Cytotoxic Prenylated Depsidones from *Garcinia parvifolia*

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Leaf extracts of *Garcinia parvifolia* provided relatively high yields of four novel, cytotoxic prenylated depsidones. The structures were determined mainly by detailed NMR spectral analysis and X-ray crystallography.

In our continuing phytochemical study of Southeast Asian plants as a source of bioactive compounds, we have found novel, cytotoxic prenylated depsidones from the leaves of *Garcinia parvifolia* Miq. (Guttiferae), the bark of which had previously furnished a cytotoxic bixanthone.[1] Four new depsidones (**1**−**4**) with the dibenzo[*b*,*e*][1,4]-dioxepin-11-one skeleton were isolated from the chloroform-soluble fraction. This is the first example of a higher plant producing substantial amounts of depsidones, as previously only the lichens were known as a rich source of depsidones.[2,3] The finding of significant quantities of depsidones from the present plant as well as a minor component from another *Garcinia* species is of interest in terms of their biogenesis in Guttiferae plants, which usually provide shikimate-derived, aromatic natural products.[4−6]

Results and Discussion

The concentrated ethanolic extract of the leaves of *G. parvifolia*, suspended in aqueous methanol, was successively re-extracted with *n*-hexane, chloroform, and *n*-BuOH. Extensive Si gel chromatography of the *n*-hexane- and chloroform-soluble fractions yielded four novel prenylated depsidones (**1**−**4**) (Figure 1), along with some known triterpenes, xanthones, and biflavanones.

Garcidepsidone A (**1**) was obtained as colorless needles and has the molecular formula $C_{28}H_{32}O_7$ deduced from HREIMS and supported by NMR data (Table 1). The IR spectrum showed absorption bands for hydroxyl groups (ν_{max} 3542, 3355 cm^{-1}) and a lactone carbonyl group chelated to an *ortho*-hydroxyl group (ν_{max} 1629 cm^{-1}). The presence of the latter functionality was confirmed by resonances at δ_C 169.2 and δ_H 11.0. The EIMS of **1** showed a molecular ion at *m*/*z* 480 as a base peak with other significant fragments at *m*/*z* 425 ($M^{\bullet+}-55$), 424 ($M^{\bullet+}-56$), 368 ($M^{\bullet+}-2 \times 56$), 312 ($M^{\bullet+}-3 \times 56$), indicating that compound **1** bore three prenyl groups. The loss of a C_4H_7 (55 amu) unit, from the allylic cleavage of a prenyl side chain, and that of a C_4H_8 (56 amu) unit, due to the prenyl group in proximity with a phenolic group, are typical of many prenylated xanthones.[4] The presence of three prenyl groups was evident from the ^{1}H NMR spectrum, which showed three sets of signals corresponding to prenyl groups ($-CH_2CH{=}CMe_2$). Other ^{1}H NMR peaks of **1** were from four D_2O-exchangeable hydroxyl protons (including the intramolecularly H-bonded OH) and one aromatic proton.

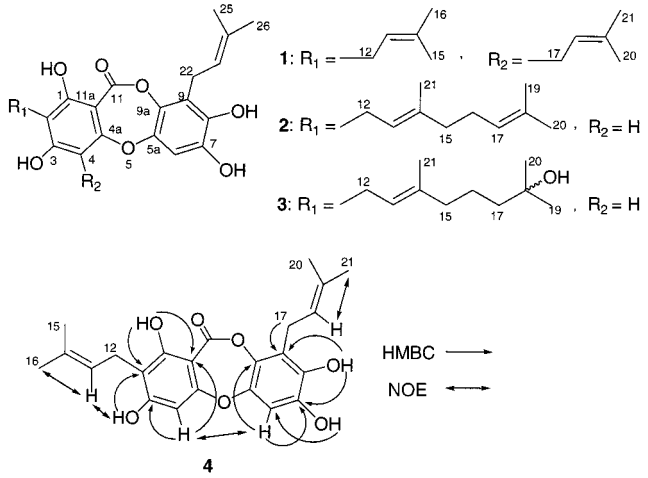

Figure 1. Structures of **1**−**4** and selected NMR correlations of **4**.

In the HMBC spectrum, the chelated proton signal at δ 11.0 corresponding to C-1-OH showed a strong cross-peak with the carbon at δ 99.4 (C-11a), which should be the aromatic carbon linked to the carbonyl group. The 1-OH proton also exhibited connectivity to δ 161.5 (oxygenated aromatic carbon C-1) and δ 112.2 (substituted aromatic carbon C-2). The cross-peaks of C-2/(H-12, -13) confirmed that C-2 was substituted with a prenyl group. The hydroxyl proton at δ 6.36, ^3J-coupled to the carbon at δ 116.0 (C-2), had to be placed at C-3. The 4-substituted prenyl group was deduced from the cross-peak between the 3-OH proton and the quaternary carbon at δ 111.3 (C-4), which also showed coupling with prenyl protons H-17 and H-18. The aromatic proton at δ 6.61 showed connectivity to four oxygenated aromatic carbons at δ 136.8 (C-9a), 143.9 (C-5a), 142.3 (C-7), and 140.6 (C-8), the latter two of which showed coupling with *ortho*- and *meta*- hydroxyl protons at δ 6.45 (OH-7) and 5.74 (OH-8). The last prenyl group was determined to be at position-9 based on the connectivity of the carbon at δ 121.0 (C-9) to the 8-OH proton and to H-22 and H-23. The complete structure could be determined from notable NOESY cross-peaks H-6/(H-17, -18). Thus, the structure of **1** was determined as 1,3,7,8-tetrahydroxy-2,4,9-tris(3-methylbut-2-enyl)-dibenzo[*b*,*e*]-[1,4]dioxepin-11-one and was confirmed by single-crystal X-ray diffraction (Figure 2).

Garcidepsidone B (**2**), an isomer of **1**, was isolated as a colorless oil and showed a molecular ion at *m*/*z* 480.2142 (calcd $C_{28}H_{32}O_7$, 480.2148) in its HREIMS. Notable fragment ions found in the EIMS were *m*/*z* 424 ($M^{\bullet+}-56$), 411 ($M^{\bullet+}-69$), 357 ($M^{\bullet+}-123$). Losses of 56 and 69 amu

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Table 1: ^{1}H and ^{13}C NMR Data of Garcidepsidones A−D (**1**−**4**) in Acetone-d_6[a]

	1[b]		**2**		**3**		**4**	
#	δ_H[c]	δ_c	δ_H[c]	δ_c	δ_H[c]	δ_c	δ_H[c]	δ_c
1	11.00 (1H, s)(OH)	161.5	11.20 (1H, s)(OH)	162.5	11.2 (1H, s)(OH)	167.7	11.21 (1H, s)(OH)	167.7
2		112.2		111.5		117.6		117.4
3	6.36 (1H, s)(OH)	160.9		162.1	9.75 (1H, s)(OH)	167.5	9.73 (1H, s)(OH)	167.5
4		111.9	6.25 (1H, s)	100.0	6.39 (1H,s)	104.4	6.39 (1H, s)	104.4
4a		157.3		159.8		165.2		165.2
5a		143.9		141.5		147.5		147.5
6	6.61(1H, s)	105.9	6.61 (1H, s)	104.8	6.67 (1H,s)	109.4	6.68 (1H, s)	109.4
7	6.45 (1H, s)(OH)	142.3		143.0	7.49 (1H, s)(OH)	147.5	7.50 (1H, s)(OH)	147.4
8	5.74 (1H, s)(OH)	140.6		140	8.75 (1H, s)(OH)	146.1	8.75 (1H, s)(OH)	146.0
9		121.0		120		126.2		126.2
9a		136.8		135.8		140.8		140.8
11		169.8		168.8		173.8		173.8
11a		99.4		98.3		102.8		98.5
12	3.30 (2H, d, 6.9)	22.3	3.36 (2H, d, 7.0)	21.8	3.31 (2H, d, 7.0)	26.5	3.29 (2H, d, 7.1)	26.6
13	5.10 (1H, t, 6.0)	121.8	5.20 (1H, m)	120.9	5.20 (1H, tq, 7.0, 1.0)	126.8	5.18 (1H, t, 7.1)	127.1
14		135.5		138.3		140.1		136.0
15	1.72 (3H, s)	18.7	2.01 (2H, t, 7.5)	39.6	1.92 (2H, t, 7.4)	45.2	1.73 (3H, s)	22.1
16	1.62 (3H, s)	26.5	2.06 (2H, m)	26.3	1.44 (2H, m)	27.5	1.64 (3H, s)	30.0
17	3.49 (2H, d, 7.0)	23.2	5.03 (1H, t, 7.0)	123.8	1.34 (2H, m)	48.5	3.49 (2H, d, 7.2)	28.3
18	5.11(1H, t, 7.0)	122.5		131.7		74.3	5.25 (1H, t, 7.2)	126.8
19		135.2	1.56 (3H, s)	17.6	1.10 (3H, s)	33.9		136.8
20	1.78 (3H, s)	18.7	1.64 (3H, s)	25.5	1.10 (3H, s)	33.9	1.81 (3H, s)	22.3
21	1.65 (3H, s)	26.5	1.77 (3H, s)	16.1	1.74 (3H, s)	20.3	1.61 (3H, s)	30.1
22	3.47 (2H, d, 7.0)	24.2	3.50 (2H, d, 7.0)	23.6	3.49 (2H, d, 7.2)	28.3		
23	5.17 (1H, t, 7.0)	121.1	5.21 (1H,m)	120.5	5.26 (1H, tq, 7.2, 1.4)	126.7		
24		135.9		134.8		136.8		
25	1.76 (3H, s)	18.7	1.80 (3H, s)	17.8	1.81 (3H, s)	22.3		
26	1.68 (3H, s)	26.5	1.69 (3H, s)	25.6	1.64 (3H, s)	30.1		

[a] Measured at 500 and 125 MHz for ^{1}H and ^{13}C, respectively. [b]measured in $CDCl_3$. [c]^{1}H chemical shift values followed by number of protons, multiplicity and coupling constant (*J*/Hz).

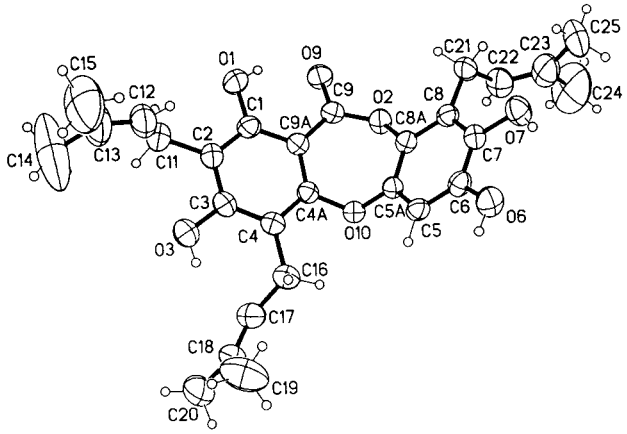

Figure 2. Perspective structure of garcidepsidone A (**1**) as a 50% thermal ellipsoid probability plot.

indicated the presence of a prenyl group, and loss of 123 amu corresponded to a geranyl group. The ^{1}H and ^{13}C NMR data in combination with the results of HMQC indicated that **2** had two aromatic protons and five methyl groups of isoprenoid origin. The presence of a geranyl group was confirmed by the multiplets at δ 1.9−2.1 (4H) corresponding to two adjacent CH_2 groups. The *E*-configuration of the $\Delta^{13,14}$ double bond in the geranyl group was deduced from the NOE correlation between Me-21 and H-12. Detailed analysis of the HMBC and NOESY spectra confirmed the structure of **2** as shown (Figure 1).

Garcidepsidone C (**3**) was isolated as a white powder, and HREIMS revealed the molecular ion as $C_{28}H_{34}O_8$, showing one H_2O more than compounds **1** and **2**. Other notable fragments were *m*/*z* 478 ($M^{\bullet+}$−18), 357 ($M^{\bullet+}$ − 18 − 123), 300 ($M^{\bullet+}$ − 18 − 124 − 56). The loss of 18 amu indicated **2** bears an aliphatic hydroxyl group, which was confirmed by the ^{13}C NMR peak at 74.3 ppm. The loss of a C_9H_{15} (123 amu) unit was produced by the allylic cleavage of a geranyl group, and the loss of 124 amu (C_9H_{16}) was evidence of the geranyl group in proximity to a phenolic OH. The loss of a C_4H_8 unit was also due to the presence of the prenyl group ortho to a phenolic OH group. From the comparison of the ^{13}C and ^{1}H NMR data with those of **1** and **2**, it was deduced that one of the double bonds in **2** was hydrated to **3**. The hydroxyl group was determined to be at the geranyl group by HMBC linkages [C-18/(H-16, -17, -19, -20)]. The final structure was confirmed by detailed HMBC and NOESY analysis and depicted as **3** (Figure 1).

Garcidepsidone D (**4**) was isolated as a white powder, and HREIMS indicated its molecular formula to be $C_{23}H_{24}O_7$. The ^{13}C NMR data (Table 1) showed peaks corresponding to the dibenzo[*b*,*e*][1,4]dioxepin-11-one skeleton as in compounds **1**− **3**. The other 10 carbons, and their associated ^{1}H NMR data, were attributed to two prenyl groups. The C-2 prenyl group was verified from HMBC correlations C-2/(H-12, -13, and OH-1); similarly, the other prenyl group was determined to be at C-9 by the correlations C-9/(H-17, -18, and OH-8). Further analysis of the detailed HMBC and NOESY data provided the structure **4** (Figure 1).

It is noteworthy that depsidones were found in relatively high yields (0.4%) in *G. parvifolia* of the Guttiferae family, which is better known for natural products from shikimate and isoprenoid pathways. The polyketide pathway has been responsible in lichens for a variety of depsidones that usually contain methyl groups substituted in positions 1 and 9 (or 6).[2,3] In view of the widespread occurrence of xanthones and benzophenone derivatives from Guttiferae, there is the interesting possibility that the new depsidones were from precursor xanthones formed by Baeyer−Villiger rearrangements from hydroperoxylation reactions. Such a biosynthetic origin appears to be supported by the presence of hydroperoxylated prenylxanthones[4] as well as instances where oxidatively rearranged benzophenone derivatives or xanthonoids were isolated from Guttiferae.[5,7]

Experimental Section

General Experimental Procedures. EIMS were run on a Micromass VG 7035 mass spectrometer at 70 ev. NMR spectra were recorded by Bruker AMX or DRX [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using acetone-d_6, with TMS as an internal standard unless otherwise stated. IR spectra were recorded on a Bio-Rad FT-IR spectrometer and UV spectra on a Hewlett-Packard 8452A diode array spectrometer. X-ray data collection was carried out on a Siemens CCD SMART system. Liquid chromatography was performed on Si gel (Kieselgel 60, particle size 0.040−0.063 mm) and Sephadex LH-20. TLC was run on Si gel precoated glass plates (Merck Si gel 60 F_{254}).

Plant Material. The leaves of *G. parvifolia* Miq. (Guttiferae) were collected from Mt. Tawai, Kinabatangan, Sabah, Malaysia, in 1996, and identified by L. Madani. A voucher specimen (SAN135189) was deposited at the herbarium of the Forest Research Centre, Sepilok, Sandakan, Sabah, Malaysia.

Cytotoxicity. Cytotoxic activity was determined as described previously.[8] ED_{50} values of compounds **1** −**3** on the P-388 cell line were 2.36, 2.42, and 3.2 μg/mL, respectively.

Extraction and Isolation. Dried and powdered leaves (1000 g) of *G. parvifolia* were successively and exhaustively extracted with cold ethanol (10 L × 2). Evaporation in vacuo reduced the extract to a residue of 60 g. This residue was suspended in aqueous methanol (10%) and then re-extracted with *n*-hexane (500 mL × 3), chloroform (500 mL × 3), and *n*-BuOH (400 mL × 3) to give 15, 20, and 18 g residues, respectively, after concentration in vacuo. The chloroform-soluble fraction was then subjected to Si gel column chromatography and eluted with a solvent mixture of increasing polarity (chloroform−methanol) to give 10 fractions. Further separations of fractions 4−8 were by Si gel flash chromatography, column chromatography on Sephadex LH-20 with $CHCl_3$−MeOH (1:1), and preparative TLC. Compounds **1**−**4** were separated in the following order on Si gel: **1** (2.0 g, 0.2%), **2** (2.0 g, 0.2%), **3** (30 mg, 0.003%), **4** (15 mg, 0.0015%).

Garcidepsidone A (**1**): colorless needles; mp 142−143 °C; UV (MeOH) λ_{max} (log ϵ) 220 (4.54), 280 (3.69) nm; IR (KBr) ν_{max} 3542, 3355, 2968, 2921, 1629, 1606, 1463, 1428, 1301, 1208, 1189, 1098, 845 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 480 $[M]^{\bullet+}$ (100), 423 (60), 424 (65), 368 (90), 312 (75); HREIMS *m*/*z* 480.2142 (calcd for $C_{28}H_{32}O_7$ 480.2148).

Crystal data for 1: $C_{28}H_{32}O_7$, M_w = 480.54, triclinic, *P*1, *a* = 8.5482(1) Å, *b* = 10.1244(2) Å, *c* = 16.9306(3) Å, α = 93.158(1)°, β = 101.419(1)°, γ = 113.362(1)°, *V* = 1303.90(4) Å^3, *Z* = 2, D_{calc} = 1.224 g/cm^3, *F*(000) = 512. Data were collected at 293(2) K in the θ range of 2.22−25.00° (−10 ≤ *h* ≤ 10, −12 ≤ *k* ≤ 12, −20 ≤ *l* ≤ 20). After application of an empirical absorption correction, SADABS, the structure was solved by direct methods. Refinement by full-matrix least-squares was performed with hydrogen atoms placed in calculated positions and allowed to ride on the atoms to which they are attached. Anisotropic thermal parameters were refined for all the ordered non-hydrogen atoms. The model converged at R_1 = 0.0484, wR_2 = 0.1261, GOF = 1.060 for 3231 reflections, with $F_o \geq 4\sigma(F_o)$; R_1 = 0.0698, wR_2 = 0.1369 for all 4481 reflections. In the final difference, the residual electron density fluctuates between 0.327 and −0.273 e Å^{-3}. Crystallographic data for **3** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk].

Garcidepsidone B (**2**): colorless oil; UV (MeOH) λ_{max} (log ϵ) 220 (4.33), 276 (4.16) nm; IR (KBr) ν_{max} 3434, 2969, 2928, 2855, 1632, 1624, 1497, 1448, 1421, 1275, 1147, 1059 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 480 $[M]^{\bullet+}$ (60), 424 (40), 411 (40), 357 (70), 300 (60); HREIMS *m*/*z* 480.2142 (calcd for $C_{28}H_{32}O_7$ 480.2148).

Garcidepsidone C (**3**): white powder; UV (MeOH) λ_{max} (log ϵ) 220 (4.42), 278 (4.05) nm; IR (KBr) ν_{max} 3425, 2924, 2855, 1624, 1466, 1429, 1270, 1154, 1070 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 498 $[M]^{\bullet+}$ (10), 480 (60), 395 (60), 300 (60); HREIMS *m*/*z* 498.2230 (calcd for $C_{28}H_{34}O_8$ 498.2254).

Garcidepsidone D (**4**): white powder; UV (MeOH) λ_{max} (log ϵ) 220 (4.38), 278 (3.98) nm; IR (KBr) ν_{max} 3447, 2922, 1622, 1497, 1462, 1430, 1267, 1153, 1066 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 412 $[M]^{\bullet+}$ (70), 370 (65), 326 (65); HREIMS *m*/*z* 412.1519 (calcd for $C_{28}H_{34}O_8$ 412.1522).

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Supporting Information Available: 2D NMR data of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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