NEW CYTOTOXIC CINNAMIC ACID DERIVATIVES FROM LEAVES OF *Bonamia trichantha*

AHMED A. HUSSEIN[1,4], DIONISIO A. OLMEDO[1], YELKAIRA VASQUEZ[1], PHYLLIS D. COLEY[2,3], PABLO N. SOLIS[1] AND MAHABIR P. GUPTA[1,2*]

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ABSTRACT

Bioassay-guided fractionation of the methanolic extract of the young leaves of *Bonamia trichantha* led to the isolation of four new cinnamic acid derivatives trichanthins **A-D** (**1-4**).Their structures were established by spectroscopic methods. All compounds were tested in cytotoxic assays against the MCF-7, H-460 and SF-268 human cancer cell lines.

Key words: *Bonamia trichantha*; Convolvulaceae; Caffeic acid ester; *p*-Coumaric acid ester: Farnesol; Z-(11)-hexadecen-1-ol; Cytotoxicity.

RESUMEN

Del extracto metanólico de las hojas jóvenes de *Bonamia trichantha*, se aislaron cuatro nuevos derivados del ácido cinámico, cuyas estructuras fueron determinadas por métodos espectroscópicos. Tres de los compuestos mostraron actividad citotóxica sobre las líneas celulares cancerosas humanas MCF-7, H-460 y SF-268.

INTRODUCTION

This work was carried out as part of an Ecologically Based Bioprospecting program in Panama funded by ICBG (International Cooperative Biodiversity Group, NIH) (Kursar *et al.*, 1999). The genus *Bonamia* (Convolvulaceae) comprises approximately 45 species in the tropics and subtropics of both hemispheres. Two species occur in Panama; *B. sulphurea* and *B. trichantha*. *Bonamia trichantha* Hallier f. is widely distributed in Panama (Haynes *et al.*, 1975). There are not traditional uses reported for this species, and neither information on its chemical constituents. In our ongoing search for new potential anticancer agents from Panamanian flora (Rodríguez *et al.*, 2003, Hussein *et al.*, 2004), *B. trichantha* methanolic extract of young leaves showed *in vitro* cytotoxic

*[1] Centro de Investigaciones Farmacognósticas de la Flora Panameña (CIFLORPAN), Facultad de Farmacia, Universidad de Panamá, Apartado 10767, Estafeta Universitaria, Panamá, República de Panamá.
[2] Smithsonian Tropical Research Institute, Box 2072, Panama, Republic of Panama.
[3] Department of Biology, University of Utah, Salt Lake City, UT 84112-0840.
[4] Present address Department of Phytochemistry, Pharmaceutical Sciences Division, National Research Centre, Cairo, Egypt.
* To whom correspondence should be addressed. Tel: (507) 269-7655. Fax: (507) 264-0789.
E-Mail: cytedqff@ancon.up.ac.pa

activity against MCF-7, H-460 and SF-268 human cancer cell lines. The present paper describes the isolation, structure elucidation and the cytotoxic activity of the compounds, named trichanthins **A–D**.

RESULTS AND DISCUSSION

Bioassay-guided fractionation of the methanolic extract of young leaves of *B. trichantha* using MCF-7 (breast), H-460 (lung) and SF-268 (CNS) human cancer cell lines afforded four new cinnamic derivatives named trichanthins **A–D**.

Compound **1** exhibited a blue fluorescent spot under UV_{365nm} light. The IR spectrum exhibited absorption bands due to –OH (3600-3000 cm^{-1}) and carbonyl group (1650 cm^{-1}). Its ^1H NMR spectrum showed signals assigned to aromatic protons at 7.06 (1H, d, J = 1.0 Hz, H-2), 6.85 (1H, d, J = 8.3 *Hz*, H-5) and 6.92 (1H, dd, J = 8.3, 1.0 Hz, H-6) characteristic of a trisubstituted aromatic ring and two olefinic proton doublets at δ 7.54 and 6.21 ppm with a J = 15.3 *Hz*, indicated a *trans* double bond. These data suggested the presence of a *trans*-caffeic acid moiety in this compound, similar to those reported by Hamerski *et al.*, 2005. Further signals in the ^1H NMR spectrum (see Table 1) indicated the presence of a farnesyl group (Inoue *et al.*, 1994), which must be esterifying the carboxyl group, since a long-range correlation is observed in the HMBC spectrum between H-1' and C-9 (δ = 168.0).

Further long-range correlations observed in the HMBC spectrum between H-1'/C-2', C-3'; H-2'/C-3', C-15' and the connectivities observed in the NOESY spectrum between H-1'/Me-15'; H-10'/Me-12'; allowed the unambiguous assignment of all methyl signals and stereochemistry of the double bonds to the farnesyl moiety. The remaining connectivities observed in the HMBC spectrum corresponded to caffeic moiety [H-7/C-9, C-8, C-1, C-6]. Based on the above data, structure **1** was assigned to the new compound trichanthin **A**.

The IR spectrum of compound **2** exhibited absorption bands due to –OH (3320 cm^{-1}) and carbonyl (1648 cm^{-1}) group. Its ^1H NMR spectrum (see Table 1) showed signals assigned to aromatic protons at 7.38 (2H, d, J = 8.5 *Hz*, H-2, H-6) and 6.92 (2H, d, J = 8.5 *Hz*, H-3, H-5) characteristic of a *p*-disubstituted aromatic ring and two olefinic proton doublets at δ 7.62 and 6.30 ppm with a J = 15.3 *Hz*, indicated a *trans* double bond.

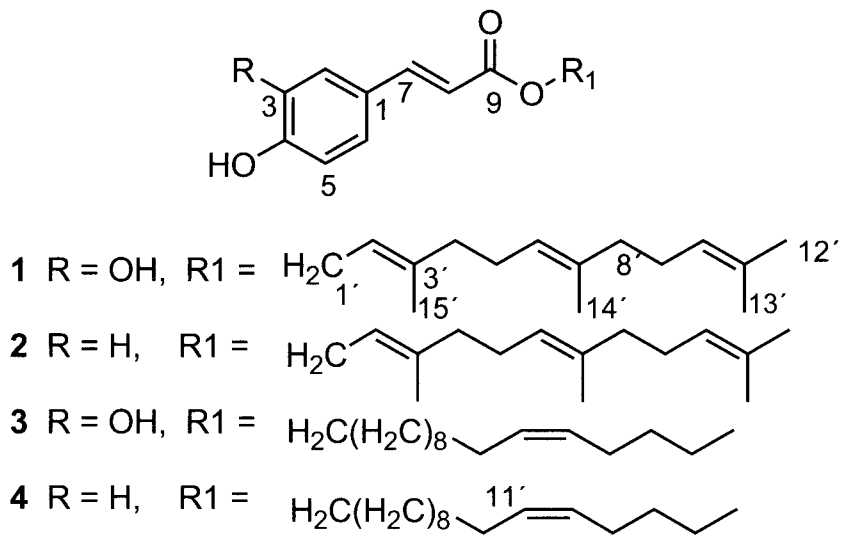

Figure 1. Structures of Compounds **1-4**.

Table 1. $^{1}H^{a}$ and $^{13}C^{b}$ NMR spectral data of Compounds **1-4**

	1		**2**		**3**		**4**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Position	δH (*J in* Hz)		δH (*J in* Hz)		δH (*J in* Hz)		δH (*J in* Hz)	
1		127.1		126.8		127.1		127.0
2	7.06 d (1.0)	114.1	7.38 d (8.5)	130.0	7.06 d (1.0)	114.5	7.38 d (8.5)	130.0
3		144.0	6.92 d (8.5)	116.0		142.2	6.92 d (8.5)	115.9
4		146.1		158.6		146.9		158.1
5	6.85 d (8.3)	115.2	6.92 d (8.5)	115.9	6.85 d (8.3)	115.5	6.92 d (8.5)	115.9
6	6.92 dd (1.0, 8.3)	122.1	7.38 d (8.5)	130.0	6.92 dd (1.0, 8.5)	122.4	7.38 d (8.5)	130.0
7	7.54 d (15.3)	145.1	7.62 d (15.3)	144.9	7.54 d (15.7)	145.6	7.62 d (16.1)	144.9
8	6.21 d (15.3)	115.0	6.30 d (15.3)	115.1	6.21 d (15.7)	115.0	6.30 d (16.1)	114.7
9		168.0		168.1		168.7		167.8
1'	4.72 d (5.4)	61.5	4.71 d (5.4)	61.6	4.24 d (6.6)	65.2	4.26 d (6.7)	64.5
2'	5.39 d (5.4)	118.0	5.41 d (5.4)	118.3	1.72 m	28.7	1.72 m	28.7
3'		142.4		142.4	1.25 m	31.9	1.25 m	31.9
4'	1.94-2.08 m	39.5	1.95-2.10 m	39.7	1.25 m	29.8	1.25 m	29.8
5'	1.94-2.08 m	26.5	1.95-2.10 m	26.9	1.25 m	29.7	1.25 m	29.7
6'	5.07 m	123.4	5.09 m	123.6	1.25 m	29.6	1.25 m	29.6
7'		135.0		131.5	1.25 m	29.5	1.25 m	29.5
8'	1.94-2.08 m	39.3	1.95-2.10 m	39.5	1.25 m	29.3	1.25 m	29.3
9'	1.94-2.08 m	26.0	1.95-2.10 m	26.0	1.25 m	29.2	1.25 m	29.2
10'	5.07 m	124.1	5.07 m	124.6	2.07 m	27.2	2.07 m	27.2
11'		131.1		131.3	5.30 dt (2.1, 6.9)	130.0	5.31 dt (2.0, 6.5)	130.0
12'	1.65 s	25.4	1.64 s	25.7	5.30 dt (2.1, 6.9)	129.8	5.31 dt (2.0, 6.5)	129.9
13'	1.57 s	17.6	1.57 s	17.7	2.07 m	27.2	2.07 m	27.2
14'	1.57 s	15.8	1.57 s	16.0	1.25 m	25.9	1.25 m	25.9
15'	1.71 s	16.3	1.73 s	16.5	1.25 m	22.7	1.25 m	22.7
16'					0.85 t (1.3)	14.1	0.85 t (1.3)	14.1

[a] δH values were obtained in $CDCl_3$, at 300 MHz, TMS, Internal Standard.

[b] ^{13}C spectra were recorded in $CDCl_3$ at 75 MHz. Assignments were made on the basis of COSY, HMBC and NOESY spectra for compounds 1-4

These data suggested the presence of a *trans* *p*-coumaric acid moiety in this compound, similar to those described by Wu *et al.*, 2005. Further long-range correlations observed in the HMBC spectrum between H-7/C-9, C-8, C-6, C-2, confirmed the presence of a p-coumaric acid moiety in the compound **2**. The remaining signals in the ^{1}H NMR (Table 1) and the observed connectivities, in the HMBC and NOESY spectra indicated the presence of a farnesyl moiety as in compound **1**. Based on the above data the structure of **2** was assigned to the new farnesyl ester of *p*-coumaric acid, named trichanthin **B**, which has been synthesized by Moeller *et al.*, 1992. However, its presence in plant kingdom is reported for the first time.

The IR spectrum of Compound **3** showed similar absorption bands to those of compounds **1** and **2**, corresponding to hydroxyl (3100 cm^{-1}) and carbonyl groups (1650 cm^{-1}). Its ^{1}H NMR spectrum (see Table 1) showed signals which suggested the presence of a *trans*-caffeic acid moiety as in compound 1. Further signals assigned to aliphatic methylene groups at δ 1.25 (18H, m, H-3 to H-9, H-14', H-15'), terminal methyl at 0.85 (3H, t, J = 1.3 *Hz*, H-16') and an oxygenated methylene at 4.24 (2H t, J = 6.6 *Hz*, H-1'), indicated a long chain alcoholic residue. The presence of a double triplet signal at 5.30 (2H, dt, J = 2.1, 6.9 *Hz*, H-11', H-12') assigned to two olefinic protons, indicated a *cis* double bond between protons H-11' and H-12'.

The *Z*-(11)-hexadecen-1-yl moiety, must be esterifying the carboxyl group in com-

pound **3**, since a long-range correlation was observed in the HMBC spectrum between H-1' (4.24 ppm) and C-9 (δ = 168.7 ppm).

The remaining connectivities observable in the HMBC spectrum corresponding to caffeic moiety as described for trichanthin **A**. Alkaline hydrolysis of **3** yielded *Z*-(11)-hexadecen-1-ol, which was identified by GC-MS analysis. Based on these data the structure of **3** was assigned to the new compound *Z*-(11)-hexadecen-1-yl ester of caffeic acid, which was named trichanthin **C**.

Compound **4** exhibited in the IR similar bands to those compounds described above in this paper. Its ^{1}H NMR spectrum (see Table 1) showed identical signals corresponding to a *trans p*-coumaric acid as in compound **2** and also displayed characteristic signals to an *Z*-(11)-hexadecen-1-yl moiety as in compound **3**. Thus, compound **4** was identified as the *Z*-(11)-hexadecenyl ester of *p*-coumaric acid, which was named trichanthin **D.**

Table 2 shows GI_{50} values of trichanthins **A-D** (**1-4**). Compounds **1**, **2** and **4** showed cytotoxic activity against the MCF-7 (breast), H-460 (lung), and SF-268 (CNS), cell lines, while compound **3** was inactive. Cytotoxic activity of phenolic acids is well documented (García *et al.*, 1998, Han *et al.*, 2003, Babich *et al.*, 2003). Antioxidant, immunostimulatory and neutrophil elastase inhibitory activities have also been reported for phenolic acids and their derivatives (Sawicka *et al.*, 1994, Löser *et al.*, 2000, Cos *et al.*, 2002). Ester derivatives of phenolic acids like farnesyl and fatty alcohol showed more potent activity than their parent compounds, which may suggest that lipid derivatives can augment the activity of these compounds (Mishima *et al.*, 1999, Saek *et al.*, 2000). Antiseborrheic activity has been reported for the compound **2** (Moeller *et al.*, 1992).

Table 2. Cytotoxic Activity of Plant Extract and Compounds **1–4**.

compound /extract	MCF-7	H-460	SF-268
		GI_{50} (μg/mL)	
B. tricantha young leaves methanolic extract	6.2	4.2	<1
trichanthin **A** (**1**)	1.1	2.3	2.5
trichanthin **B** (**2**)	4.5	6.1	6.4
trichanthin **C** (**3**)	>10	>10	>10
trichanthin **D** (**4**)	1.8	1.9	2.6
adriamycin	0.37	0.42	0.50

EXPERIMENTAL PART

General

Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. NMR spectra were recorded using a Brüker Avance 300 spectrometer in CDCl3 at 300 MHz for ^{1}H and 75.0 MHz for ^{13}C NMR. Mass spectra were obtained on a Kratos MS50TC mass spectrometer. GC-MS Agilent Technologies 6890N gas chromatograph with a 5973 electronic impact (EI) detector and HP-5MS fused silica capillary column with 30 m X 0.25 mm I. D. and 25 μm film thickness. The samples were introduced via all-glass injector working in the splitless mode, with He as carrier gas, linear velocity 43 cm/s. The GC conditions were: Column temperature programmed from 40 °C (no holding time) to 150 °C at 10 °C/min., holding 1 min. and increased to 250 °C at 5 °C/min., holding 4 min. The injector temperature was 250 °C. The identification of components was accomplished using the Wiley7n.l database with a 95% minimum quality. Silica gel [Merck, Kieselgel 60 (0.063–0.200 mm) and (0.015–0.040 mm)] was used for column chromatography. Silica gel plates (Merck, Kieselgel 60 F_{254s}) were used for TLC.

Cytotoxicity bioassays

The cytotoxic activity was determined according to the method of Monks *et al.*, 1997.

Plant Material

Young leaves of *B. trichantha* were collected from Camino de Cruces (N 9° 0´ 20´´, W 79° 35' 35", in May 2003). Voucher specimen number 54935 was deposited in the Herbarium of the University of Panama (PMA).

Extraction and isolation

Fresh young leaves (274 g) were extracted and subjected to solvent partitioning as described before (Hussein *et al.*, 2003, 2004). Briefly, fresh young leaves of *B. trichantha* were homogenized with cold methanol for 30 s in a Waring blender followed by treatment with a Polytron homogenizer (Brinkmann Instruments, Inc. Westbury) for least 2 min or until the suspension of leaf material was homogenous. The mixture was filtered under a vacuum through Whatman # 4 filter paper, and the mark was washed with EtOAc. The MeOH and EtOAc extracts were combined and filtered through Whatman #1 filter paper. The extracts was concentrated *in vacuo* at < 40 °C in a rotary evaporator and stored at – 80 °C until for use. The residue (15.1 g) was partitioned between CH_2Cl_2 and H_2O, and the CH_2Cl_2 partition was concentrated to dryness, redissolved in 90% MeOH, and extracted with hexane. The aqueous partition was extracted with EtOAc. The activity was retained in hexane and 90% MeOH fractions, which were combined together because of similar TLC profiles (4.5 g). Column chromatography on a Silica gel using a gradient of EtOAc in hexane (0 to 100% EtOAc) yielded 14 fractions. Re-chromatography of fraction 4 (355 mg) on a Silica gel column using as a solvent system hexane/EtOAc (65:35, 1500 ml) yielded pure compound **1** (80 mg, 0.029 %). Fraction 6 (420 mg) was chromatographed under the same conditions and yielded pure compound **2** (10 mg, 0.004 %). Fraction 3 (250 mg) was chromatographed on Silica gel column using 10 % EtOAc in hexane to afford compound **3** (70 mg, 0.025 %) and compound **4** (13 mg, 0.005 %).

Trichanthin A (1): Colourless crystals. mp 80 °C . IR (KBr): υ_{max} 3600–3000 (*br*), 2950, 1650, 1616, 1580, 1505, 1433, 1368, 1245, cm^{-1}. ^1H- and ^{13}C- NMR, $CDCl_3$ (see Table 1). EIMS *m/z* (rel. int.) $[M]^+$ 384.2(20.5), 179.9(87.8), 163.0(61.5), 136.0(100) HR-FABMS found *m/z* 384.22971 (calcd for $C_{24}H_{32}O_4$, 384.23006).

Trichanthin B (2): Colourless crystals. mp 135 °C. IR (KBr): υ_{max} 3320, 2850, 1648, 1570, 1490, 1415, 1340, 1240, 1140 cm^{-1}; ^1H- and ^{13}C- NMR, $CDCl_3$ (see Table 1). EIMS molecular peak was not seen, *m/z* (rel. int.) 255(100), 240(13), 225(13), 162(36) 120(36).

Trichanthin C (3): Viscous oil; IR (KBr): υ_{max} 3100, 2760, 1610, 1510, 1430, 1380, 1290, 1220, 1130 cm^{-1}. ^1H- and ^{13}C- NMR, $CDCl_3$ (see Table 1). EIMS molecular peak could not detected, *m/z* (rel. int.) 179.9(100), 163(83.3), 136.0(19.2).

Trichanthin D (4): Viscous oil; IR (KBr): υ_{max} 3300, 2755, 1615, 1519, 1438, 1384, 1290, 1220 cm^{-1}. ^1H- and ^{13}C- NMR, $CDCl_3$ (see Table 1). EIMS, molecular peak could not detected, *m/z* (rel. int.) 194(100), 162(72.5), 137(70.4), 120(36.5), 109(58.4), 97.

Alkaline hydrolysis of compounds 3 and 4.

Compounds **3** (30 mg) and **4** (10 mg) were dissolved separately in KOH (10%, 8 ml) and $CHCl_3$ (20 ml) and the solution was left for 2 hrs at room temperature while stirring. The $CHCl_3$ layer was concentrated and chromatographed on silica gel column using Hexane:EtOAc (8:2). The resulting fractions **3a** (5 mg) and **4a** (1 mg) were analyzed by GC-MS and the compound *Z*-(11)-hexadecen-1-ol was identified (R_t 24.45, M^+ 240.40). (calcd $C_{16}H_{32}O$, 240.50)

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