), F52–F53 (each 2.6 l each)], 97:3 (eluates F55–F57, each 2.0 l), 1:1 (eluate F58, 1.6 l), respectively]. Subfraction F55 (0.54 g) was further fractionated by a Si gel RP-18 column (84 g) using the mixtures of MeOH–H_2O (3:7, 4:6, 1:1) as eluents to give additional subfractions F59–F68 [MeOH–H_2O 3:7 [eluates F59–F60 (each 25 ml), F61 (200 ml), F62 (100 ml), F63 (600 ml)], 4:6 (eluate F64, 500 ml), 1:0 (eluate F65–F68, 100 ml), respectively]. Subfractions F61–63 were combined and subjected to reversed-phase preparative HPLC (MeCN-H_2O, 25:75) separation to afford **1** (3.87 mg), **2** [70.32 mg, $[\alpha]_D^{20}$ +34.0° (*c* 0.10, MeOH)], **3** [(2.03 mg), $[\alpha]_D^{20}$ +7.0° (*c* 0.10, MeOH)], **6** (2.72 mg), **7** [1.46 mg, $[\alpha]_D^{20}$ +14.0° (*c* 0.10, MeOH)], **8** [6.83 mg, $[\alpha]_D^{20}$ +3.3° (*c* 0.61, MeOH)], dihydrodehydrodiconiferyl alcohol (**9**) [30.47 mg, $[\alpha]_D^{20}$ +0.0° (*c* 0.10, MeOH)], and 5-methoxy-*trans*-dihydrodehydrodiconiferyl alcohol (**10**) [3.86 mg, $[\alpha]_D^{20}$ +4.7° (*c* 0.34, MeOH)].

3.5. Burselignan (*1*)

White powder; $[\alpha]_D^{20}$: −64.2° (MeOH; *c* 0.01); UV nm (MeOH) λ_{max} (log ε): 222 (4.0), 284 (3.5); IR cm^{-1} (dried film) ν_{max}: 3350, 2938, 1512, 1456, 1370, 1249, 1119, 1027; for ^1H and ^{13}C NMR spectra, see Table 1; positive HRTOFESIMS *m/z*: 383.1475 $[M + Na]^+$, calcd 383.1471 for $C_{20}H_{24}O_6Na$.

3.6. Bursephenylpropane (*6*)

Colorless syrup; UV nm (MeOH) λ_{max} (log ε): 216 (3.8); IR cm^{-1} (dried film) ν_{max}: 3375, 2939, 1590, 1505, 1458, 1422, 1228, 1123, 1053, 1032; for ^1H and ^{13}C NMR spectra, see Table 1; positive HRTOFESIMS *m/z*: 309.1311 $[M + Na]^+$, calcd for 309.1314, $C_{14}H_{22}O_6Na$.

3.7. Burseneolignan (*7*)

Colorless syrup; $[\alpha]_D^{20}$: +14.0° (MeOH; *c* 0.1); UV nm (MeOH) λ_{max} (log ε): 217 (4.0); IR cm^{-1} (dried film) ν_{max}: 3354, 2939, 2841, 1592, 1508, 1458, 1422, 1270, 1238, 1225, 1123, 1034; for ^1H and ^{13}C NMR spectra see Table 1; positive HRTOFESIMS *m/z*: 461.1780 $[M + Na]^+$, calcd 461.1788 for $C_{22}H_{30}O_9Na$.

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