A New Prenylated Hydroquinone from the Roots of *Garcinia atroviridis* Griff ex T. Anders (Guttiferae)

Dharma Permana[a], Nordin Hj. Lajis[a], Khozirah Shaari[a], Abdul M. Ali[b], Mukram M. Mackeen[b], Mariko Kitajima[c], Hiromitsu Takayama[c], and Norio Aimi[c]

[a] Laboratory of Phytomedicines, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Department of Biotechnology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[c] Faculty of Pharmaceutical Sciences, Chiba University, I-33 Yayoi-cho, Inage-ku, Chiba 263–8522, Japan

Reprint requests to Prof. Dr. N. H. Lajis. E-mail: nhlajis@ibs.upm.edn.my

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A continuing study on the phytochemical constituents of *Garcinia atroviridis* Griff ex T. Anders (Guttiferae) has led to the isolation of a new prenylated hydroquinone from the roots of the plant. The compound has been elucidated to be 4-methylhydroatrovirinone, primarily by the use of gradient NMR and mass spectroscopy. The roots also yielded the known morelloflavone and its 7-*O*-β-D-glucopyranoside, fukugiside, together with 14-*cis*-docosenoic acid.

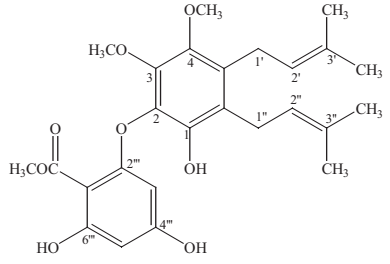
Key words: Prenylated Hydroquinone, *Garcinia atroviridis*

Introduction

Garcinia atroviridis Griff ex T. Anders (Guttiferae) is a medium-sized tree, widely distributed throughout Peninsular Malaysia. The species either grows wild or is cultivated on a small scale by villagers for its acidic fruits, much used for medicinal and culinary purposes. We have previously reported the identification of atrovirinone and atrovirisidone, a prenylated quinone and prenylated depsidone respectively, isolated from the roots of the species (Permana *et al*., 2001). In this paper, we further describe the isolation and identification of a new prenylated hydroquinone, 4-methylhydroatrovirinone (**1**), recently isolated from the roots of the species and believed to be the intermediate involved in the biosynthesis of atrovirisidone (**2**) to atrovirinone (**3**).

Results and Discussion

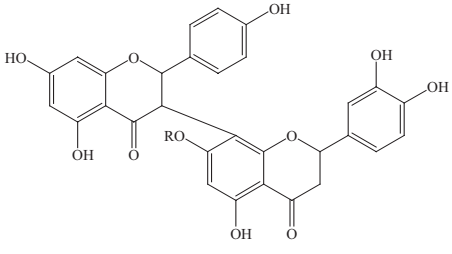
Compound **1** was obtained as a yellow amorphous solid with a melting point of 120–121 °C. The molecular formula was determined to be $C_{26}H_{33}O_8$ by HRFAB-MS which showed a $[M+1]^+$ peak at *m/z* 473.2292. The IR spectrum exhibited bands at 3356 cm^{-1} and 1674 cm^{-1}, attributable to the presence of hydroxyl and car-



4-Methylhydroatrovirinone (**1**)

CH_3-(CH_2)6-CH=CH-$(CH_2)_{12}$-COOH

14-*cis*-Docosenoic acid (**4**)



Morelloflavone, R = H (**5**)
Fukugiside, R = Glucose (**6**)

Fig. 1. Structures of 4-methylhydroquinone and other constituents isolated from the roots of *G. atroviridis*.

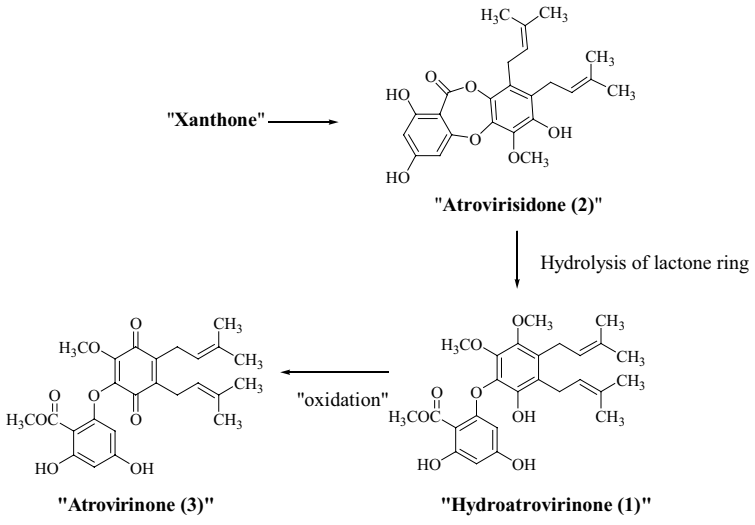
bonyl groups, respectively. The ^{13}C NMR spectrum also supported the presence of 26 carbons in the molecule, one of which is an ester carbonyl (δ_C 169.4).

Careful analysis of the ^{1}H and ^{13}C NMR of **1** indicated a similar molecular skeleton to atrovirinone (5) except for the absence of the 1,4-benzoquinone carbonyl groups. In their place there were two oxygenated quaternary carbons at δ_C 144.0 (C-1) and 143.9 (C-4). An additional methoxyl signal was also observed at δ 3.64 (δ_C 60.7). This led us to deduce that compound **1** was a prenylated hydroquinone, where one of the quinone carbonyls (C-4) had been replaced by a methoxyl group and the other (C-1) by a hydroxyl group. A full assignment of the prenylated hydroquinone and the benzoate partial structures of the molecule was again made possible by following the HMQC and HMBC correlations (Table 1). The C2–O–C2‴ ether linkage was again deduced from the non-coincidences of the C-2‴ and C-6‴ NMR signals, as well as the H-3‴ and H-5‴ proton signals, which rule out the possibility of a symmetrical benzoate. Thus, compound **1** was characterized as 2-*O*-(1-methoxycarbonyl-4,6-dihydroxyphenoxy)-3-methoxy-5,6-di-(3-methyl-2-butenyl)-*p*-4-methylhydroquinone or 4-methylhydroatrovirinone which appears to be a new natural product. In addition, four known compounds, atrovirisidone (**2**), atrovirinone (**3**) (Permana *et al.* (2001), 14-*cis*-docosenoic acid (**4**) (Hoffman *et al*. (1996); Kling *et al*. (1993)), morelloflavone (**5**) and its glucosidic derivative, fukugiside (**6**) (Duddeck *et al*. (1978); Chen *et al*. (1974); Babu *et al*. (1988); Lin *et al*. (1997)) were also isolated. Both morelloflavone and fukugiside were present as a pair of atropisomers at the relative ratios of 7:3 and 3:7, respectively

Hydroquinone was previously suggested to be the intermediate in the biogenesis of benzoquinones *via* depsidones (or xanthone) precursors (Scheme 1). This compound has never been reported in the previous isolations of depsidones or hydroquinones from natural sources. Thus, this

Table 1. ^{1}H (500 MHz, DMSO-d_6), ^{13}C (125 MHz, DMSO-d_6) NMR data of compound **1** and its short (^{1}J) and long-range (^{2}J & ^{3}J) C–H connectivities established by FGHMQC and FGHMBC, respectively.

Position	^{1}H NMR	^{13}C NMR	^{2}J	^{3}J
1	8.61 (OH, s)	144.0	144.0 (C-1)	133.6 (C-2), 122.9 (C-6)
2		133.6		
3		143.6		
3-OCH_3	3.66 (3H, s)	60.4		143.6 (C-3)
4		143.9		
4-OCH_3	3.64 (3H, s)	60.7		143.9 (C-4)
5		130.2		
6		122.9		
1′	3.24 (2H, d, J = 6.1)	25.2	130.2 (C-5), 123.4 (C-2′)	143.9 (C-4), 122.9 (C-6), 130.6 (C-3′)
2′	5.00 (H, m)	123.4	25.2 (C-1′), 130.6 (C-3′)	143.9 (C-4, ^{4}J), 130.2 (C-5), 17.7, 25.4
3′		130.6		
3′-CH_3	1.71 (3H, s)	17.7	130.6 (C-3′)	123.4(C-2′), 25.4
-CH_3	1.66 (3H, s)	25.4	130.6 (C-3′)	123.4 (C2′), 17.7
1″	3.24 (2H, d, J = 6.1)	24.9	122.9 (C-6), 123.2 (C-2″)	144.0 (C-1), 130.2 (C-5), 130.4 (C-3″)
2″	5.00 (H, m)	123.2	24.9 (C-1″), 130.4 (C-3″)	130.2 (C-5), 17.7, 25.4
3″		130.4		
3″-CH_3	1.67 (3H, s)	17.7	130.4 (C-3″)	123.2 (C-2″), 25.4
-CH_3	1.65 (3H, s)	25.4	130.4 (C-3″)	123.2 (C-2″), 17.7
1‴		98.4		
2‴		159.7		
3‴	5.55 (H, d, J = 2.1)	93.7	159.7 (C-2‴), 161.4 (C-4‴)	98.4 (C-1‴), 96.7 (C-5‴)
4‴	10.02 (OH, s)	161.4	161.4 (C4‴)	93.7 (C-3‴), 96.7 (C-5‴)
5‴	5.95 (H, d, J = 2.1)	96.7	161.4 (C-4‴), 162.1 (C-6‴)	98.4 (C-1‴), 93.7 (C-3‴)
6‴	10.97 (OH, s)	162.1	162.1 (C-6‴)	98.4 (C-1‴), 96.7 (C-5‴)
C$OOCH_3$		169.4		
$COOCH_3$	3.81 (3H, s)	51.9		169.4



Scheme 1. Postulated relationship atrovirinone, hydroatrovirinone and atrovirinone.

finding supports the previous postulate on the biogenesis of these benzoquinones from *Garcinia* species.

Experimental Section

General experimental procedures

Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. IR spectra were recorded on Perkin-Elmer 1650 FTIR spectrometer and the UV spectra were obtained with a Shimadzu UV-VIS 160 spectrophotometer. NMR spectra were determined on a JEOL JNM-A 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C). HRFABMS were taken on a JEOL JMS HX-110A mass spectrometer. Merck silica gel 9385, and Sephadex-LH-20 were used for column chromatography. Analytical TLC was run on Merck DC-Plastikfollen 60 F_{254}.

Plant material

The roots of *Garcinia atroviridis* were collected from the Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Selangor, Malaysia, during the month of April 1999. A voucher specimen (MM-1) has been deposited at the herbarium of the Biology Department, Universiti Putra Malaysia.

Extraction and isolation.

The dried and pulverized roots of *Garcinia atroviridis* (1 kg) were extracted with MeOH (3 × 5 l) by successive overnight soakings. The combined extracts were evaporated *in vacuo* to give a brown gum (115 g). This was re-dissolved in 750 ml MeOH/water, 1:2 and re-extracted with EtOAc (3 × 250 ml) to yield a brownish gum (31 g) after concentration *in vacuo*. This final extract (30 g) was then subjected to silica gel column chromatography (5 × 15 cm) and eluted with 100% hexane followed by *n*-hexane/EtOAc mixtures, 2:1, 1:1 and 1:2, and finally with 100% EtOAc to give twenty five (100 ml) fractions. Fractions with similar TLC pattern were combined to give six combined fractions A (1–2), B (3–7), C (8–13), D (14–20), E (21–22) and F (23–25).

The combined fraction B was rechromatographed on a silica gel column (2.5 × 15 cm) and eluted with *n*-hexane/EtOAc (7:3) to give forty (15 ml) fractions. The combined fractions 18–30 (1.1 g) was again rechromatographed on sephadex LH-20 column (1.5 × 30 cm) and eluted with MeOH to give thirty (15 ml) fractions of which fractions 10–15 were combined and recrystallized from $CHCl_3$/*n*-hexane to give 20 mg of compound **3**.

Further column chromatography of combined fraction C (2.4 g) on silica gel (2.5 × 15 cm) using *n*-hexane/MeOH (2:1) as eluant gave twenty-five (15 ml) fractions of which fractions 8–10 were combined and recrystallized from $CHCl_3$/*n*-hexane to give 15 mg of compound **2**.

Rechromatography of the combined fraction A (1.4 g) on silica gel column 2.5 × 15 cm) and eluted with *n*-hexane/EtOAc (4:1) gave twenty (15 ml)

fractions. From this batch of fractions, compound **1** (9.0 mg) was obtained from the fractions 11 and 12 after recrystallisation from $CHCl_3$. Repeated column chromatography of fractions 3–9 (0.6 g) first on silica gel eluted with *n*-hexane/EtOAc, 9:1, followed by further purification on Sephadex LH-20 gave 14-*cis*-docosenoic acid (**4**) as a yellow oil (100 mg). Further column chromatography of combined fraction D (6.0 g, silica gel and eluted with hexane/EtOAc, 1:3) and fraction E (1.0 g, silica gel eluted with EtOAc/MeOH, 19:1), yielded 45 mg of morelloflavone (**5**) and 15 mg of fukugiside (**6**), respectively. Both compounds were recrystallized from MeOH. All the known compounds were identified by spectral data and also by comparison with literature data. Both morelloflavone and fukugiside were present as pairs of atropisomers at the relative ratios of 7:3 and 3:7, respectively. This was revealed by variable temperature ^{1}H and ^{13}C NMR data at 28 °C and 120 °C.

4-Methylhydroatrovirinone (**1**)

Yellow amorphous powder ($CHCl_3$/hexane); m.p. 120–121 °C; UV (MeOH): λ_{max} (log ε) = 205 (3.59), 270 (3.13) nm; IR (KBr): ν_{max} = 3356 (OH), 2940 (C–H), 1674 (C=O), 1590, 1460, 1166 cm^{-1}; HR-FABMS: *m/z* 473.2292 $(M+H)^+$, calcd. for $C_{26}H_{33}O_8$; EI-MS (rel int.): 472 (100), 440 (20), 409 (12), 384 (60), 369 (55), 341 (30), 153 (45); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.97 (H, s, 6‴-OH), 10.02 (H, s, 4‴-OH), 8.61 (H, s, 1-OH), 5.00 (2H, m, H-2′, H-2″), 5.95 (H, d, *J* = 2.1, H-5‴), 5.55 (H, d, *J* = 2.1, H-3‴), 3.81 (3H, s, COO*CH*$_3$, 3.66 (3H, s, 3-OCH_3), 3.64 (3H, s, 4-OCH_3), 3.24 (4H, d, *J* = 6.1, H-1′, H-1″), 1.71 (3H, s, 3′-CH_3), 1.66 (3H, s, 3′-CH_3), 1.67 (3H, s, 3″-CH_3), 1.65 (3H, s, 3″-CH_3). ^{13} C {^{1}H} NMR (125 MHz, DMSO-d_6) δ = 144.0 (C-1), 133.6 (C-2), 143.6 (C-3), 130.2 (C-5), 122.9 (C-6), 25.2 (C-1′), 123.4 (C-2′), 130.6 (C-3′), 24.9 (C-1″), 123.2 (C-2″),130.4 (C-3″), 98.4 (C-1‴), 159.7 (C-2‴),161.4 (C-4‴), 93.7 (C-3‴), 96.7 (C-5‴), 162.1 (C-6‴), 169.4 (*C*OOCH$_3$), 51.9 (COO*C*H$_3$), 17.7, 25.4 (4 × CH_3).

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