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DNA strand-scission by phloroglucinols and lignans from heartwood of *Garcinia subelliptica* Merr. and *Justicia* plants

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Abstract

Five 2,4,6-prenylated phloroglucinols, garcinielliptones HA (**1**), HB (**2**), HC (**3**), HD (**4**) and HE (**5**), were isolated from the heartwood of *Garcinia subelliptica* Merr. Their structures, including relative configurations, were elucidated by means of spectroscopic data analysis. The ability of phloroglucinols, **1**–**5** and lignans, tuberculatin (**8**), justicidin A (**9**), procumbenoside A (**10**) and ciliatosides A (**11**) and B (**12**), isolated from *Justicia ciliata* and *Justicia procumbens*, to induce DNA-cleavage activity was examined using pBR322, a supercoiled, covalently closed circular DNA, and it was analyzed by agarose gel electrophoresis. In the presence of Cu (II), compounds **3**, **8**, **10** and **11** caused significant breakage of supercoiled plasmid pBR322. The products were relaxed circles with no detectable linear forms. In the Cu(II)-mediated DNA damage of **3** and selective compound **8**, Cu(I) was shown not to be an essential intermediate by using the Cu(I)-specific sequestering reagent neocuproine.

Keywords: *Garcinia subelliptica*; *Justicia*; Guttiferae; Acanthaceae; Heartwood; DNA strand-scission activity; Phloroglucinol; Lignan

1. Introduction

The isolation and characterization of various xanthones, benzophenone and phloroglucinol derivatives from *Garcinia subelliptica* Merr. (Clusiaceae) have been reported (Iinuma et al., 1995; Minami et al., 1996, 1998; Fukuyama et al., 1993, 1998). Several xanthones possess inhibitory activity against DNA topoisomerases I and II (Tosa et al., 1997), whereas some xanthone derivatives have antioxidant activities (Minami et al., 1994). In previous papers, we isolated several new terpenoids and phloroglucinols from seeds of *G. subelliptica* and reported their anti-inflammatory activities (Lin et al., 1996; Chung et al., 1998; Weng et al., 2003a,b, 2004). Two of the phloroglucinols isolated from seeds of *G. subelliptica*, garcinielliptones F and I (Lin et al., US Pat. application), may be valuable in the therapeutical treatment or prevention of peripheral and certain central inflammatory diseases associated with the increase of chemical mediators.

Recently, a cytotoxic phloroglucinol, garcinielliptone FB, was isolated from the pericarp of this plant (Wu et al., 2005). In a previous paper, we also reported isolation of lignans from *Justicia* plants (Acanthaceae) and their antiplatelet, cytotoxic and anti-inflammatory effects (Day et al., 1999, 2000, 2002; Weng et al., 2004).

Bleomycin, a DNA strand scission agent, is used as an anticancer drug. Its usage suggests that novel natural products capable of DNA degradation may be used to guide design and/or discovery of active compounds for treating cancer. Additionally, the metal ion, Cu(II), can stimulate redox cycling of several kinds of natural products, such as catechins (Furukawa et al., 2003), leading to production of reactive oxygen species, which can also induce DNA degradation.

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An investigation on cytotoxic constituents with DNA strand-scission activity of various Formosan plants, led to isolation of five new prenylated phloroglucinols, garcinielliptones HA (**1**), HB (**2**), HC (**3**), HD (**4**) and HE (**5**) from the heartwoods of *Garcinia subelliptica*. Several lignans also possessed Cu(II)-mediated DNA strand-scission activity and including tuberculatin (**8**), justicidin (**9**) and procumbenoside (**10**), and two anti-inflammatory lignans, ciliatosides A (**11**) and B (**12**), which were isolated from *Justicia ciliata* and *J. Procumbens*, respectively (Day et al., 1999, 2000, 2002).

2. Results and discussion

The molecular formula of **1**, $[\alpha]_D^{26}$ −36.7 (*c* 0.25, acetone), was determined to be $C_{20}H_{28}O_6$ by HRESIMS (364.1885, Δ 0.0001 mmu), which was consistent with its ^1H and ^{13}C NMR spectroscopic data. The IR absorption of **1** indicated the presence of OH (3417 cm^{-1}), α,β-unsaturated β-hydroxyl ketone (1630 cm^{-1}) (Bellamy, 1958) and aromatic (1607 cm^{-1}) moieties. The UV spectrum of **1** [λ_{max} 236 (4.13), 290 (4.16) and 346 (3.87) nm] established the presence of an *o*-hydroxyl aromatic carbonyl moiety (Scatt, 1964). The ^{13}C (Table 1) spectrum of **1** disclosed the presence of a fully substituted aromatic ring (δ 101.5, 101.7, 105.9, 157.5, 161.9 and 164.3). The structure of **1** was completely assigned by a combination of one- and two-dimensional NMR methods. Its ^1H NMR spectrum (Table 1) contained signals for an isopropyl group, four tertiary methyls, two methylene groups, three methine groups including two oxymethines at δ 3.78 (*t*, $J = 6.8$ Hz) and 4.77 (*dd*, $J = 10.0$, 8.8 Hz), and a lowfield shifted signal of an hydroxyl group (δ 13.82). Analysis of ^1H–^1H COSY (bold lines, Fig. 1) and HMQC spectra for **1** established the connectivities of four ^1H–^1H and ^1H–^{13}C spin systems.

The IR spectrum and the HMBC correlations (Fig. 2) established that the lowfield shifted signal of hydroxyl and 1-oxoisobutyl groups were linked to C-5 and C-4, respectively. The HMBC correlations (Fig. 2) also confirmed that a 2-hydroxyisopropyl group, the C-16 and a 2-methyl-2-oxygenated-3-hydroxy-butyl group, were linked to C-17, C-6 and C-2, respectively. The above results and the NOESY correlations of Me-20/$H_β$-7, with C-1 and C-3 presented as quaternary oxygenated carbons suggested that **1** possessed a new furochromane skeleton.

The relative stereochemistry of **1** was elucidated on the basis of NOESY experiments. The NOESY cross-peaks between Me-20/$H_β$-7, $H_β$-7/H-8, and $H_α$-16/H-17 suggested that HO-8 and H-17 are on the α-side of **1**. To further clarify the relative configuration of **1**, a computer-assisted 3D structure was obtained by using the molecular modeling program CS CHEM 3D Ultra 8.0, with MM2 force-field calculations for energy minimization (Fig. 3). The calculated distances between $H_α$-16/H-17 (2.357 Å), Me-20/$H_β$-7 (3.846 Å) and $H_β$-7/H-8 (2.584 Å), are all less than 4 Å. This is consistent with the NOESY interactions between each of these proton pairs. Thus, garcinielliptone HA is characterized as **1**. The chemical shift values of C-1 and C-3 (Table 1) were assigned by comparison with those of garcinielliptones B and I (Weng et al., 2003a,b).

The molecular formula of compound **2** was established as $C_{26}H_{36}O_6$ (*m/z* 451.2463, Δ 0.0003 mmu) by HRESIMS. The IR spectrum of **2** implied the presence of OH (3431 cm^{-1}), conjugated ketone (1665 cm^{-1}) and α,β-unsaturated β-hydroxyl ketone (1597 cm^{-1}) moieties (Bellamy, 1958). The UV spectrum of **2** showed maximum absorption at 326 (3.87) and 228 (4.16) nm. Its ^1H NMR spectrum (Table 1) exhibited the signals for an isopropyl group, six tertiary methyls, a methoxyl group, two methylene groups, four methines including three olefinic protons and an unusually low-field shifted resonance (δ 17.85) that suggested the presence of an hydroxyl proton that participates in strong hydrogen bonding. The ^{13}C NMR spectrum showed signals for 26 carbons, which could be sorted by DEPT experiments into 8 methyls, 2 methylenes, 4 methines, 11 quaternary carbons including two carbonyl carbons and an enolic carbon at δ 202.9, 197.7 and 192.6, consist with the IR spectrum described above and one methoxyl carbon. The ^1H–^1H and ^1H–^{13}C spin systems are represented as bold lines (Fig. 1). Analysis of 1D NMR, HMBC (Fig. 2) and NOESY spectroscopic data confirmed that two prenyl (3-methylbut-2-enyl) groups were linked at C-2 and the connectivities between C-1/C-2 and C-2/C-3. The HMBC correlations (Fig. 2) and NOESY correlations of OMe/Me-20 and Me-21 confirmed a 2-(2-methoxylisopropyl) furan ring fused on C-4 and C-5, linkages between C-3/C-4 and the appearance of an α,β-unsaturated β-hydroxyl ketone moiety in **2**. The above evidence and the NOESY correlation of H-23/Me-20 or Me-21 and OH-22/Me-24 and Me-25 enabled structure **2** to be characterized as such and supported the assignment of carbon signals of C-1, C-3 and C-22.

The molecular formula of **3**, $[\alpha]_D^{25}$ 51 (*c* 1.34, acetone), was determined to be $C_{25}H_{36}O_5$ by HRESIMS ($[M+1]^+$, *m/z* 417.2640, Δ 0.0001 mmu), which was consistent with its ^1H and ^{13}C NMR spectroscopic data. The IR absorption of **3** implied the presence of OH (3403 cm^{-1}) and α,β-unsaturated β-hydroxyl ketone (1594 cm^{-1}) (Bellamy, 1958) moieties. The UV spectrum was similar to that of **2**. The ^1H and ^{13}C NMR spectra are almost identical to those of corresponding data for **2** except for the absence of signals due to 2-methoxylisopropylfuran ring and the appearance of resonances due to a 2-hydroxyisopropyl-2,3-dihydrofuran ring (Table 1). The ^1H–^1H COSY and HMQC data for **3** established the connectivities of three ^1H–^1H and ^1H–^{13}C spin systems represented as bold lines (Fig. 1). The HMBC correlations of Me-20 and Me-21/C-18 and C-19, H-18/C-4 and C-5, H_2-17/C-4, C-5, C-3 and C-19 established the 2-hydroxyisopropyl-2,3-dihydrofuran ring was fused on C-4–C-5 bond. Thus, the

Table 1
^{1}H and ^{13}C NMR spectroscopic data (δ in ppm, J in Hz) of **1**–**2**, **5** in acetone-d_6, and **3**, **4** in $CDCl_3$

Position	**1**		**2**		**3**		**4**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		157.5		202.9		204.5		204.6		163.2
2		101.5		64.4		61.4		61.6		100.4
3		164.3		192.6		191.6		191.8		164.5
4		101.7		118.1		108.8		108.8		101.3
5	OH 13.82*s*	161.9		161.7		170.1		170.1	OH 13.52*s*	160.4
6		105.9		104.9		102.2		102.3		106.1
7	α 2.82*dd* (16.4, 5.2) β 2.47*dd* (16.4, 7.2)	27.0	2.65*d* (7.6)	39.4	2.66*d* (7.5)	39.0	2.60*d* (7.7)	39.1	α 3.02*m*, β 3.07*m*	26.6
8	β 3.82 (*t*, 6.4)	69.3	4.78*bt* (7.6)	119.5	4.79*m*	118.0	4.79*m*	118.1	4.81*t* (9.6)	92.7
9		80.1		136.3		134.4		134.9	OH 3.85*s*	71.2
10	1.29*s*	21.9	1.54*s*	18.6	1.56*s*	25.5	1.55*s*	25.7	1.26*s*	25.9
11	1.36*s*	26.6	1.54*s*	18.6	1.56*s*	17.6	1.55*s*	17.9	1.33*s*	26.1
12		209.3	2.65*d* (7.6)	39.4	2.66*d* (7.6)	37.1	2.68*d* (6.0)	37.3		209.3
13	3.78*m*	39.5	4.78*bt* (7.6)	119.5	4.79*m*	117.6	4.79*m*	117.7	3.78*m*	38.7
14	1.15*d* (6.4)	20.2		136.3		134.6		134.7	1.15*d* (6.8)	19.5
15	1.14*d* (6.4)	20.0	1.48*s*	26.5	1.50*s*	17.4	1.50*s*	17.6	1.13*d* (6.8)	19.3
16	α 3.05*dd* (14.8, 8.8) β 2.96*dd* (14.8, 10.0)	28.1	1.48*s*	26.5	1.50*s*	25.5	1.50*s*	25.7	α 2.98*m*, β 3.07*m*	27.3
17	4.77*dd* (10.0, 8.8)	92.3	6.62*s*	105.1	2.90*d* (8.8)	26.2	3.09*m*	26.4	4.76 t (9.6)	93.0
18		72.0		159.2	4.79*m*	92.2	4.79*m*	93.2	3.84*s*	71.4
19	1.25*s*	26.6		74.0	OH 3.43*s*	71.2		71.6	1.05*s*	25.7
20	1.32*s*	26.8	1.54*s*	26.0	1.29*s*	25.1	1.26*s*	24.7	1.19*s*	25.5
21			1.54*s*	26.0	1.25*s*	23.8	1.26*s*	24.5		
22			OH 17.85*s*	197.7	OH 18.31*s*	198.8	OH 18.31*s*	198.9		
23			3.73*m*	36.3	3.68*m*	34.5	3.66*m*	34.7		
24			1.26*d* (6.8)	19.9	1.19*d* (6.8)	18.5	1.19*d* (6.8)	18.7		
25			1.26*d* (6.8)	19.9	1.21*d* (6.8)	19.8	1.21*d* (6.8)	20.0		
OMe			3.04*s*	51.5						

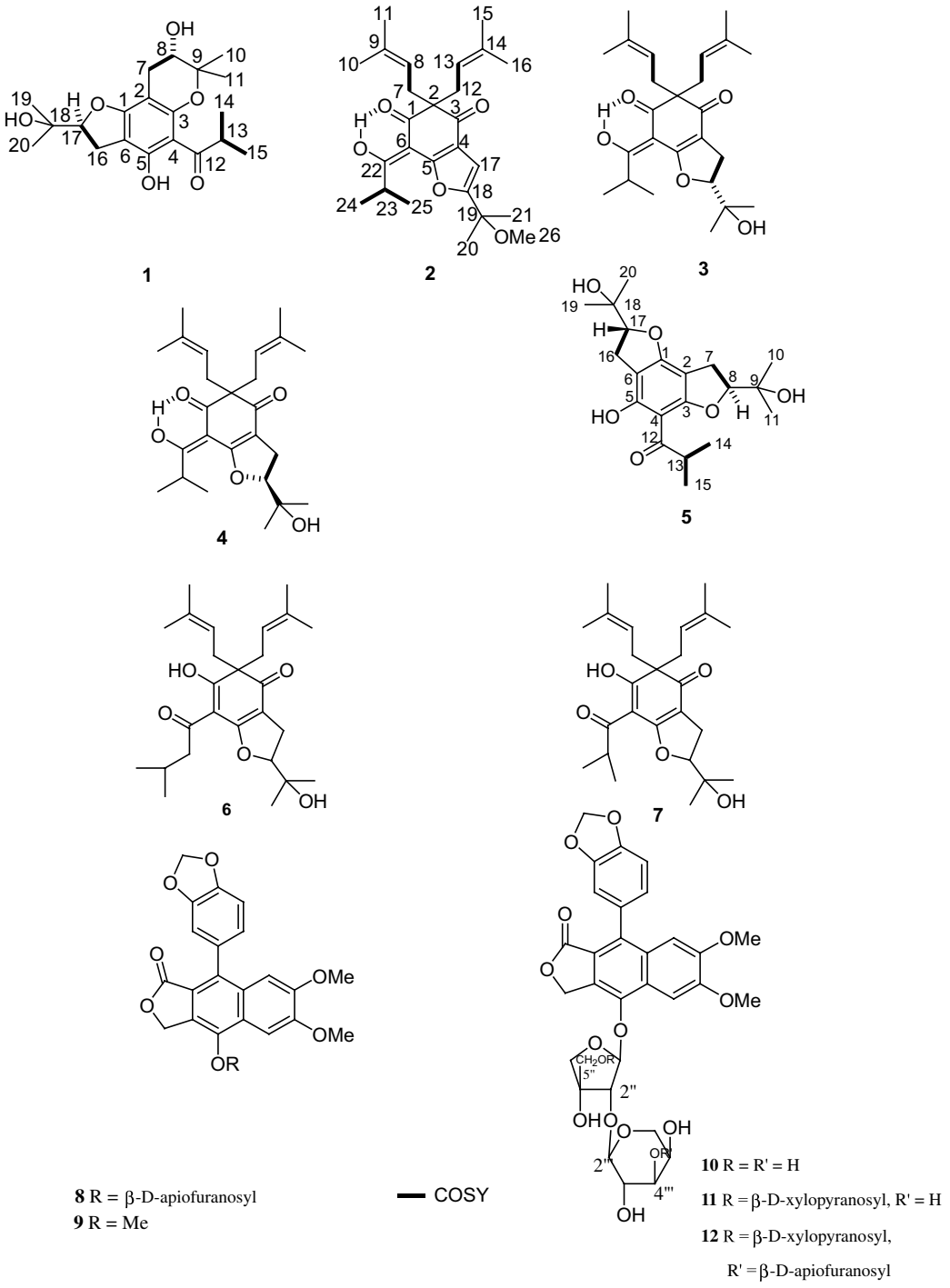

Fig. 1. Structures of **1**–**12** and key ^{1}H–^{1}H COSY correlations of **1**–**5**.

structure of garcinielliptone HC (**3**) was characterized as **3** (Fig. 1).

The molecular formula of **4**, $[\alpha]_D^{25}$ −54 (*c* 0.49, acetone), was determined to be $C_{25}H_{36}O_5$ by HRESIMS ($[M]^+$, *m/z* 417.2639, Δ 0 mmu), which was consistent with its ^{1}H and ^{13}C NMR spectroscopic data. Its IR spectra showed absorptions due to hydroxyl (3405 cm^{-1}) and α,β-unsaturated β-hydroxyl ketone (1593 cm^{-1}) moieties (Bellamy, 1958). The UV spectrum of **4** was identical to that of **3**. The ^{1}H and ^{13}C NMR spectra were almost identical to the corresponding data for **3**. The NOESY experiment of **4** identifies cross-peaks between H_β-17/H-18 and H_β-17/Me-21 or Me-20 while that of **3** only established a cross-peak between H_2-17/H-18. On the basis of the above evidence, garcinielliptone HD (**4**) was characterized as the C-18 stereoisomer of **3**.

In previous literature, two compounds, **6** (lupulone C) and **7**, oxidative derivatives of lupulone and colupulone,

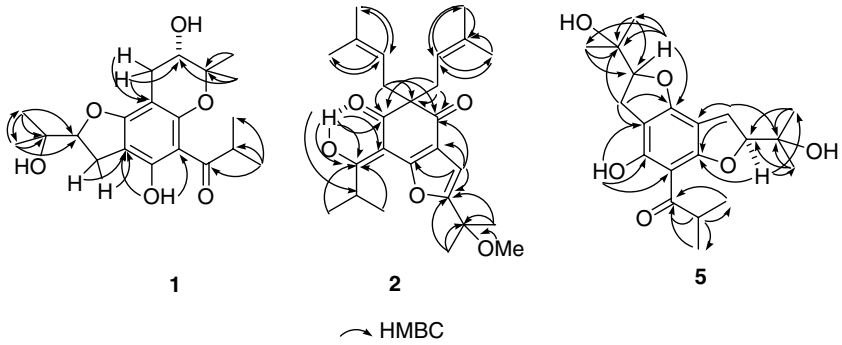

Fig. 2. Key HMBC correlations of **1**, **2** and **5**.

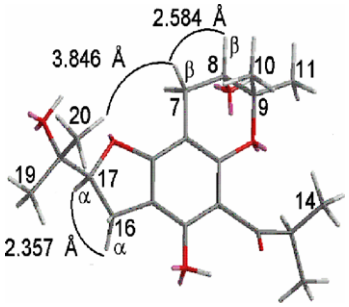

Fig. 3. Selective NOESY correlations and relative stereochemistry for **1**.

related to compounds **2**–**4** were reported (Zhao et al., 2003, 2005; Cahill and Shannon, 1969). In the ^{13}C NMR spectrum of **2**–**4**, the chemical shift values of C-1, C-3 and C-22 were significantly different from those of corresponding carbon signals of **6** (Zhao et al., 2005). It further supported the characterization of **2**–**4**.

The HRESIMS of **5** indicated a $[M + Na]^+$ peak at m/z 387.1786, Δ 0.0002 mmu, which corresponded to a molecular formula of $C_{20}H_{28}O_6$. The IR absorption of **5** implied the presence of hydroxyl (3432 cm^{-1}) and α,β-unsaturated β-hydroxyl ketone (1627 cm^{-1}) (Bellamy, 1958) groups. The UV spectrum of **5** was shown to be similar to that of **1**. Its ^1H NMR spectrum (Table 1) contained signals for an isopropyl group, four tertiary methyls, two methylene groups, three methine groups including two oxymethines at δ 4.76 (t, $J = 9.6$ Hz) and 4.81 (t, $J = 9.6$ Hz) and a low-field shift signal of hydroxyl group at δ13.52. The DEPT spectrum (Table 1) revealed the presence of six methyl, two methylene, three methine (including two oxymethine) and nine quaternary carbons. Analysis of ^1H–^1H COSY and HMQC experiment for **5** established connectivities for four ^1H–^1H and ^1H–^{13}C spin systems represented as bold lines (Fig. 1).

The HMBC correlations of OH-5/C-4, C-5 and C-6, Me-14 and Me-15/C-12 and H-13/C-12, together with the pre-

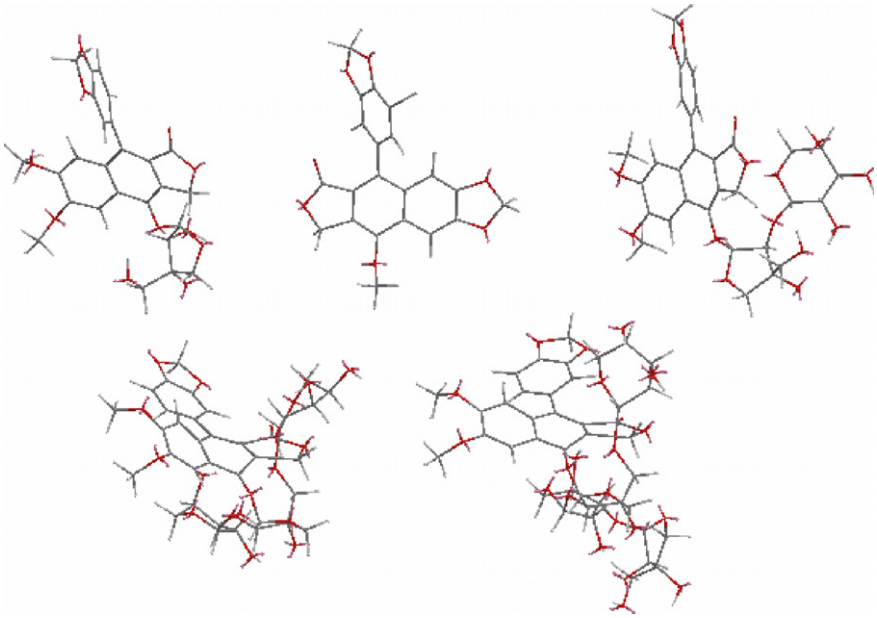

Fig. 8. Energy-minimized (mm_2) molecular structures of **8**–**12**.

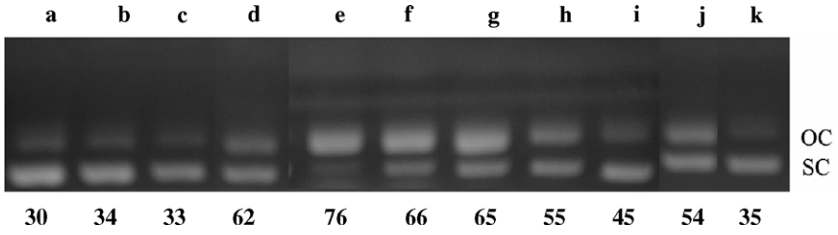

Fig. 4. DNA strand scission by compounds **1**–**5**, quantified after agarose gel electrophoresis. pBR322 plasmid DNA (500 ng) was incubated for 30 min at 37°C in the presence of the following additive: (a) no addition (DNA control); (b) Cu(II) (300 μM); (c) 300 μM **1** + Cu(II) (300 μM); (d) 300 μM **2** + Cu(II) (300 μM) ; (e–i) 300, 200, 100, 50, 25 μM **3** + Cu(II) (300 μM); (j) 300 μM **4** + Cu(II) (300 μM); (k) 300 μM **5** + Cu(II) (300 μM). OC, open circular DNA; SC, supercoiled DNA. The percent OC DNA present is shown below each lane.

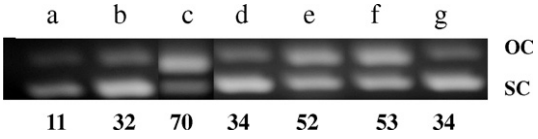

Fig. 5. Agarose gel electrophoretic patterns of plasmid DNA strand with compounds **8**–**12** in the presence of Cu(II) ion. pBR322 plasmid DNA (500 ng) was incubated for 30 min at 37 °C in the presence of following additive: (a) no addition (DNA control); (b) Cu(II) (300 μM); (c) 300 μM **8** + Cu(II) (300 μM); (d) 300μM **9** + Cu(II) (300μM); (e) 300 μM **10** + Cu(II) (300 μM); (f) 300 μM **11** + Cu(II) (300 μM); (g) 300 μM **12** + Cu(II) (300 μM). The percent OC DNA present is shown below each lane.

sentation of an *o*-hydroxyl aromatic carbony1 moiety in the IR spectrum, established that the 1-oxoisobutyl group and lowfield shifted hydroxyl group (δ13.52) were linked at C-4 and C-5, respectively. The HMBC correlations (Fig. 2) established that two 2-hydroxyisopropyl-2,3-dihydrofuran rings were fused on C-1–C-6 and C-2–C-3 bonds, respectively. The above result suggested **5** possessed a new benzo[1, 6-*b*:3,2-*b*′]di-2,3-dihydrofuran skeleton. The NOESY correlations of H_β-16/H-17 and H_α-7/H-8 confirmed that H-17 and H-8 are on the β- and α-sides of **5**. Thus, the structure of garcinielliptone HE (**5**) was characterized as **5** (Fig. 1).

Compounds **1**–**5** and **8**–**12** were tested for converting supercoiled plasmid pBR322 DNA to relaxed open circles in the presence of Cu(II). As shown in Figs. 4–6 compounds **3**, **8**, **10** and **11** gave a significant level of Cu(II)-mediated DNA breakage in a concentration-dependent manner. Compounds **2** and **4** at 300 μM also displayed slight Cu(II)-mediated DNA breakage properties, while compounds **1** and **5** at 300 μM did not (Fig. 4). Compounds **8** and **10** at 300–50 μM significantly converted the supercoiled DNA to open circle DNA, while compound **11** showed weaker DNA strand scission activity than those of **8** and **10** in the Cu(II)-mediated DNA breakage reaction. Compounds **9** and **12** each at 300 μM did not show DNA strand scission activity (Figs. 5 and 6). Compound **8** (justicidin A) has potent cytotoxic activity against several human cancer cells in vitro (Day et al., 1999). It decreases the level of cytosolic ku70 leading to apoptosis in human colorectal cancer cells (Lee et al., 2005) and induced apoptosis in human hepatoma cells proceeding via caspase-8 and followed by mitochondrial disruption (Su et al., 2006) but did not show Cu(II)-mediated DNA breakage. This may be due to low DNA binding by **9**. As shown in Figs. 6 and 7, the conversion of supercoiled DNA to a relaxed form induced by selective compounds **3** and **8** in the presence of Cu(II) was not inhibited with neocuproine (600 μM), a Cu(I)-specific sequesting agent (Ahsan and Hadi, 1998). It is thus suggested that Cu(I) is

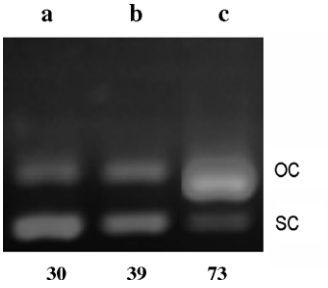

Fig. 7. Effect of neocuproine (600 μM) on **3** (300 μM)–Cu(II)(300 μM)-induced breakage of pBR322 DNA. (a) DNA along; (b) DNA + Cu(II) + neocuproine; (c) DNA + Cu(II) + **3** + neocuproine. OC, open circular DNA; SC, supercoiled DNA. The percent OC DNA present is shown below each lane.

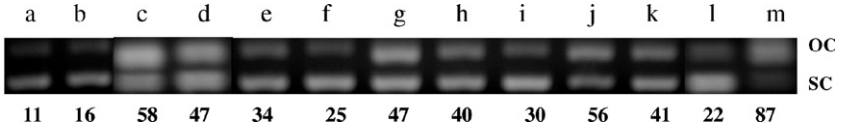

Fig. 6. Agroase gel electrophore patterns of plasmid DNA strand with compounds **8**, **10** and **11** in the presence of Cu(II) ion and effects of neocuproine (600 μM) on **8**-Cu(II) (300 μM)-induced breakage of pBR322 DNA. pBR322 plasmid DNA (500 ng) was incubated for 30 min at 37 °C in the presence of following additive: (a) no addition (DNA control); (b) Cu(II) (300 μM); (c) 200 μM **8** + Cu(II) (200 μM); (d) 100 μM **8** + Cu(II) (100 μM); (e) 50 μM **8** + Cu(II) (50 μM); (f) 25 μM **8** + Cu(II) (25 μM); (g) 200 μM **10** + Cu(II) (200 μM); (h) 100 μM **10** + Cu(II) (100 μM); (i) 50 μM **10** + Cu(II) (50 μM); (j) 200 μM **11** + Cu(II) (200 μM); (k) 100 μM **11** + Cu(II) (100 μM); (l) DNA + Cu(II) + neocuproine; (m) DNA + **8** (300 μM) + Cu(II) + neocuproine. The percent OC DNA present is shown below each lane.

not an essential intermediate in the **3** or **8**-mediated DNA cleavage reaction. Other metal ions tested (Mg(II), Mn(II), Ca(II), Co(II), Ni(II), Fe(II)) and Fe(III) were ineffective or less effective in the DNA breakage reaction of **3** and **8** (data not shown). Taken together, this study was the first report of 2,4,6-prenylated phloroglucinols with DNA strand-scission activity.

As shown in Figs. 1 and 6, the *O*-glycosidation of **8** or **11** with more than one sugar unit led to less DNA strand-scission activity. As shown in Fig. 8, the deshielding of the γ-lactone ring of **8** and **10** by the sugar molecules established significant DNA strand scission activity, while the shielding of the γ-lactone ring of **11** and **12** by sugar molecule led to less or no DNA strand scission activity. It has been shown that several compounds that contain a catechol moiety undergo Cu(II)-mediated oxidation to yield reactive oxygen species (ROS), with these being capable of causing DNA strand scission. On the other hand, lignan glycosides, such as **8**, **10** and **11** do not contain a catechol moiety, but yet also can induce DNA strand scission in the presence of Cu(II). It was evident that the γ-lactone ring in lignan glycoside may be important for inducing Cu(II)-mediated DNA strand scission reactions.

3. Conclusions

In the present study, we have isolated five new prenylated phloroglucinols from the heartwood of *G. subelliptica*. Among them, compounds **1** and **5** possessed an unprecedented skeleton. In the presence of Cu(II), compound **3** caused significant breakage of supercoiled plasmid pBR322. Lignan glycosides, **8**, **10** and **11** caused significant breakage of supercoiled plasmid pBR322 in the presence of Cu(II). The interaction between the γ-lactone ring of lignan glucoside **8**, **10** and **11** and DNA in the presence of Cu(II) may induce DNA damage. If copper ion in vivo was available, phloroglucinol **3** and lignan glycosides, **8**, **10** and **11** may lead to DNA damage and induce cell death.

4. Experimental

4.1. General

Optical rotations were recorded with a JASCO-370 polarimeter using acetone as solvent. UV spectra were obtained in MeOH on a Jasco-UV–VIS spectrophotometer. IR spectra were measured on a Hitachi 260-30 spectrometer. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra, ^{1}H–^{1}H-COSY, NOESY, HMQC and HMBC experiments were recorded on a Varian-Unity-400 spectrometer. MS were obtained on a JMS-HX-100 mass spectrometer. Supercoiled plasmid pBR322 DNA was obtained from ABgene (ABgene House, Epsom, Surrey, UK). Agarose and other reagents used in electrophoresis were obtained from Sigma (St. Louis, MO). TBE buffer was obtained from MP Biomedicals, Inc., Germany. The purity (>95%) of compounds **1**–**5** used for DNA strand scission assay, was determined by HPLC.

4.2. Plant material

The heartwood of *G. subelliptica* (10 kg) was collected at Ping-Tung Hsien, Taiwan in August 2004. A voucher specimen (2004-GH) is deposited in the Laboratory of Medicinal Chemistry, School of Pharmacy, Kaohsiung Medical University.

4.3. Extraction and isolation of compounds

The heartwood of *G. subelliptica* (10 kg) was chipped and extracted with CH_2Cl_2 at room temperature. The resultant CH_2Cl_2 extract (100 g) was subjected to silica gel (open column, 3000 g, 10 × 70 cm, 1 ml/min) cc and eluted with *n*-hexane containing increasing amounts of EtOAc and final washing with MeOH to yield 33 fractions. Fraction 20 was further purified on Si gel (open column, 300 g, 3 × 50 cm, 1 ml/min) and eluted with *n*-hexane–acetone (2:1) to yield **1** (55.6 mg). Fraction 22 was further purified on a RP18 column (100 g, 20 × 2 cm, 10 ml/min) and eluted with acetone–H_2O (1:1) to yield **2** (9.9 mg), **3** (250.1 mg) and **4** (99.0 mg), respectively. Fraction 23 was further purified on a RP18 column (100 g, 20 × 2 cm, 10 ml/min) and eluted with acetone–H_2O (1:1) to yield **5** (100.5 mg).

4.3.1. Garcinielliptone HA (*1*)

Colorless oil; $[\alpha]_D^{26}$ −36.7 (*c* 0.25, acetone); UV (MeOH) λ_{max} (log ε) 346 (3.87), 290 (4.16), 236 (4.13) nm; IR (KBr) 3417, 1630, 1607 cm^{-1}; for ^{1}H and ^{13}C NMR, spectroscopic data, see Table 1; FABMS (positive) *m*/*z* 365 $[MH]^+$ (50); HRESIMS: 387.1785 for $C_{20}H_{27}O_6Na$ (387.1784, Δ 0.0001 mmu).

4.3.2. Garcinielliptone HB (*2*)

Colorless oil; $[\alpha]_D^{26}$ 0 (*c* 0.01, acetone); UV (MeOH) λ_{max} (log ε) 326 (3.87), 228 (4.16) nm; IR (KBr) 3431, 1665, 1597 cm^{-1};for ^{1}H and ^{13}C NMR, spectroscopic data, see Table 1; ESIMS (positive) *m*/*z* 451 $[MNa]^+$; HRESIMS: 451.2463 for $C_{26}H_{36}O_5Na$ (451.2460, Δ 0.0003 mmu).

4.3.3. Garcinielliptone HC (*3*)

Yellow oil; $[\alpha]_D^{26}$ 51 (*c* 1.34, acetone); UV (MeOH) λ_{max} (log ε) 376 (3.47), 356 (4.01), 236 (4.13) nm; IR (KBr) 3403, 1594 cm^{-1}; for ^{1}H and ^{13}C NMR, spectroscopic data, see Table 1; EIMS *m*/*z* 416 $[M]^+$ (3), 361 (3), 347 (10), 305 (10), 79 (100) ; HRESIMS: 417.2640 for $C_{25}H_{37}O_5Na$ (417.2641, Δ 0.0001 mmu).

4.4. Garcinielliptone HD (*4*)

Yellow oil; $[\alpha]_D^{26}$ −54 (*c* 0.49, acetone); UV (MeOH) λ_{max} (log ε) 374 (3.47), 356 (4.00), 236 (4.10) nm; IR (KBr) 3405,

1593 cm^{-1}; for ^1H and ^{13}C NMR, spectroscopic data, see Table 1; EIMS *m/z* 416 $[M]^+$ (3), 361 (3), 347 (13), 189 (33), 79 (100) ; HRESIMS: 417.2639 for $C_{25}H_{37}O_5$ (417.2641, Δ 0.0002 mmu).

4.5. Garcinielliptone HE (5)

Colorless oil; $[\alpha]_D^{26}$ −42 (*c* 0.50, acetone); UV (MeOH) λ_{max} (log ε) 348 (3.92), 264 (3.98), 242 (4.03) nm; IR (KBr) 3432, 1627 cm^{-1}; for ^1H and ^{13}C NMR, spectroscopic data, see Table 1; ESIMS *m/z* 387 $[MNa]^+$; HRESIMS: 387.1786 for $C_{20}H_{28}O_6Na$ (387.1784, Δ 0.0002 mmu).

4.6. Justicia materials, extraction, isolation and compound identification

Described as in the previous reports (Day et al., 1999, 2000, 2002).

4.7. DNA strand-scission assay

Reaction mixtures (25 μL) contained 10 mM Tris-HCl buffer (pH 8.0), supercoiled pBR322 plasmid DNA (500 ng), compounds **1**–**5** (dissolved in DMSO, with final DMSO concentration of no more than 5% in the 25 μL reaction solution), $CuCl_2$ and other components as described in the figures. Neocupronine or divalent metal ions were included in some experiments. Each batch of experiments included one blank control (DNA alone) and one metal control (DNA + Cu^{2+}). After being incubated at 37 °C for 30 min, the reaction mixture was treated with 5 μL of 30% glycerol–0.01% bromophenol blue and was analyzed by electrophoresis in a 1.0% agarose gel containing 0.7 μg/mL ethidium bromide. The electrophoresis was carried out in TBE buffer (89 mM Tris, 89 mM boric acid, containing 2 mM EDTA, pH 8.3) at 110–120 V for 2–3 h. Following electrophoresis, the DNA was imaged by ethidium bromide fluorescence which was photographed under ultraviolet light. (Ahsan and Hadi, 1998; Chaudhuri et al., 1995). The fluorescence of the relaxed pBR322 band was measured through the use of a PDI scanning densitometer with UN-SCAN-IT gel software (Version 6.1), Silk Scientific Inc., Orem, Utah, USA.

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