Atrovirisidone B, a New Prenylated Depsidone with Cytotoxic Property from the Roots of *Garcinia atroviridis*

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A new prenylated depsidone, atrovirisidone B (**2**), together with naringenin (**3**) and 3,8″-binaringenin (**4**) were isolated from the roots of *Garcinia atroviridis*. Their structures were determined on the basis of spectral data interpretation. Compound **2** showed cytotoxic activity against human breast (MCF-7), human prostate (DU-145) and human lung (H-460) cancer cells.

Key words: Garcinia atroviridis, Cytotoxic Activity, Atrovirisidone B

Introduction

Garcinia atroviridis Griff. ex T. Anderson (Guttiferae) is a medium-sized fruit tree, which may be found growing wild or cultivated, and widely distributed throughout the Malaysian Peninsula. The fruits of *G. atroviridis* are highly acidic, and their thinly sliced dried form is available commercially as a seasoning. In folkloric medicine, this plant has been used for the treatment of cough, dandruff, earache, stomach pains associated with pregnancy, and throat irritation. In a preliminary investigation of the biological activities of *G. atroviridis*, the roots were found to exhibit antibacterial and antioxidant activities (Mackeen *et al.*, 2000).

In our previous report, we described the isolation, characterization and biological activities of atrovirisidone (**1**), atrovirinone, 4-methylhydroatrovirinone, morelloflavone, fukugiside and 14-*cis*-docosenoic acid from this species (Permana *et al.*, 2001, 2003). Since in our preliminary anti-inflammatory assay strong activity was observed for atrovirinone, we attempted to isolate this compound further in order to carry out a detailed evaluation. In the current investigation on the roots of *G. atroviridis*, a new prenylated depsidone, named atrovirisidone B (**2**), together with naringenin (**3**) and 3,8″-binaringenin (**4**) were isolated. We describe herewith the isolation and structure elucidation of these compounds, and the evaluation of their cytotoxic activity against a panel of three human tumor cell lines.

Results and Discussion

Structure elucidation

Atrovirisidone B (**2**) (Fig. 1) was obtained as white crystals with a melting point of 156–158 °C; it gave a molecular ion peak at *m/z* 426.1673 by HREIMS, which is consistent with the molecular formula $C_{24}H_{26}O_7$. The UV spectrum showed an absorption bands at λ_{max} 210 and 311 nm. The IR spectrum exhibited absorption bands due to hydroxyl and lactone carbonyl groups at 3449 cm^{-1} and 1630 cm^{-1}, respectively. The ^{13}C NMR spectrum (Table I) also supported the presence of a lactone carbonyl group by the signal at δ_C 168.4 (C-11). In the ^1H NMR spectrum, a signal at δ_H 11.01 (OH, s) indicated the presence of a chelated proton. The HMBC spectrum further confirmed that the signal (δ 11.01) correlated with the aromatic carbon atom at δ_C 165.9 (C-1) as well as with that at δ_C 100.6 (C-2), and with a quaternary carbon atom at δ_C 99.7 (C-11a). The ^1H NMR and HSQC spectra showed the signals assignable to two *meta* coupled aromatic protons at δ_H 6.34 (δ_C 101.0, C-4) and δ_H 6.28 (δ_C 100.6, C-2). In the

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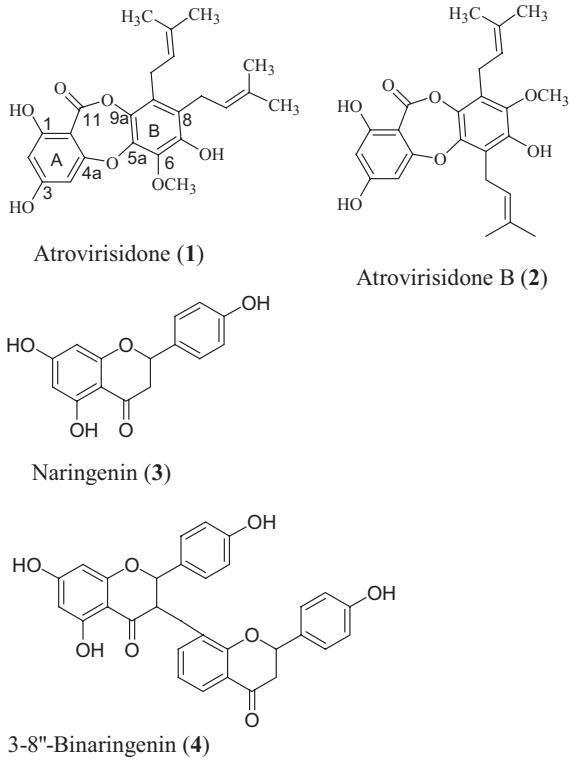

Fig. 1. The constituents isolated from the roots of *G. atroviridis*.

HMBC experiment, the aromatic proton signal at δ_H 6.34 showed connectivity to two of the hydroxylated/oxygenated aromatic carbon atoms at δ_C 163.1 (C-3) and δ_C 162.3 (C-4a). Further, the proton signal at δ_H 6.34 also showed ^3J correlations to the carbon atoms at δ_C 100.6 (C-2) and δ_C 99.7 (C-11a). Another aromatic proton signal at δ_H 6.28 correlated with the carbon atoms at δ_C 101.0 (C-4), δ_C 99.7 (C-11a), in addition to correlations with the hydroxylated aromatic carbon atoms at δ_C 165.9 (C-1) and δ_C 163.1 (C-3). These assignments confirmed the location of hydroxyl groups at C-1 and C-3. The presence of two prenyl units was shown by the ^{1}H NMR, HSQC and HMBC spectra, which indicated the presence of two sets of signals, one at δ_H 3.53 (δ_C 23.4), δ_H 5.22 (δ_C 121.9), δ_C 133.2, δ_H 1.74 (δ_C 26.0), δ_H 1.87 (δ_C 18.3), and the other at δ_H 3.48 (δ_C 24.1), δ_H 5.22 (δ_C 121.7), δ_C 133.1, δ_H 1.71 (δ_C 26.0) and δ_H 1.83 (δ_C 25.9). Observation on ^{1}H-^{13}C correlations of the protons signal at δ_H 3.53 to two of the oxygenated aromatic carbon atoms at δ_C 145.1 (C-5a) and δ_C 145.4 (C-7), and the quaternary carbon atom at δ_C 118.6 (C-6) indicated that one of the prenyl moieties is attached at C-6. Furthermore, the hydroxyl proton signal at δ 5.65 was coupled to the carbon signals at δ_C 145.4 (C-7) as well as to those at δ_C 118.6 (C-6) and δ_C 142.5 (C-8). The methoxyl signal at δ_H 3.78 was found to correlate with the carbon signal at δ_C 142.5 (C-8). The HMBC spectrum further suggested that the hydroxyl and methoxyl functionalities are attached to C-7 and C-8, respectively. The assignment of the second prenyl unit at C-9 was based on the HMBC spectrum which indicated ^{1}H-^{13}C correlations of the proton signal at δ_H 3.48 with the oxygenated aromatic carbon atom at δ_C 136.3 (C-9a) and the methoxylated carbon atom at δ_C 142.5 (C-8), as well as the quaternary carbon atom at δ_C 125.4 (C-9). Based on the above considerations, compound **2** is therefore deduced as a new prenylated depsidone, atrovirisidone B [1,3,7-trihydroxy-8-methoxy-6,9-di-(3-methyl-2-butenyl)-1*H*-dibenzo [*b*,*e*]-[1,4]dioxepin-11-one].

Naringenin (**3**) and 3,8″-binaringenin (**4**) were identified based on analysis of the NMR data and comparison with literature data (Duddeck *et al.*, 1978; Lin *et al.*, 1997). These compounds are commonly found in *Garcinia* species (Waterman and Hussain, 1983).

Cytotoxic activity

The results depicted in Table II summarize the cytotoxic effects of all isolates on human breast cancer (MCF-7), human prostate cancer (DU-145) and lung cancer (H-460) cell lines. Compounds **1** and **2** showed cytotoxic activity toward all cell lines tested. Compound **3** only exhibited weak cytotoxic activity against DU-145 cells (IC_{50} = 30.9 ± 1.9 μM), while compound **4** was inactive toward all cell lines tested.

Experimental

General experimental procedures

Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. The UV and IR spectra were recorded on Shimadzu UV-VIS 160 and Perkin-Elmer 1650 FTIR spectrometers, respectively. NMR spectra were determined on a Varian Unity 500 spectrometer Varian Inc, Palo Alto, CA; 500 MHz for ^{1}H and 125 MHz for ^{13}C. EIMS and HR-MS were taken on a Finnigan MAT95XL-T mass spectrometer. Merck silica

Table I. ^{13}C NMR (125 MHz, $CDCl_3$),^{1}H NMR (500 MHz, $CDCl_3$) data and HMBC of atrovirisidone B (**2**).

C	^{13}C NMR (ppm)	^{1}H NMR (ppm)	HMBC
1	165.9	11.01 (OH, s)	C-2, C-11a
2	100.6	6.28 (H, d, J = 2.0 Hz)	C-1, C-3, C-4, C-11a
3	163.1		
4	101.0	6.34 (H, d, J = 2.0 Hz)	C-2, C-11a
4a	162.3		
5a	145.1		
6	118.6		
7	145.4	5.65 (OH, s)	C-6, C-8
8	142.5		
8-OCH_3	62.0	3.78 (3H, s)	
9	125.4		
9a	136.3		
11	168.4		
11a	99.7		
1′	23.4	3.53 (2H, d, J = 6.5 Hz)	C-5a, C-6, C-7, C-3′
2′	121.9	5.22 (H, m)	C-6, C-4′, C-5′
3′	133.2		
4′	26.0	1.74 (3H, s)	C-2′, C-3′, C-5′
5′	18.3	1.87 (3H, s)	C-2′, C-3′, C-4′
1″	24.1	3.48 (2H, d, J = 7.0 Hz)	C-8, C-9a, C-3″
2″	121.7	5.22 (H, m)	C-9, C-4″, C-5″
3″	133.1		
4″	26.0	1.71 (3H, s)	C-2″, C-3″, C-5″
5″	25.9	1.83 (3H, s)	C-2″, C-3″, C-4″

Table II. Cytotoxic activity of compounds **1**, **2**, **3**, and **4**[a].

Compound	MCF-7	DU-145	H-460
1	11.02 ± 6.4	24.78 ± 13.4	16.11 ± 1.2
2	22.93 ± 6.8	9.34 ± 7.4	16.47 ± 1.2
3	113.5 ± 3.6	30.9 ± 1.9	nd
4	85.10 ± 2.9	nd	88.18 ± 1.4

nd, IC_{50} value is higher than the range of concentrations tested.

[a] Results are expressed as IC_{50} values (μM) ± SD of 3 experiments performed in triplicate.

gel 9385 was used for column chromatography. Analytical TLC was run on Merck DC-Plastikfolien 60 F_{254}.

Plant material

The main and the small roots of *Garcinia atroviridis* were collected in February 2003 from the Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Selangor, Malaysia. A voucher specimen (MM-1) was deposited at the herbarium of the Biology Department, University of Putra Malaysia.

Extraction and isolation

The ground air-dried sample of the small roots (0.5–1 cm diameter, attached to main roots) of *Garcinia atroviridis* (0.8 kg) was extracted three times with MeOH, each time by soaking in 4 l of solvent overnight. The combined extracts were evaporated under reduced pressure to give a brownish gum (97.0 g). The extract (90.0 g) was shaken with 1000 ml of water/MeOH (7:3 v/v) mixture and fractionated with EtOAc (3 × 250 ml). Removal of the solvents from the EtOAC fraction under reduced pressure gave a brownish gum (14.3 g). The extract (14.0 g) was then subjected to silica gel column chromatography (5.0 × 15 cm) and successively eluted with *n*-hexane followed by *n*-hexane/EtOAc (9:1 v/v), *n*-hexane/EtOAc (4:1 v/v), *n*-hexane/EtOAc (2:1 v/v), *n*-hexane/EtOAc (1:1 v/v), EtOAc, and finally with MeOH to give fourty five fractions (50 ml each) designated as

fractions A (1–4), B (5–6), C (7–11), D (12–13), E (14–21), F (22–27), G (28–31), and H (32–45).

Purification of fraction C (2.6 g) using silica gel column chromatography (*n*-hexane/EtOAc, 2:1 v/v) yielded atrovirisidone (**1**). Fraction D (0.6 g) was rechromatographed on a silica gel column (2.5 × 15 cm) and eluted with *n*-hexane/EtOAc (2:1 v/v) to give twenty six fractions (15 ml each). The combined fractions 8–15 (0.3 g) were further rechromatographed on a silica gel column (2.5 × 10 cm) (*n*-hexane/EtOAc, 2:1 v/v) to give twenty fractions (5 ml each). Fractions 10–14 were combined and recrystallized from dichloromethane/*n*-hexane to give atrovirisidone B (**2**) as white crystals (13 mg).

The ground air-dried main roots (5–8 cm diameter, attached to trunk, 1.5 kg) were extracted three times with MeOH, each time by soaking in 6 l of solvent for overnight. The combined extracts were evaporated under reduced pressure to give a brownish gum (130.0 g). The extract (125.0 g) was shaken with 1000 ml of water/MeOH (7:3 v/v) mixture and fractionated with EtOAc (3 × 400 ml). Removal of the solvent from the EtOAC fraction under reduced pressure gave a brownish gum (21.7 g). Purification of the ethyl acetate fraction by silica gel column chromatography (*n*-hexane/EtOAc, 3:2 v/v) gave naringenin (**3**) (17 mg) and 3,8″-binaringenin (**4**) (50 mg), both as a yellow amorphous solid.

*Atrovirisidone B (**2**)*

White crystals (dichloromethane/*n*-hexane); m.p. 156–158 °C. – UV (MeOH): λ_{max} (log ε) = 210 (4.87), 311 nm (4.49). – IR (KBr): λ_{max} = 3449, 2929, 2972, 1630, 1458, 1432, 1303, 1169, 1057 cm^{-1}. – ^1H and ^{13}C NMR: see Table I. – MS (EI, 70 eV): *m/z* (rel. int.) = 426 (14), 357 (100), 339 (32), 325 (30), 297 (13), 285 (69), 273 (13), 205 (10), 153 (12), 121 (7), 91 (7), 69 (6). – HREIMS: *m/z* = 426.1673 [M^+], calcd. for $C_{24}H_{26}O_7$ 426.1673.

Cytotoxicity assay

The *in vitro* cytotoxicity assay was carried out according to the procedures described previously (Stanslas *et al*., 2000). The absorbance of the formazan solution was determined at 550 nm using a microplate reader (Spectramax Plus, USA). The IC_{50} values (concentration of a drug that produces 50% reduction in the absorbance compared with untreated controls) were determined from the dose-response curves.

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