Isolation and Bioactivities of Constitutents of the Roots of *Garcinia atroviridis*

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Received November 30, 2000

Two new prenylated compounds, the benzoquinone atrovirinone (**1**) and the depsidone atrovirisidone (**2**), were isolated from the roots of *Garcinia atroviridis.* Their structures were determined on the basis of the analysis of spectroscopic data. While compound **2** showed some cytotoxicity against HeLa cells, both compounds **1** and **2** were only mildly inhibitory toward *Bacillus cereus* and *Staphylococcus aureus*.

Garcinia atroviridis Griff ex T. Anders (Guttiferae), a medium-sized fruit tree, which may be found growing wild or cultivated, is widely distributed throughout Peninsular Malaysia. 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.[1] In a preliminary investigation of the biological activities of *G. atroviridis*, the roots were found to exhibit antibacterial and antioxidant activities.[1] Previous chemical studies on this species have reported the isolation of garcinia acid (hydroxycitric acid) and its dibutyl methyl ester and β- and γ-lactone derivatives from the fruits, and a tetraoxygenated xanthone, atroviridin, from the stem bark.[2−4] In this paper, we describe the isolation and structure elucidation of atrovirinone (**1**), a new prenylated benzoquinone, and atrovirisidone (**2**), a new prenylated depsidone from the methanol extract of the dried roots of *G. atroviridis*. Both compounds were also assayed for antimicrobial and cytotoxic activities.

Compound **1**, obtained as red needles, was assigned the molecular formula $C_{25}H_{28}O_8$ by HRFABMS. The IR spectrum exhibited absorption bands at 3382 and 1654 cm^{-1} for hydroxyl and carbonyl functionalities, respectively. The UV spectrum showed an absorption band at λ_{max} 204, 221, and 273 nm, which was consistent with the absorptions of a 1,4-benzoquinone chromophore, as previously reported.[5,6] The ^{13}C NMR spectrum (in $CDCl_3$) showed resonances for all 25 carbons present in the molecule (Table 1). The carbon signals at δ 183.4, 182.2, and 170.8 were assigned to two *p*-benzoquinone carbonyls and an ester carbonyl, respectively. The ^1H NMR proton and HMQC spectra indicated the presence of two methoxyls from the signals at δ 4.04 (δ_C 60.4) and 3.90 (δ_C 52.5). The HMBC spectrum further suggested that the former is attached to an olefinic carbon (δ_C 135.3), and the latter is located at a carbonyl carbon (δ_C 170.8).

The ^{13}C NMR data of **1** indicated the presence of three hydroxylated or alkoxylated aromatic carbons at δ 165.5, 161.2, and 160.1. The ^1H NMR and HMQC spectra showed signals due to two *meta*-coupled aromatic proton signals at δ 6.11 (δ_C 98.0) and δ 5.72 (δ_C 95.0). The HMBC spectrum revealed that correlations occurred between C-6‴ (δ 165.5), C-1‴ (δ 97.2), and C-5‴ (δ 98.0) with the HO-6‴ proton signal at δ 11.90, while the signals of C-4‴ (δ 161.2), C-3‴ (δ 95.0), and C-5‴ (δ 98.0) correlated with the HO-4‴ proton signal at δ 5.59, suggesting that the two hydroxyls are located at C-4‴ and C-6‴. In the aromatic region of the spectrum, the proton signal at δ 6.11 (H-5‴) further showed correlations with the carbon signals at δ 165.5 (C-6‴), 161.2 (C-4‴), 97.2 (C-1‴), and 95.0 (C-3‴), while another aromatic proton signal at δ 5.72 (H-3‴) correlated with the carbon signals at δ 161.2 (C-4‴), 160.1 (C-2‴), 98.0 (C-5‴), and 97.2 (C-1‴), establishing the partial structure **a** (Figure 1).

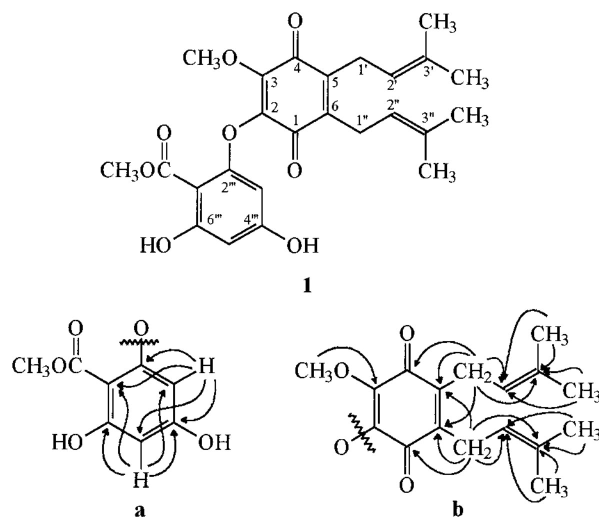

Figure 1. Compound **1** and its partial structures (**a** and **b**) with selected HMBC correlations.

Although it was not possible to unambiguously assign their ^1H and ^{13}C NMR signals, the presence of two prenyl side chains in **1** was indicated by the occurrence of two sets of signals [δ_H 3.22 (δ_C 25.8); δ_H 4.95 (δ_C 119.3); δ_C 134.3; δ_H 1.69 (δ_C 25.9); δ_H 1.76 (δ_C 18.1), and δ_H 3.19 (δ_C 25.8); δ_H 4.95 (δ_C 119.2); δ_C 134.2; δ_H 1.66 (δ_C 25.8)]; δ_H 1.69 (δ_C 18.1)] in its ^1H and ^{13}C NMR spectra. The HMBC spectrum further showed that the proton signal at δ 3.22 correlated with the carbonyl carbon at δ 183.4, the olefinic carbon signals at δ 142.2 and 141.7, and those at δ 134.3 and 119.3. The proton signal at δ 3.19 was found to correlate with the carbonyl carbon at δ 182.2, the olefinic carbons at δ 142.2 and 141.7, and those at δ 134.2 and 119.2. In this manner, it was apparent that the two prenyl units are

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10.1021/np000563o CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 06/28/2001

Table 1. ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) Data of Compounds **1** (in $CDCl_3$) and **2** (in CD_3OD)[a,b]

	compound **1**			compound **2**	
position	δ_C[a]	δ_H	position	δ_C	δ_H
1[c]	183.4 *(184.7)*		1	166.6	
2[b]	135.3		2	101.1	6.18 (1H, d, J = 2.4)
3	147.4 *(148.7)*		3	166.7	
CH_3O-3	60.4 *(61.0)*	4.04 (3H, s)	4	101.5	6.31 (1H, d, J = 2.4)
4[c]	182.2 *(183.7)*		4a	163.5	
5[d]	142.2 *(143.4)*		5a	143.0	
6[d]	141.7 *(143.0)*		6	138.4	
1′[e]	25.8 *(26.3)*	3.22 (2H, brd, J = 7.0)	CH_3O-6	62.9	3.94 (3H, s)
2′[f]	119.3 *(120.9)*	4.95 (1H, m)	7	147.5	
3′[g]	134.3 *(134.8)*		8[j]	129.0	
4′[h]	25.9 *(25.9)*	1.69 (3H, s)	9[j]	126.0	
5′[i]	18.1 *(18.1)*	1.76 (3H, s)	9a	137.3	
1″[e]	25.8 *(26.2)*	3.19 (2H, brd, J = 7.0)	11	169.2	
2″[f]	119.2 *(120.8)*	4.95 (1H, m)	11a	99.0	
3″[g]	134.2 *(135.0)*		1′	26.2	3.32 (2H, brd, J = 6.4)
4″[h]	25.8 *(25.9)*	1.66 (3H, s)	2′	124.0	5.00 (1H, m)
5″[i]	18.1 *(18.1)*	1.69 (3H, s)	3′	132.2	
1‴	97.2 *(97.6)*		4′[k]	25.8	1.64 (3H, s)
2‴	160.1 *(161.1)*		5′[k]	18.0	1.71 (3H, s)
3‴	95.0 *(96.4)*	5.72 (1H, d, J = 2.4)	1″	26.5	3.39 (2H, brd, J = 6.4)
4‴	161.2 *(164.8)*		2″	123.6	4.96 (1H, m)
5‴	98.0 *(98.8)*	6.11 (1H, d, J = 2.4)	3″	132.9	
6‴	165.5 *(166.1)*		4″[l]	25.9	1.64 (3H, s)
$-$*C*OOCH$_3$	170.8 *(172.0)*		5″[l]	18.3	1.76 (3H, s)
$-$COO*CH$_3$*	52.5 *(52.6)*	3.90 (3H, s)			
HO-4‴		5.59 (1H, s)			
HO-6‴		11.90 (1H, s)			

[a] Values in italics are for the sample run in CD_3OD. [b] C-2 was not detected in CD_3OD. [c−l] Assignments may be interchanged within the same column.

located adjacent to each other on the 1,4-benzoquinone ring. A correlation of the methoxyl protons resonating at δ 4.04 with the carbon signal at δ 135.3 was also observed. All these data established the partial structure **b** (Figure 1).

The connectivity between partial structures **a** and **b** was established for **1** based on the observation that the ^{13}C NMR signals for C-2‴ and C-6‴ did not coincide. Similarly, the noncoincidence of the H-3‴ and H-5‴ signals also was observed. These observations exclude the possibility of a C-2/C-4‴ connectivity since if this were the case, a symmetrical benzoate would be produced. Furthermore, an NOE enhancement of 4% was observed only on the H-3‴ (δ 5.72) proton signal upon irradiation of the CH_3O-3 (δ 4.04) protons to indicate a C-2/C-2‴ ether linkage. Thus, compound **1** (atrovirinone) was deduced to be a new prenylated benzoquinone and assigned as 2-(1-methoxycarbonyl-4,6-dihydroxyphenoxy)-3-methoxy-5,6-di(3-methyl-2-butenyl)-1,4-benzoquinone.

Compound **2**, obtained as colorless crystals, was assigned the molecular formula $C_{24}H_{26}O_7$ by HRFABMS. This formula was CH_2O less than that of compound **1**, corresponding to the loss of a methoxyl group, as also supported by ^{1}H and ^{13}C NMR data. The expected 24 carbon signals were observed in the ^{13}C NMR spectrum (Table 1). Only one signal (δ 169.2) attributable to an ester carbonyl was detected at low field, suggesting the absence of the benzoquinone moiety found in compound **1**. This ester carbonyl was found to be that of a lactone on the basis of the IR absorption band at 1670 cm^{-1}. The IR spectrum also showed significant absorption at 3388 cm^{-1} (hydroxyl). The pattern of signals (δ 6.31: 1H, d, J = 2.4 Hz and δ 6.18: 1H, d, J = 2.4 Hz) in the aromatic region of the ^{1}H NMR spectrum resembled that of compound **1**, indicating a similar substitution pattern of two *meta*-coupled aromatic protons. The results of the HMQC experiment showed that the signals at δ 6.31 and 6.18 correlated with the signals at δ 101.5 and 101.1, respectively. This was confirmed by HMBC correlations of the ^{1}H NMR signal at δ 6.31 with

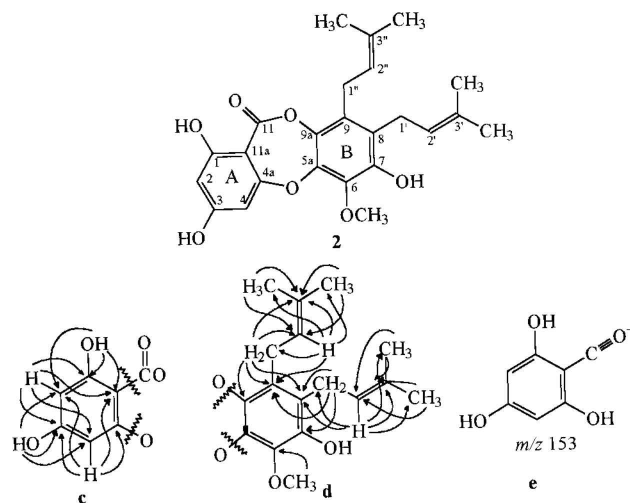

Figure 2. Compound **2**, its partial structures (**c** and **d**) with selected HMBC correlations, and its major mass spectral fragmentation ion (**e**).

the ^{13}C NMR signals at δ 166.7, 163.5, 101.1, and 99.0 and the ^{1}H NMR signal at δ 6.18 with the carbon signals at δ 166.7, 166.6, 101.5, and 99.0. Therefore, by comparison with compound **1**, the signals at δ 166.7 and 166.6 were assigned to two hydroxyl-bearing carbons in ring A to form the partial structure **c** (Figure 2). The resonances at δ 147.5, 143.0, 138.4, 137.3, 129.0, and 126.0 in the ^{13}C NMR spectrum together with the absence of signals for additional aromatic protons in the ^{1}H NMR spectrum also suggested the presence of a fully substituted aromatic ring (ring B). Of these carbon signals, those at δ 147.5 (−OH), 143.0 (−O−), 138.4 (−O−), and 137.3 (−O−) were oxygen bearing. The single methoxyl group present (δ_H 3.94, δ_C 62.9) was attached to ring B, as shown by the HMBC correlation between the proton signal at δ 3.94 and the carbon signal at δ 138.4.

Table 2. Antimicrobial and Cytotoxic Activities of Atrovirinone (**1**) and Atrovirisidone (**2**)

compound	diameter of inhibition (mm) at 10 μg/disk			HeLa (IC_{50} μg/mL)
	S. aureus	*B. cereus*	*E. coli*	
atrovirinone (**1**)	6.5	6.5	−ve	15
atrovirisidone (**2**)	8	7.5	−ve	>30
streptomycin	13.5	21	12.5	
doxorubicin				11
colchicine				21

The occurrence of two prenyl units in **2** was concluded from the ^{1}H and ^{13}C NMR and HMQC spectra by the presence of two sets of signals comparable to the analogous signals observed in **1**. The HMBC spectrum showed that correlations occurred between the ^{1}H NMR signal at δ 3.39 with the signals at 137.3, 132.9, 129.0, 126.0, and 123.6 as well as the ^{1}H NMR signal at δ 3.32 with the ^{13}C NMR signals at 147.5, 132.2, 129.0, 126.0, and 124.0 and indicated that the two prenyl units were located at adjacent carbons in ring B to suggest the partial structure **d** (Figure 2). In an NOE experiment, irradiation of the methoxyl protons at δ 3.94 gave a peak enhancement (7%) at δ 6.31. The linkage between rings A and B was determined from this result and by comparison with the spectral data of garcinisidone A[7] and garcidepsidones A−D.[8] Hence compound **2** was established as a new depsidone (atrovirisidone) and assigned as 1,3,7-trihydroxy-6-methoxy-8,9-di(3-methyl-2-butenyl)-1*H*-dibenzo[*b*,*e*]-[1,4]dioxepin-11-one. The mass fragment ion at *m*/*z* 153 (100%) in the EIMS was suggestive of the fragment **e** (Figure 2) since depsidones exhibit significant cleavage of the depside and ether linkage to give a product ion corresponding to the ring A fragment.[9] Only very few depsidones from higher plants have so far been reported, although many such isolations from lichens and fungi have been reported.[7,8,10,11] In view of the common occurrence of xanthones in the family Guttiferae, their possible role as the precursor for the biogenesis of depsidones has also been suggested.[8]

Structurally, atrovirisidone (**2**) is closely related to atrovirinone (**1**). Atrovirinone could be formed from atrovirisidone by hydrolysis of the lactone ring in the latter followed by oxidation to 1,4-benzoquinone (Scheme 1; Supporting Information).

The results of the antimicrobial and cytotoxic activities of compounds **1** and **2** are shown in Table 2. At the dose of 10 μg per disk, compounds **1** and **2** exhibited significant inhibitory activity only against *Staphylococcus aureus* and *Bacillus cereus* (both Gram positive) but not against *Escherichia coli* (Gram negative), *Aspergillus ochraceus* (fungus), and *Candida albicans* (yeast). The antibacterial activity of both compounds was less than the control, streptomycin sulfate. Only compound **1** showed cytotoxicity toward HeLa cells with an IC_{50} of 15 μg/mL, which was comparable to the standards doxorubicin (IC_{50} 11 μg/mL) and colchicine (IC_{50} 21 μg/mL).

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. UV (in absolute ethanol) and IR (KBr) spectra were recorded on a JASCO V-560 spectrophotometer and a Perkin-Elmer 1650 FTIR spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra ($CDCl_3$ and/or CD_3OD as indicated) were determined on a JEOL JNM-A 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. The spectra were interpreted with the aid of the ^{1}H−^{1}H COSY, HMBC, and HMQC techniques. EIMS were recorded using a JEOL JMS-AM20 spectrometer, with ionization being induced by electron impact at 70 eV. HRFABMS were obtained on a JEOL JMS HX-110A spectrometer. For column chromatography silica gel Merck 9385 and Sephadex LH-20 were used. Analytical TLC was performed with Merck DC-Plastikfollen 60 F_{254}.

Bioassays. The antimicrobial assay was performed at the test dose of 10 μg/disk using the disk diffusion method. Cytotoxicity toward the human cervical carcinoma cell line (HeLa) was determined using an MTT assay after a 72 h incubation period. Both these assays were performed as described previously.[1]

Plant Material. The roots of *Garcinia atroviridis* were collected in Apirl 1999 at Serdang, Selangor, Malaysia, and air-dried as well as ground before extraction. A voucher specimen (MM-1) was lodged at the herbarium of the Biology Department, Universiti Putra Malaysia.

Extraction and Isolation. The ground roots of *Garcinia atroviridis* (1 kg) were extracted with MeOH (3 × 5 L) by successive overnight soakings. The combined extracts were evaporated under reduced pressure to give a brown gum (115 g). The gum was shaken with H_2O/MeOH (2:1) (750 mL) and extracted with EtOAc (3 × 250 mL). The EtOAc extract was concentrated under reduced pressure to give a brownish gum (31 g). This extract was then subjected to Si gel column chromatography and successively eluted with *n*-hexane followed by *n*-hexane/EtOAc (2:1), *n*-hexane/EtOAc (1:1), *n*-hexane/EtOAc (1:2), and finally EtOAc, to give 20 (100 mL) fractions. Combination afforded fraction B (3−7) and fraction C (8−13). Fraction B (3.7 g) was rechromatographed on a Si gel column and eluted with *n*-hexane/EtOAc (7:3) to give 40 (15 mL) fractions, of which fractions 18−30 were pooled. These pooled fractions (1.1 g) were subsequently chromatographed on a Sephadex LH-20 column and eluted with MeOH to give 30 (15 mL) fractions. Fractions 10−15 were combined and recrystallized with $CHCl_3$/*n*-hexane to afford compound **1** as red needles (20 mg). Fraction C (2.4 g) was chromatographed on a Si gel column and eluted with *n*-hexane/EtOAc (2:1) to give 25 (15 mL) fractions. Fractions 8−10 were combined and recrystallized with $CHCl_3$/*n*-hexane to give compound **2** as colorless crystals (15 mg).

Atrovirinone (1): red needles ($CHCl_3$/*n*-hexane); mp 124−125 °C; UV (EtOH) λ_{max} (log ϵ) 204 (5.14), 221 (5.10), 273 (5.04) nm; IR (KBr) ν_{max} 3382, 2936, 1654, 1614, 1444, 1160, 1034 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 456 [M^+] (60), 441 (100), 409 (36), 341 (22), 153 (24), 129 (13), 97 (16), 91 (16), 83 (18), 73 (23) 69 (72), 55 (38); HRFABMS *m*/*z* 457.1831 [M + H]$^+$ calcd for $C_{25}H_{29}O_8$, 457.1862.

Atrovirisidone (2): colorless crystals ($CHCl_3$/*n*-hexane); mp 75−76 °C; UV (EtOH) λ_{max} (log ϵ) 207 (4.93), 269 (4.36) nm; IR (KBr) ν_{max} 3388, 2974, 1670, 1636, 1592, 1460, 1240, 1166 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 426 [M^+] (88), 395 (9), 370 (34), 355 (24), 339 (73), 327 (14), 311 (17), 235 (18), 193 (29), 153 (100), 136 (14), 119 (18), 105 (21), 91 (40), 69 (75); HRFABMS *m*/*z* 427.1675 [M + H^+] calcd for $C_{24}H_{27}O_7$, 427.1757.

Acknowledgment. The authors thank the Ministry of Science, Technology and the Environment, Kuala Lumpur, Malaysia, for financial support under the research grant IRPA 03-02-04-0043.

Supporting Information Available: Scheme showing the postulated biogenetic relationship of atrovirinone (**1**) and atrovirisidone (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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