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Phloroglucinols with Antioxidant Activity and Xanthonolignoids from the Heartwood of *Hypericum geminiflorum*

Chien-Chang Wu,[†] Ming-Hong Yen,*[,‡] Shyh-Chyun Yang,[†] and Chun-Nan Lin*[,†,‡]

School of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China, and Institute of Life Science, National Taitung University, Taitung 950, Taiwan, Republic of China

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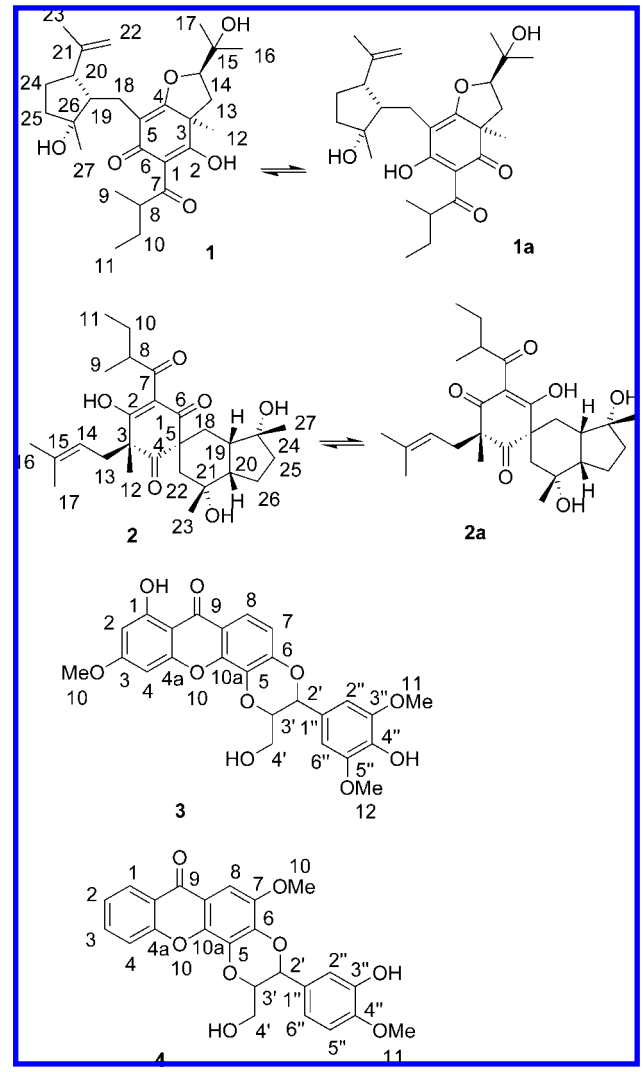
A new phloroglucinol, hyperielliptone HA (**1/1a**), a new spirophloroglucinol possessing an unprecedented skeleton, hyperielliptone HB (**2/2a**), and two new xanthonolignoids, hyperielliptones HC (**3**) and HD (**4**), were isolated from the heartwood of *Hypericum geminiflorum*. Compounds **1/1a** and **2/2a** were obtained as tautomeric pairs. The structures and relative configurations of these compounds were elucidated by spectroscopic methods. In biological testing, compound **2/2a** revealed significant inhibition of oxidative DNA damage and an inhibitory effect on xanthine oxidase.

Several years ago, we reported the isolation and biological activity of constituents from *Hypericum geminiflorum* Hemsl. (Clusiaceae).[1–4] In the present study on constituents, as part of a continuing investigation on the antioxidant constituents of Taiwanese plants, a new phloroglucinol, hyperielliptone HA (**1/1a**), a new spirophloroglucinol, hyperielliptone HB (**2/2a**), two new xanthonolignoids, hyperielliptones HC (**3**) and HD (**4**), and 12 known constituents, 1,3,8-trihydroxy-4-methoxyxanthone,[5] 1,3,8-trihydroxy-2-methoxyxanthone,[6] 1,7-dihydroxyxanthone,[7] 2,3-methylenedioxyxanthone,[8] 1,5-dihydroxy-6-methoxyxanthone,[3] 1-hydroxyxanthone,[9] 2,3-dimethoxyxanthone,[10] cadensin D,[9] 1,8-dihydroxy-3-methoxyxanthone,[11] β-sitosteryl-3β-glucopyranoside-6′-*O*-palmitate,[12] 7β-hydroxystigmast-4-en-3-one,[13] and 2,6,2′,6′-tetramethoxy-4,4′-bis(2,3-epoxy-1-hydroxypropyl)biphenyl,[14] were isolated from the heartwood of *H. geminiflorum*. The structure elucidation of **1/1a**, **2/2a**, **3**, and **4** and the antioxidant activity of all constituents isolated from this plant are reported herein.

Results and Discussion

The molecular formula of **1/1a**, [α] −6.2 (*c* 0.1, acetone), was determined to be $C_{27}H_{40}O_6$ by HRESIMS ($[M + Na]^+$, 483.2722, Δ −0.0004 mmu), which was consistent with its ^1H and ^{13}C NMR data. The UV maxima were 214 (4.30), 228 (4.20), and 278 (4.10) nm. The IR absorption of **1/1a** implied the presence of OH (3417 cm^{-1}), carbonyl ketone (1727 cm^{-1}), and α,β-unsaturated β-hydroxyl ketone (1642 cm^{-1}) functionalities.[15] The ^1H and ^{13}C NMR spectra of **1/1a** (Table 1) showed signals for 14 methyl groups, 12 methylene groups, eight methine groups including two oxymethine groups, and 20 quaternary carbons. However, double ^1H and ^{13}C NMR patterns in a ratio of approximately 2:1 (derived from the ^1H NMR signal intensity) and only one pseudomolecular peak in the positive HRESIMS enabled the conclusion to be made that this compound occurs in isomeric forms. This was later shown to represent two enol tautomers, **1** and **1a**, in solution ($CDCl_3$) in this same ratio, with **1** being the preferred tautomer. The 2D NMR experiments on **1/1a** gave evidence for the presence of two enol tautomers by revealing two independent networks of correlations between the more intensive signals on one bond and the less intensive signals on the other. The assignments presented below refer to the preferred tautomeric structure **1**. Analysis of $^1H-^1H$ COSY and HMQC experiments for **1** established the connectivities of eight $^1H-^1H$ and $^1H-^{13}C$ spin systems represented as bold lines (Figure 1). The HMBC correlations (Figure 1) established a 2-hydroxyisopropyl-2,3-dihydrofuran ring and a 2-hydroxy-5-isopropenyl-2-methylcyclopentylmethyl group fused on the C-3−C-4 bond and linked to C-5, in turn. The HMBC correlations also revealed the presence of a 1-oxo-2-methylbutyryl group in **1**. In the ^{13}C and ^1H NMR spectra of **1**, the chemical shift values of C-1, C-2, C-6, C-7 to C-11 and of H-8 to H-11 were similar to those of the corresponding carbon and proton signals, respectively, of ialibinone C.[16] Thus, the 1-oxo-2-methylbutyryl group was linked to C-1 and the structure of hyperielliptone HA (**1**) was elucidated



* To whom correspondence should be addressed. Tel: +886 7 3121101, ext. 2163. Fax: +886 7 5562365. E-mail: lincna@cc.kmu.edu.tw (C.-N.L.); yen@kmu.edu.tw (M.-H.Y.).

[†] Kaohsiung Medical University.

[‡] National Taitung University.

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **1/1a** (in d_6-acetone) and **2/2a** (in $CDCl_3$)

	1		**1a**		**2**		**2a**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		107.8		109.8		111.7		112.0
2		198.1		194.7		198.6		193.6
3		57.7		57.7		56.0		60.0
4		179.1		179.1		201.1		201.1
5		106.6		106.5		64.8		59.5
6		194.6		198.0		193.6		197.6
7		203.3		203.6		206.5		204.5
8	3.63 (m)	41.6	3.71 (m)	41.2	3.46 (m)	41.0	3.40 (m)	40.7
9	1.15 (d, 6.8)	18.9	1.07 (d, 6.8)	17.4	1.10 (d, 6.8)	15.9	1.09 (d, 6.8)	16.7
10	α1.41 (m)	28.1	α1.40 (m)	28.1	α1.47 (m)	27.6	α1.47 (m)	27.6
	*β*1.68 (m)		*β*1.70 (m)		*β*1.80 (m)		*β*1.80 (m)	
11	0.83 (t, 7.2)	12.9	0.94 (t, 7.2)	12.7	0.98 (t, 7.6)	11.6	0.87 (t, 7.6)	12.0
12	1.51 (s)	27.0	1.51 (s)	27.0	1.56 (s)	22.9	1.42 (s)	21.9
13	α2.09 (m)	33.4	α2.09 (m)	33.5	α2.42 (dd, 13.6, 9.2)	40.7	α2.42 (dd, 13.6, 9.2)	40.7
	*β*2.29 (m)		*β*2.29 (m)		*β*2.70 (dd, 13.6, 6.8)		*β*2.70 (dd, 13.6, 6.8)	
14	4.79 (dd, 10.8, 5.2)	92.5	4.78 (m)	92.5	4.58 (m)	117.2	4.58 (m)	118.3
15		71.2		71.2		137.5		134.6
15-OH	3.79		3.79					
16	1.36 (s)	27.3	1.36	27.4	1.51 (s)	25.8	1.59 (s)	28.1
17	1.20 (s)	26.7	1.20	26.7	1.41 (s)	17.6	1.40 (s)	17.9
18	2.46 (d, 8.4)	22.1	2.46 (d, 8.4)	22.2	α1.71 (m)	24.0	α1.71 (m)	24.0
					*β*1.98 (d, 12.0)		*β*1.98 (d, 12.0)	
19	1.97 (m)	51.1	1.97 (m)	51.1	1.80 (m)	48.1	1.80 (m)	48.1
20	2.65 (m)	53.5	2.65 (m)	53.5	1.80 (m)	51.6	1.80 (m)	51.6
21		149.8		149.8		73.8		73.8
22	α4.51 (s)	111.4	α4.51 (s)	111.4	α1.52 (m)	49.6	α1.52 (m)	49.5
	*β*4.68 (s)		*β*4.68 (s)		*β*2.20 (m)		*β*2.20 (m)	
23	1.64 (s)	19.6	1.64 (s)	19.6	1.00 (s)	21.7	1.00 (s)	21.7
24-OH				2.17			2.17	
24	α1.37 (m)	29.7	α1.37 (m)	29.6		79.3		79.3
	*β*1.89 (m)		*β*1.89 (m)					
25	1.68 (m)	42.4	1.68 (m)	42.4	1.81 (m)	39.7	1.81 (m)	39.8
26		80.8		80.8	α1.42 (m)	21.5	α1.42 (m)	21.5
					*β*1.80 (m)		*β*1.80 (m)	
26-OH	2.98		2.96					
27	1.23 (s)	28.0	1.22 (s)	28.2	1.39 (s)	26.7	1.39 (s)	26.7

as shown **1**. The NOESY correlations of H_α-13/H-14 and Me-12, H_β-24/H-20, H-20/H-19, and H-22, and H-19/H-22 and Me-27 suggested Me-12 and H-14 are α-oriented and that H-19, Me-27, and H-20 are *β*-oriented. Therefore, compound **1** was characterized as 4-hydroxy-7-(2-hydroxy-5-isopropenyl-2-methylcyclopentylmethyl)-2-(1-hydroxy-1-

methylethyl)-3a-methyl-5-(2-methylbutyryl)-3,3a-dihydro-2*H*-benzofuran-6-one (**1**). The tautomeric form **1a** was identified as

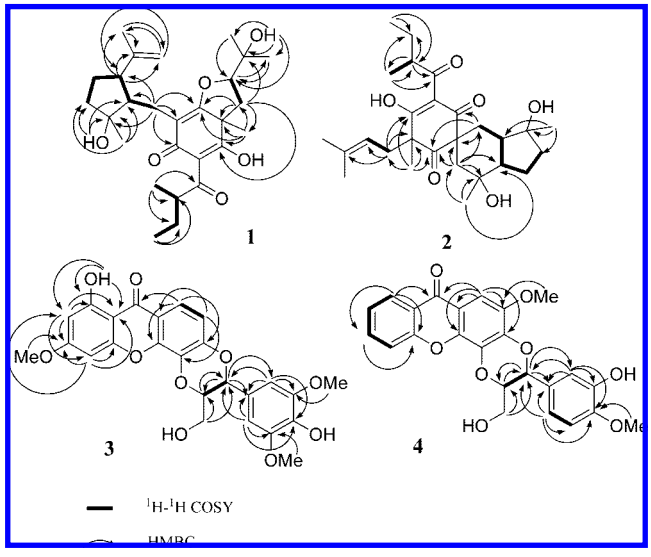

Figure 1. Structures and key ^{1}H−^{1}H COSY and HMBC correlations of compounds **1**−**4**.

6-hydroxy-7-(2-hydroxy-5-isopropenyl-2-methylcyclopentylmethyl)-2-(1-hydroxy-1-methylethyl)-3a-methyl-5-(2-methylbutyryl)-3,3a-dihydro-2*H*-benzofuran-4-one (**1a**). From the ^{1}H NMR, COSY, and NOESY spectra, a computer-generated 3D structure of **1** was obtained from the molecular modeling program CS CHEM 3D V 3.5.1, with MM2 force-field calculations for energy minimization (Figure S1, Supporting Information). The calculated distances between H_α-13/H-14 (2.953 Å), H_β-13/Me-12 (2.550 Å), H_β-24/H-20 (2.418 Å), H-20/H-19 (2.463 Å), H-20/H-22 (2.957 Å), H-19/H-22 (2.343 Å), and H-19/Me-27 (2.886 Å) are all less than 4.00 Å. This is consistent with the well-defined NOESY interactions observed for each of these proton pairs.

Compounds **2/2a** showed a pseudomolecular peak at *m/z* 483.2726 $[M + Na]^+$ in the HRESIMS. The UV maxima were 215 (4.20), 226 (4.10), and 280 (4.30) nm. The IR spectrum of **2/2a** implied the presence of OH (3417 cm^{-1}), carbonyl ketone (1714 cm^{-1}), α*β*-unsaturated ketone (1673 cm^{-1}), and α*β*-hydroxyl ketone (1589 cm^{-1}) groups.[15] The ^{1}H and ^{13}C NMR spectrum of **2/2a** (Table 1) showed signals for 14 methyl groups, 12 methylene groups, eight methine groups including two olefinic protons, and 20 quaternary carbons. The similar ^{1}H and ^{13}C NMR patterns to compounds **1/1a** and presence of only one pseudomolecular peak in the positive HRESIMS suggested that compounds **2/2a** also existed in two enol tautomeric forms. The assignment presented below refers to the preferred tautomeric structure **2**.

^{1}H−^{1}H COSY and HMQC spectra for **2** established the connectivities of six ^{1}H−^{1}H and ^{1}H−^{13}C spin systems represented as bold lines (Figure 1). The HMBC correlations as shown in Figure 1 established a spiro 4-methyloctahydroindene-1,4-diol unit linked at C-5 with a prenyl and a methyl linked together at C-3, respectively. The HMBC correlations also indicated the presence

of a 1-oxo-2-methylbutyryl group in **2** and a carbonyl ketone group substituted at C-4. In addition, the ^{13}C NMR signals of C-1, C-2, and C-6 to C-11 and the ^1H NMR signals of H-8, Me-9, H_2-10, and Me-11 were similar to the corresponding data obtained for **1**. Thus, the chemical structure of hyperielliptone HB (**2**) was elucidated as a spirophloroglucinol with an unprecedented skeleton, **2/2a**. The NOESY correlations of H-14/H_β-13, H_β-13/Me-12, H_β-18/H-19, H-19/Me-27, H_β-22/Me-23, and Me-23/H-20 suggested that Me-12, Me-23, H-20, H-19, and Me-27 are β-oriented and a prenyl group at C-3 is on the β-side of **2**. The relative configuration at C-3 and C-5 was established by the fact that the bridge between C-3 and C-5 can only exist in the *cis* form.[16] Thus, the bond between C-4 and C-5 must be α-oriented. From the ^1H NMR, COSY, and NOESY spectra, a computer-generated 3D structure of **2** was obtained similar to that of **1** (Figure S1, Supporting Information). The calculated distances between H_β-13/Me-12 (2.568 Å), H_β-18/H19 (2.147 Å), H-19/Me-27 (3.557 Å), H_β-22/Me-23 (2.401 Å), and Me-23/H-20 (2.479 Å) were all less than 4.00 Å. This is consistent with the well-defined NOESY interactions observed for each of these proton pairs.

The molecular formula of **3**, $[\alpha]^{25}_D$ 0 (*c* 0.1, acetone), was determined to be $C_{25}H_{22}O_{10}Na$ (505.1113, Δ 0.0002 mmu) by HRESIMS. The UV spectrum showed the presence of a xanthone moiety [λ_{max} (log ϵ) 210 (4.20), 248 (4.20), and 317 (3.70) nm].[17] The bathochromic shift induced by addition of $AlCl_3$ suggested the presence of a hydroxyl group at either C-1 or C-8. The IR absorption of **3** implied the presence of OH (3439 cm^{-1}), conjugated carbonyl (1649 cm^{-1}), and aromatic ring (1614 and 1580 cm^{-1}) units. The ^1H NMR spectrum displayed signals of a hydrogen-bonded hydroxyl group [δ 13.60 (1H, s)], six aromatic protons [δ 6.51 (1H, d, J = 2.0 Hz), 6.60 (1H, d, J = 2.0 Hz), 7.17 (1H, d, J = 8.8 Hz), 7.19 (2H, s), and 7.97 (1H, d, J = 8.8 Hz)], three methoxyl groups [δ 3.71 (3H, s) and 3.83 (6H, s)], two oxygenated methines [δ 5.62 (1H, d, J = 8.1 Hz) and 4.57 (1H, m)], and a hydroxymethyl group [δ 4.37 (1H, d, J = 12.7 Hz) and 3.99 (1H, dd, J = 12.7, 2.9 Hz)]. The observation of a deshielded doublet [δ 5.62] measured in pyridine-d_5 suggested a benzylic methylene substituted by oxygen, and its *trans*-diaxial relationship (J = 8.1 Hz) indicated the occurrence of a *trans*-substituted 1,4-dioxane ring between the xanthone framework and the phenyl ring.[18,19] The HMBC correlations as shown in Figure 1 suggested that a tetrasubstituted benzene ring and a methoxy group were linked at C-2′ and C-3, respectively, and a 1,4-dioxane ring was fused on C-5 and C-6. The $^1H-^1H$ COSY of the cross-peak between H-7 and H-8 and the bathochromic shift induced by addition of $AlCl_3$ in its UV spectrum suggested that the hydrogen-bonded hydroxy group at δ 13.60 was linked to C-1. The HMBC correlations (Figure 1) of H-4′/C-2′ and C-3′ and C-4′ as a secondary oxygenated carbon suggested a hydroxymethy group was linked to C-3′. Therefore, the structure of hyperielliptone HC was determined as 3′-hydroxymethyl-2′-(4″-hydroxy-3″,5″-dimethoxyphenyl)-5′,6′:5,6-(1-hydroxy-3-methoxyxanthone)-1′,4′-dioxane (**3**). The optical inactivity and *trans* relationship of the dioxane protons suggested that **3** was obtained as a racemic mixture of 2′*R*, 3′*R* and 2′*S*, 3′*S* enantiomers.[18]

The molecular formula of **4**, $[\alpha]^{25}_D$ 0 (*c* 0.1, acetone), was determined to be $C_{24}H_{20}O_8Na$ (489.1053, Δ 0.0003 mmu) by HRESIMS. The UV spectrum showed the presence of a xanthone moiety as in that for **3**. The IR absorption of **4** implied the presence of OH (3421 cm^{-1}), conjugated carbonyl (1638 cm^{-1}), and aromatic ring (1607 cm^{-1}) moieties. The ^1H NMR spectrum revealed five aromatic protons [δ 7.37 (1H, td, J = 7.2, 2.0 Hz), 7.50 (1H, d, J = 7.6 Hz), 7.67 (1H, s), 7.68 (1H, td, J = 7.2, 2.0 Hz), and 8.61 (1H, dd, J = 7.2, 2.0 Hz)], aromatic proton signals of an ABX system [δ 7.31 (1H, d, J = 8.0 Hz), 7.37 (1H, dd, J = 8.0, 3.2 Hz), and 7.45 (1H, d, J = 3.2 Hz)], two methoxyl groups [δ 3.68 (3H, s) and 3.83 (3H, s)], two oxygenated methines [δ 5.64, (1H, d, J = 8.4 Hz) and 4.61 (1H, m)], and a hydroxymethyl group [δ 4.38, (1H, d, J = 12.8 Hz) and 3.99 (1H, dd, J = 12.8, 2.9 Hz)]. Compound **4** was found to possess also a *trans*-substituted 1,4-dioxane ring between the xanthone framework and the phenyl ring, similar to that of **3**.[18,20] The HMBC correlations as shown in Figure 1 suggested that a trisubstituted benzene ring and a methoxy group are linked at C-2′ and C-7, respectively, and a 1,4-dioxane ring and a hydroxymethyl group are fused to C-5 and C-6 and linked to C-3′, respectively. In addition, the $^1H-^1H$ COSY cross-peaks between H-1/H-2, H-2/H-3, H-3/H-4, and H-5″/H-6″ (Figure 1) and the NOESY cross-peak between OMe-4″/H-5″ supported that the structure of hyperielliptone HD, which was determined as 3′-hydroxymethyl-2′-(3″-hydroxy-4″-methoxyphenyl)-5′,6′:5,6-(7-methoxyxanthone)-1′,4′-dioxane (**4**). The optical inactivity and *trans* relationship of the dioxane protons suggested that **4** is also a racemic mixture of 2′*R*, 3′*R* and 2′*S*, 3′*S* enantiomers.[18]

The structurally similar benzodioxan moiety present in dehydrosilybin was reported to racemize under basic conditions.[21] Compounds **3** and **4** were isolated as a racemic mixture. This indicated that the possibility of racemization in the experimental conditions employed for isolation of **3** and **4** may be impossible.

The activity of constituents isolated from the heartwood of this plant in the inhibition of DNA damage caused by free radicals ($O_2^{-\bullet}$ generated by xanthine/xanthine oxidase) was investigated by an agarose gel electrophoresis method. As shown in Figure S2 (Supporting Information), compound **2/2a** inhibited the DNA damage caused by free radicals. The radical scavenging activities of α-tocopherol (positive control) and **2/2a** were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent. Compound **2/2a** showed weak DPPH scavenging effect with a 4.5% inhibition at 300 μM. The positive control, α-tocopherol, showed DPPH scavenging activity with an IC_{50} value of 18.1 ± 1.5 μM. As shown in Figure 2, compound **2/2a** and allopurinol (positive control) inhibited xanthine oxidase in a concentration-dependent manner with IC_{50} values of 42.1 ± 5.8 and 2.0 ± 0.7 μM, respectively.

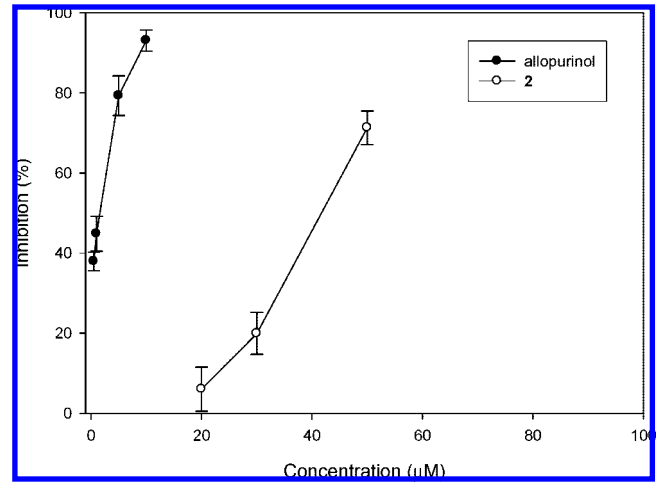

Figure 2. Dose-dependent inhibition of xanthine oxidase by **2/2a** and allopurinol. Data are presented as means ± SEM, n = 3−6.

Experimental Section

General Experimental Procedures. 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. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra and $^1H-^1H$-COSY, NOESY, HMQC, and HMBC experiments were recorded on a Varian Unity-400 NMR spectrometer. MS were obtained on a JMS-HX-100 mass spectrometer. Silica gel (Merck), particle size 15−40 μM, was used for column chromatography. Silica gel 60 F_{254} precoated aluminum sheets (0.2 mm, Merck) were employed for TLC. All solvents were HPLC grade. Ethidium bromide, bromophenol blue, Trizma, xanthine, xanthine oxidase, superoxide dismutase, α-tocopherol, allopurinol, and 1,1-

diphenyl-2-picrylhydrazyl were purchased from Sigma Chemicals. Tris-acetate-ethylenediamine-tetraacetic acid disodium salt was purchased from J. T. Baker; cupric chloride and glycerol were from Mallinckrodt, Inc. Supercoiled pBR322 plasmid DNA was purchased from Abgene House, Advanced Biotechnologies Ltd., Epsom, UK.

Plant Material. The heartwood of *H. geminiflorum* (9.8 kg) was collected at Ping Tung Hsieng, Taiwan, in October 2003. A voucher specimen (2003-5) has been deposited at the Department of Medicinal Chemistry, School of Pharmacy, Kaohsiung Medical University.

Extraction and Isolation. The air-dried heartwood of *H. geminiflorum* (9.8 kg) was extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated under reduced pressure to give a dark brown residue (40 g). This residue was fractionated by chromatography over silica gel (0.015−0.04 mm) and eluted with a gradient of *n*-hexane−EtOAc (4:1) to *n*-hexane−EtOAc (1:2) to yield four fractions. Fraction 1 was subjected to repeated chromatography on silica gel to give 1,3,8-trihydroxy-4-methoxyxanthone (7 mg), 1,3,8-trihydroxy-2-methoxyxanthone (9 mg), 1,7-dihydroxyxanthone (40 mg), 2,3-methylenedioxyxanthone (12 mg), 1,5-dihydroxy-6-methoxyxanthone (9 mg), 1-hydroxyxanthone (3 mg), and 2,3-dimethoxyxanthone (5 mg). Fraction 2 was subjected to repeated chromatography on silica gel (0.015−0.04 mm) to give **1** (6 mg), **2** (56 mg), **3** (2 mg), **4** (4 mg), cadensin D (20 mg), 1,8-dihydroxy-3-methoxyxanthone (6 mg), α-sitosteryl-3α-glucopyranoside-6′-*O*-palmitate (16 mg), 7α-hydroxystigmast-4-en-3-one (8 mg), and 2,6,2′,6′-tetramethoxy-4,4′-bis(2,3-epoxy-1-hydroxypropyl)biphenyl (5 mg). The known compounds were identified using spectroscopic methods and gave values consistent with data reported in the literature.[3,5–14]

Hyperielliptone HA (1/1a): colorless oil; $[\alpha]^{25}_D$ −6.2 (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ϵ) 214 (4.3), 228 (4.2), 278 (4.1) nm; IR (KBr) ν_{max} 3416, 1721, 1642 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m/z* 460 ($[M]^+$, 3), 442 ($[M - H_2O]^+$, 23), 427 ($[442 - Me]^+$, 13), 385 ($[442 - CH_3(CH_2)_2CHCH_3]^+$, 20), 357 ($[385 - CO]^+$, 3), 321 ($[357 - 2\ H_2O]^+$, 28), 179 (45), 121 (47); HRESIMS *m/z* 483.2726 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_6Na$ 483.2722).

Hyperielliptone HB (2/2a): colorless oil; $[\alpha]^{25}_D$ 1.0 (*c* 1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.2), 226 (4.1), 280 (4.3) nm; IR (KBr) ν_{max} 3417, 1714, 1673, 1589 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 483 ($[M + Na]^+$), 437, 381, 288; HRESIMS *m/z* 483.2720 $[M + Na]^+$ (calcd for $C_{27}H_{40}O_6Na$ 483.2722).

Hyperielliptone HC (3): white powder; $[\alpha]^{25}_D$ 0 (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ϵ) 210 (4.2), 248 (4.2), 317 (3.7) nm; IR (KBr) ν_{max} 3439, 1649, 1614, 1580 cm^{-1}; ^1H NMR (pyridine-d_5) δ 3.71 (3H, s, OMe-3), 3.83 (6H, s, OMe-3″ and OMe-5″), 3.99 (1H, dd, J = 12.7, 2.9 Hz, H-4′), 4.37 (1H, d, J = 12.7 Hz, H-4′), 4.57 (1H, m, H-3′), 5.62, (1H, d, J = 8.1 Hz, H-2′), 6.51 (1H, d, J = 2.0 Hz, H-4), 6.60 (1H, d, J = 2.0 Hz, H-2), 7.17 (1H, d, J = 8.8 Hz, H-7), 7.19 (2H, s, H-2″ and H-6″), 7.97 (1H, d, J = 8.8 Hz, H-8), 13.60 (1H, s, OH-1); ^{13}C NMR (pyridine-d_5) 180.6 (C-9), 166.8 (C-3), 164.0 (C-1), 158.1 (C-4a), 150.1 (C-6), 149.4 (C-3″), 149.4 (C-5″), 146.8 (C-10a), 138.7 (C-4″), 132.8 (C-5), 126.4 (C-1″), 117.6 (C-8), 115.4 (C-8a), 114.3 (C-7), 106.4 (C-2″), 106.4 (C-6″), 103.9 (C-9a), 97.9 (C-2), 93.1 (C-4), 79.9 (C-3′), 78.1 (C-2′), 61.1 (C-4′), 56.5 (OMe-3″), 56.5 (OMe-5″), 56.0 (OMe-3); ESIMS *m/z* 505 ($[M + Na]^+$), 437, 413, 381; HRESIMS *m/z* 459.1053 $[M + Na]^+$ (calcd for 459.1056).

Hyperielliptone HD (4): white powder; $[\alpha]^{25}_D$ 0 (*c* 0.1, acetone); UV (MeOH) λ_{max} (log ϵ) 210 (4.2), 235 (4.1), 312 (3.7) nm; IR (KBr) ν_{max} 3421, 1638, 1607 cm^{-1}; ^1H NMR (pyridine-d_5) δ3.68 (3H, s, OMe-4″), 3.83 (3H, s, OMe-7), 3.99 (1H, dd, J = 12.8, 2.9 Hz, H-4′), 4.38 (1H, d, J = 12.8 Hz, H-4′), 4.61 (1H, m, H-3′), 5.64, (1H, d, J = 8.4 Hz, H-2′), 7.31 (1H, d, J = 8.0 Hz, H-5″), 7.37 (1H, dd, J = 8.0, 3.2 Hz, H-6″), 7.37 (1H, td, J = 7.2, 2.0 Hz, H-2), 7.45 (1H, d, J = 3.2 Hz, H-2″), 7.50 (1H, d, J = 7.6 Hz, H-4), 7.67 (1H, s, H-8), 7.68 (1H, td, J = 7.2, 2.0 Hz, H-3), 8.61 (1H, dd, J = 7.2, 2.0 Hz, H-1); ^{13}C NMR (pyridine-d_5) 175.8 (C-9), 156.3 (C-4a), 149.2 (C-4″), 148.9 (C-3″), 146.9 (C-7), 142.3 (C-10a), 140.7 (C-6), 134.4 (C-3), 133.7 (C-5), 126.7 (C-1), 124.2 (C-2), 122.0 (C-1″), 121.8 (C-6″), 118.3 (C-4), 116.6 (C-5″), 115.2 (C-8a), 112.3 (C-9a), 112.3 (C-2″), 97.6 (C-8), 79.7 (C-3′), 77.6 (C-2′), 61.1 (C-4′), 55.8 (OMe-4″), 55.8 (OMe-7); ESIMS *m/z* 459 ($[M + Na]^+$), 437, 413, 381; HRESIMS *m/z* 459.1053 $[M + Na]^+$ (calcd for 459.1056).

Inhibition of Oxidative DNA Damage. A mixture of supercoiled plasmid pBR322 DNA (1 μg/μL) and xanthine (2 mM)/xanthine oxidase (0.7 U/mL) in 10 mM phosphate buffer (pH 7.4) was incubated for 20 min with 500 μM superoxide dismutase, quercetin, or **2/2a** in a total volume of 20 μL in a 1.5 mL microfuge tube at 37 °C, respectively. Quercetin was used as positive control. After incubating for 20 min, a 15 μL aliquot of mixture was loaded into 1.0% agarose gel containing ethidium bromide (0.05 μg/mL) in Tris-acetate-ethylenediaminetetraacetic acid (EDTA) buffer. The electrophoresis was carried out for 30 min at 100 V. Then the gels were illuminated with UV light and photographed. Plasmid DNA subjected to electrophoresis without superoxide dismutase, quercetin, or **2/2a** served as the control. The gel electrophoretic motility of the various forms of DNA was compared with the control.[22]

Free Radical Scavenging Activity. Radical scavenging activity of **2/2a** and α-tocopherol (standard) was determined using 1,1-diphenyl-2-picrylhydrazyl as a reagent[20] with modification by using 96-well plates. A 0.1 mM solution of 1,1-diphenyl-2-picrylhydrazyl radical in MeOH was prepared, and then 150 μL of this solution was mixed with 50 μL of sample solution. The mixture were incubated for 30 min in a dark room at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 490 nm using a MRX microplate reader (Dynex MRX II, Dynex Technologies, Chantility, VA). The percent 1,1-diphenyl-2-picrylhydrazyl scavenging effect was calculated using the following equation:

$$\text{1,1-diphenyl-2-picrylhydrazyl scavenging effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.[23]

Assay of Xanthine Oxidase Activity. The xanthine oxidase activity with xanthine as the substrate was measured at 25 °C, according to the protocol of Kong and associates[24] with modification. The assay mixture consisting of 50 μL of test solution, 60 μL of 70 mM phosphate buffer (pH 7.5), and 30 μL of enzyme solution [0.1 units/mL in 70 mM phosphate buffer (pH 7.5)] was prepared immediately before use. After preincubation at 25 °C for 15 min, the reaction was initiated by addition of 60 μL of substrate solution (150 μM xanthine in the same buffer). The reaction was monitored for 5 min at 295 nm. The xanthine oxidase activity was expressed as micromoles of uric acid per minute.

Statistical Analysis. Data were expressed as means ± SD. Statistical analysis were performed using the Bonferroni *t*-test method after ANOVA for multigroup comparison and the student's *t*-test method for two group comparison, with $p < 0.05$ considered to be statistically significant.

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Supporting Information Available: Selective NOESY correlations and relative configurations of **1** and **2**, figure of inhibitory effects of **2/2a** on DNA damage caused by free radicals, and 1D and 2D NMR spectra of **1/1a** and **2/2a**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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