Goniolactones A−F, Six New Styrylpyrone Derivatives from the Roots of *Goniothalamus cheliensis*

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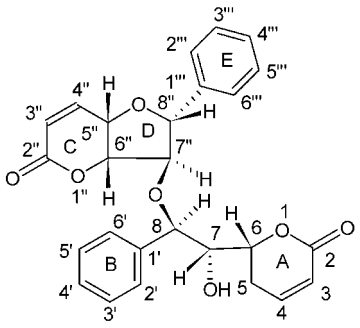
Six new styrylpyrone derivatives, goniolactones A−F (**1**−**6**), have been isolated from the roots of *Goniothalamus cheliensis*. The structures and stereochemistry of the new compounds were elucidated by interpretation of spectroscopic data and chemical methods. The relative configuration of goniolactone A (**1**) was determined by X-ray crystallography analysis, and the absolute configurations of goniolactones A (**1**) and B (**2**) were established by Mosher's method. Goniolactone B (**2**) exhibited significant cytotoxicity against A2780, HCT-8, and KB cells with IC_{50} values of 7.40, 4.43, and 7.23 μM, respectively.

The genus *Goniothalamus* (Annonaceace) consists of 50 species distributed in the tropics and subtropics, of which 10 are found in the People's Republic of China.[1] Several acetogenins, styrylpyrones, and alkaloids have been isolated from the plants in the genus, and their cytotoxic activity against a number of human cancer cell lines has been reported in previous literature.[2−4] *Goniothalamus cheliensis* Hu was chosen as the subject of the present investigation due to the significant cytotoxicity observed for the 95% EtOH extract against mouse lymphocytic leukemia cells in a preliminary biological screening procedure. Bioassay-guided fractionation of an EtOH extract of the roots of the title plant led to the isolation of six new compounds, goniolactones A−F (**1**−**6**). In the present paper, we describe the isolation and structure elucidation of these compounds as well as their cytotoxicity evaluation.



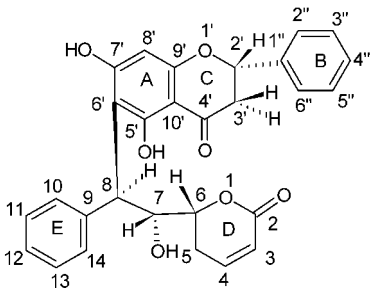
1

Results and Discussion

Goniolactone A (**1**) was obtained as needles. Its HREIMS gave a protonated molecular ion peak $[M + H]^+$ at *m/z* 449.1596, consistent with the molecular formula $C_{28}H_{24}O_7$ (calcd for $C_{28}H_{24}O_7$, 449.1600). The IR spectrum for **1** displayed absorption bands attributable to hydroxyl (3427 cm^{-1}), carbonyl (1736 and 1687 cm^{-1}), and benzene ring (1650 and 1590 cm^{-1}) absorptions. The ^1H NMR spectrum showed signals for 10 aromatic protons at δ 7.16−7.37, four olefinic protons at δ 6.00−7.00, seven oxygenated methine protons at δ 3.60−5.00, and a pair of methylene germinal protons at δ 2.20−2.90, which were very similar to those of goniothalenol and goniodiol.[2,5,6] This suggested that **1** included two fragments, namely, goniothalenol and goniodiol moieties. Further comparison of the ^{13}C NMR data of **1** with those of goniothalenol and goniodiol revealed that C-8 and C-7″ were shifted downfield by 5.5 and 5.1 ppm, respectively. This, in combination with the observed molecular formula, indicated that these two carbons were linked to each other through an ether bond. The HREIMS of **1** gave a base peak at *m/z* 97.0282 in accordance with the characteristic peak of α,β-unsaturated δ-lactone, and the strong fragment ion peak at *m/z* 321.1193 supported a cleavage between C-7 and C-8.

To confirm the structure and relative stereochemistry, **1** was subjected to a single-crystal X-ray diffraction analysis (Figure 1). Rings A and C adopt the twisted chair conformations, ring D adopts the envelope conformation, and rings C and D are fused in a *cis* relationship, while H-7 and H-7″ are *trans* to H-8 and H-6″, respectively. The dihedral angle between the planes of the two benzene rings is 42.65° in the solid state.

For determination of the absolute stereochemistry of **1**, (*S*)- and (*R*)-methoxyfluoromethylphenylacetic acid (MTPA) esters of **1** (**1r** and **1s**) were prepared. The ^1H NMR data (see Table 1) of **1r** and **1s** indicated the absolute configuration of C-7 to be *S*.[7] According to the above analysis, the absolute configuration of **1** was determined as 6*R*, 7*S*, 8*R*, 5″*R*, 6″*S*, 7″*S*, and 8″*R*, which is consistent with those of goniothalenol and goniodiol.[8,9]



2

Goniolactone B (**2**) was obtained as an amorphous powder. The HREIMS revealed a molecular ion peak at *m/z* 472.1519, corresponding to the molecular formula $C_{28}H_{24}O_7$. The absorption bands at 3419, 1697, 1633, and 1495 cm^{-1} in the IR spectrum indicated the presence of hydroxyl,

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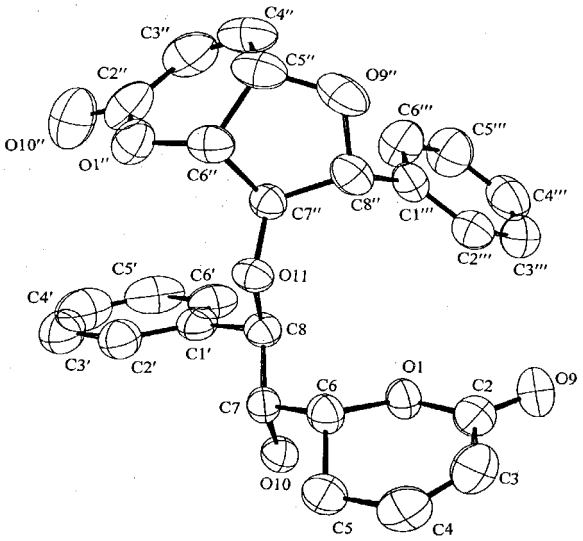


Figure 1. ORTEP diagram of goniolactone A (**1**).

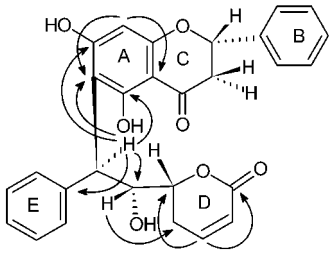


Figure 2. HMBC correlations for **2**.

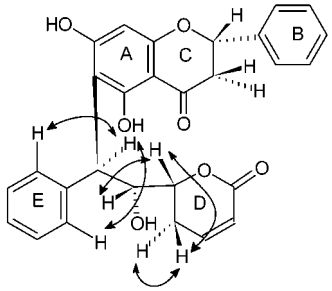
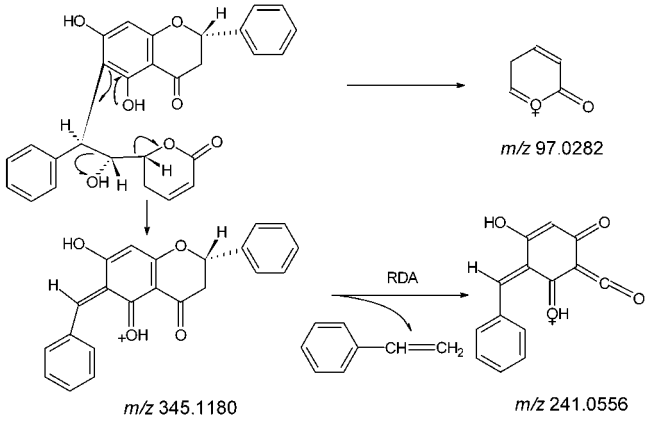


Figure 3. NOE correlations for **2**.

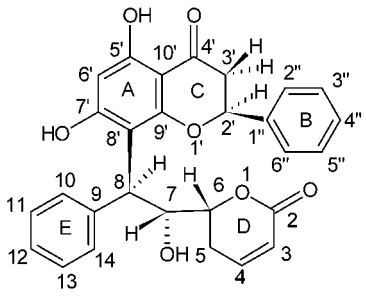
carbonyl, and benzene ring absorptions. The UV spectrum exhibited characteristic absorptions for a dihydroflavone unit at 294 and 221 nm.[10] The absorption band at 294 nm showed a slow bathochromic shift (+20 nm) with the addition of $AlCl_3$/HCl, after 4 h. This suggested that a free hydroxyl was substituted at the C-5′ position and that the *ortho*-position to the C-5′ hydroxyl had a functionality hindering chelation of $AlCl_3$ with the C-4′ carbonyl and the C-5′ hydroxyl.[10] The ^{1}H NMR spectrum of **2** showed signals for 11 aromatic protons, two olefinic protons, four methine protons, and four methylene protons. Moreover, the signals at δ 7.20−7.60 (5H, m) were assigned to the protons of the ring B of a dihydroflavone unit, with the signals at δ 5.51 (1H, dd, J = 13.3, 3.0 Hz), 2.74 (1H, dd, J = 17.0, 3.0 Hz), and 3.14 (1H, dd, J = 17.0, 13.3 Hz) consisting of an ABX system to H-2′, H-3′α, and H-3′β, and that at δ 6.03 (1H, s) to H-6′ or H-8′, by comparison of the ^{1}H NMR data of **2** with those of pinocembrin.[12] The signals for the remaining protons (see Table 2) were closely comparable to those of goniodiol.[2] The H-7 signal was shifted downfield 1.25 ppm and the H-8 signal upfield 0.40 ppm compared to the analogous signals for goniodiol. Comparison of the ^{13}C NMR data of **2** with those of pinocembrin and goniodiol further

Scheme 1. EIMS Fragmentation of Goniolactone B (**2**)



revealed some significant differences. Thus, **2** lacked the signals at δ 96.4 in pinocembrin and at δ 73.6 in goniodiol and had two additional signals, an aromatic carbon at δ 110.6 and a methine carbon not bearing oxygen at δ 44.7, which suggested that **2** contains fragments of both pinocembrin and 8-deoxygoniodiol in its molecule. The linkage of C-6′ of the pinocembrin moiety with C-8 of the 8-deoxygoniodiol moiety through a carbon−carbon bond was verified by a long-range correlation of the proton at δ 4.89 (H-8) with the carbon at δ 110.6 (C-6′) in the HMBC experiment. The HREIMS also supported the above linkage from the fragment ion at *m*/*z* 345.1180 by the cleavage between C-7 and C-8 and the daughter ion at *m*/*z* 241.0556 through *retro*-Diels−Alder (RDA) cleavage (see Scheme 1).

The relative stereochemistry of the 8-deoxygoniodiol moiety was determined from coupling constants and by the NOESY experiment. In the NOESY spectrum, the signal for the proton at δ 4.49 (H-6) correlating with the protons at δ 2.34 (H-5β) and 4.96 (H-7) indicated that the three protons were β-oriented. While the J value between H-7 and H-8 of 10.0 Hz approximated that of the goniodiol moiety in **1**, this suggested that H-7 was in a *trans* relationship with H-8. On the basis of the above discussion, the relative stereochemistry of this moiety was in accordance with that of goniodiol.[2] The absolute configuration of the chirality at C-7 in **2** was determined as *R* by the Mosher ester method (see Table 2). Accordingly, the chiral centers of C-6 and C-8 were assigned as *R* and *S*, respectively.[9,11] Assuming that the pinocembrin moiety in **2** is derived from (−)-pinocembrin ($[\alpha]_D^{24}$ −51° in EtOH) also isolated from this plant, the absolute configuration of C-2′ was tentatively assigned as *S*.[12]



3

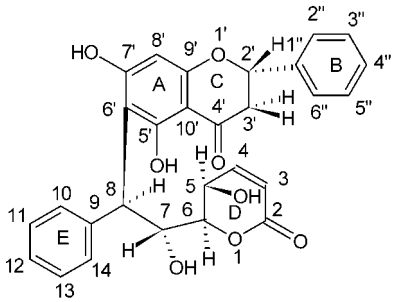
Goniolactone C (**3**) was isolated as an oil, and its molecular formula was assigned as $C_{28}H_{24}O_7$ on the basis of its HREIMS ([M]$^+$ *m*/*z* 472.1516), which indicated it was an isomer of **2**. A preliminary examination of the spectral data for **3** suggested this compound is very similar to **2** in

Table 1. ^{1}H and ^{13}C NMR Spectral Data for **1**, **1r**, and **1s** in $CDCl_3$[a]

position	**1** (δ_H)	**1** (δ_C)	**1s** (δ_H)	**1r** (δ_H)	$\Delta\delta = \delta$**1s** $-$ δ**1r**
2		163.4			
3	6.01 dd (9.7, 2.6)	120.9	5.92 dd (9.7, 2.6)	5.99 dd (9.7, 2.6)	−0.07
4	6.92 ddd (9.7, 5.0, 2.5)	145.6	6.83 m	6.86 m	−0.03
5α	2.82 m	25.9	2.16 m	2.28 m	−0.12
5*β*	2.21 m		2.08 m	2.24 m	−0.16
6	4.82 dd (13.2, 1.5)	75.8	4.85 dd (13.1, 1.5)	4.93 dd (13.1, 1.5)	−0.07
7	3.67 dd (8.8, 1.5)	74.6	5.55 dd (8.8, 1.5)	5.55 dd (8.8, 1.5)	0.00
8	4.58 d (8.8)	80.1	4.87 d (8.8)	4.83 d (8.8)	+0.04
1′		139.5			
2′(6′)	7.16 m	126.8	7.27 m	7.23 m	+0.04
3′(5′)	7.28 m	129.1	7.37 m	7.34 m	+0.03
4′	7.37 m	128.8	7.20 m	7.17 m	+0.03
2″		160.9			
3″	6.14 d (9.8)	124.0	6.14 d (9.7)	6.14 d (9.7)	0.00
4″	6.89 dd (9.8, 5.0)	139.5	6.89 dd (9.7, 5.0)	6.90 dd (9.7, 5.0)	−0.01
5″	4.47 t (5.5, 5.0)	68.6	4.41 t (5.5, 5.0)	4.43 t (5.5, 5.0)	−0.02
6″	4.70 dd (5.5, 2.0)	85.8	4.55 dd (5.5, 2.2)	4.58 dd (5.5, 2.2)	+0.03
7″	4.21 dd (5.0, 2.0)	89.2	4.21 dd (5.0, 2.2)	4.19 dd (5.0, 2.2)	+0.02
8″	4.86 d (5.0)	84.2	4.86 d (5.0)	4.84	+0.02
1‴		137.9			
2‴(6‴)	7.16 m	126.8	7.28 m	7.28 m	0.00
3‴(5‴)	7.25 m	128.6	7.32 m	7.32 m	0.00
4‴	7.35 m	128.3	7.15 m	7.15 m	0.00

[a] Chemical shift values are given in ppm, and *J* values in parentheses are given in Hz. Assignments were confirmed by ^{1}H−^{1}H COSY, HSQC, and HMBC experiments.

structure, with both compounds containing dihydroflavone and 8-deoxygonidiol moieties. In the UV spectrum of **3**, the absorption at 292 nm showed a rapid bathochromic shift (+28 nm) on the addition of $AlCl_3$ within 5 min, indicating that no modifying group was substituted *ortho* to the 5′-hydroxyl group. In the ^{13}C NMR spectrum, C-6′ and C-8′ were observed with a upfield shift (−12.5 ppm) and a downfield shift (+13.0 ppm), respectively. These differences revealed that an 8-deoxygonidiol moiety was linked at C-8′ of a pinocembrin unit. The HMBC experiment clearly exhibited the linkage between C-8′ and C-8 by a long-range correlation of the proton at δ 4.90 (H-8) with the carbon at δ 108.5 (C-8′). Since **3** is probably produced by the same biogenetic pathways as **2**, the absolute configuration of **3** was assigned as 6*R*, 7*R*, 8*S*, and 2′*S*.[2,9,11]



4

Goniolactone D (**4**) was obtained as needles. Its HREIMS gave a molecular ion peak at *m*/*z* 488.1470, in agreement with the molecular formula $C_{28}H_{24}O_8$. Similarities in the UV spectrum and variations in the absorption bands with diagnostic reagents (NaOAc and $AlCl_3$/HCl) between **2** and **4** suggested the presence of a 5′,7′-dihydroxyldihydroflavone segment in **4**[10] and a substituted modifying group located at C-6′. However, there were some significant differences between the two compounds, with **4** having one more oxygen atom than **2**, indicating the presence of an additional hydroxyl in the structure. In the ^{1}H NMR spectrum of **4**, the signals for H-5α at δ 2.34 (1H, m) and H-5*β* at δ 2.83 (1H, m) in **2** were not observed in the upfield region and the signal for the proton at δ 4.52 (1H, dd, J = 5.8, 3.0 Hz) appeared more downfield as compared to **2**, which is a characteristic signal of a styrylpyrone unit bearing oxygen at C-5.[9,12] By comparison of the ^{1}H NMR data of the styrylpyrone moiety of **4** with those of goniotriol, the signals at δ 5.97 (1H, d, J = 9.7 Hz), 6.99 (1H, dd, J = 9.7, 5.8 Hz), 4.35 (1H, t, J = 5.8, 3.0 Hz), 5.28 (1H, m), and 5.04 (1H, d, J = 8.0 Hz) were ascribed to H-3, H-4, H-6, H-7, and H-8, respectively. On the basis of analysis of its spectral data, **4** consisted of two fragments, a pinocembrin moiety and an 8-deoxygoniotriol moiety. A significant cross-peak in the HMBC experiment between H-8 (δ 5.04) and C-6′ (δ 108.6) confirmed the linkage between C-6′ and C-8. The HREIMS, which gave the same fragment ion peak at *m*/*z* 345.1167 and 241.0573 as **2**, also helped to confirm that the pinocembrin moiety was linked to the C-8 position of the 8-deoxygoniotriol unit through a carbon−carbon bond.

The relative stereochemistry of **4** was established by the inspection of NOE correlations and the observed coupling constants. The signals for H-5α and H-8 showed NOE enhancements when the proton at δ 4.35 (H-6) was irradiated, so H-5α, H-6, and H-8 were α-oriented. The coupling constant (8.0 Hz) between H-7 and H-8 approximated that of goniotriol, which indicated that H-7 was on the opposite side of the molecule of H-8 in **4**. This was also deduced from a NOE difference experiment which showed no enhancement of the signal for H-8 (δ 5.04) when the proton at δ 5.28 (H-7) was irradiated. Thus, the relative stereochemistry of this unit was in accordance with that of goniotriol.[13] On the basis of the absolute configuration of goniotriol[14] and from biogenetic considerations, the absolute configuration of **4** was assigned as 5*S*, 6*R*, 7*R*, 8*S*, and 2′*S*.

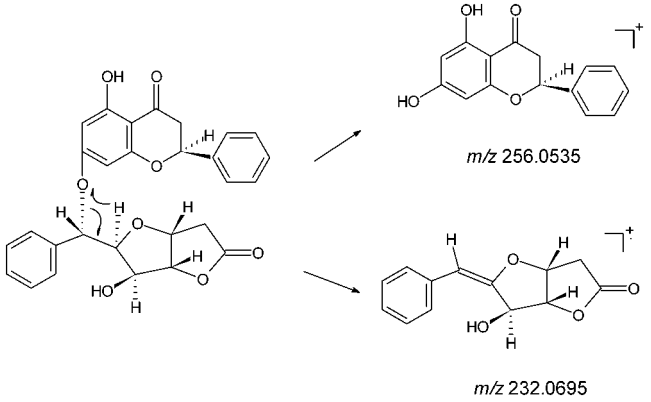
Goniolactone E (**5**) was isolated as a white powder. Its HREIMS gave a protonated molecular ion peak ($[M + H]^+$) at *m*/*z* 489.1548, consistent with the molecular formula $C_{28}H_{25}O_7$. The UV absorption band at 285 nm was shifted bathochromically 20 nm on the addition of $AlCl_3$, indicating the presence of a free C-5′-hydroxyl group in a dihydroflavone unit.[10] In the ^{1}H NMR spectrum, two doublets at δ 6.02 and 6.05 with a small coupling constant (J = 2.0 Hz) were characteristic of two *meta*-coupled H-6′ and H-8′ protons of ring A of a dihydroflavone unit, indicating a 5,7-

Table 2. ^{1}H and ^{13}C NMR Spectral Data for **2**, **2r**, and **2s** in $CDCl_3$[a]

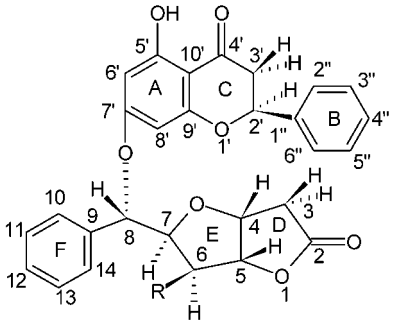
position	**2** (δ_H)	**2** (δ_C)	**2s** (δ_H)	**2r** (δ_H)	$\Delta\delta = \delta$**2s** $- \delta$**2r**
2		165.2			
3	5.82 dd (9.8, 2.0)	121.1	5.94 dd (9.5, 2.0)	5.97 dd (9.5, 2.0)	−0.03
4	7.01 ddd (9.8, 6.5, 1.5)	147.2	6.99.m	7.05.m	−0.06
5α	2. 83 m	27.2	2.23 m	2.45 m	−0.02
5*β*	2. 34 m		2.85 m	2.87 m	−0.02
6	4.49 ddd (12.5, 4.5, 1.5)	79.2	4.42 m	4.44 m	−0.02
7	4.96 d (10.0)	72.6	4.98 d (10.0)	4.90 d (10.0)	+0.08
8	4.89 d (10.0)	44.7	4.98 d (10.0)	4.90 d (10.0)	+0.08
9		144.1			
10(14)	7.37 m	129.4	7.25.m	7.17 m	+0.08
11(13)	7.58 m	129.9	7.41 m	7.39 m	+0.02
12	7.12 m	127.3	6.90 m	6.89 m	+0.01
2′	5.51 dd (13.3, 3.0)	79.9	5.36 dd (13.5, 3.0)	5.38 dd (13.5, 3.0)	−0.02
3′α	2.74 dd (17.0, 3.0)	43.6	2.84 dd (17.0, 3.0)	2.85 dd (17.0, 3.0)	−0.01
3′*β*	3.14 dd (17.0, 13.3)		3.13 dd (17.0, 13.3)	3.14 dd (17.0, 13.5)	−0.01
4′		197.1			
5′		162.8			
6′		110.6			
7′		164.0			
8′	6.03 s	95.5	6.50 s	6.39 s	−0.01
9′		162.3			
10′		103.1			
1″		140.0			
2″ (6″)	7.47m	126.3	7.42 m	7.42 m	0.00
3″ (5″)	7.61 m	128.5	7.44 m	7.44 m	0.00
4″	7.22 m	128.5	7.20 m	7.20 m	0.00
PhOH	12.72 s				

[a] Chemical shift values are given in ppm, and *J* values in parentheses are given in Hz. Assignments were confirmed by ^{1}H−^{1}H COSY, HSQC, and HMBC experiments.

Scheme 2. EIMS Fragmentation of Goniolactone E (**5**)



disubstituted pattern for ring A. The signals for three protons of an ABX system at δ 5.34 (1H, dd, J = 13.5, 3.0 Hz), 2.77 (1H, dd, J = 17.0, 3.0 Hz), and 3.03 (1H, dd, J = 17.0, 13.5 Hz) were assigned to H-2′, H-3′α, and H-3′*β* of ring C, respectively. The signals at δ 7.34−7.43 (10H, m) were ascribed to those of two monosubstituted phenyls, rings B and F.[6] There were remaining five oxygenated methine protons at δ 4.23−5.05 and two methylene protons at δ 2.59 (1H, d, J = 19.0 Hz) and 2.68 (1H, d, J = 19.0, 5.0 Hz), which were similar to those of goniofufurone or 8-*epi*-goniofufurone.[2] By comparison of the ^{13}C NMR data with those of pinocembrin and goniofufurone, **5** exhibited data similar to both these compounds, but C-8 was shifted downfield about 4.40 ppm, which suggested that a pinocembrin moiety was linked to the C-8 position of a goniofufurone moiety in **5**. The HREIMS of **5** confirmed the presence of the two fragments from the fragment ion peaks at *m*/*z* 256.0535 (calcd for $C_{15}H_{12}O_4$, 256.0702) and 232.0695 (calcd for $C_{13}H_{12}O_4$, 232.0736) (see Scheme 2). A linkage from the C-7 position of pincembrin to the C-8 position of goniofufurone through an ether bond was also supported by the HMBC correlation of the proton at δ 5.46 (H-8) with the carbon at δ 165.2 (C-7′). The relative stereochemistry of **5** was inferred from the NOESY spectrum and the coupling constant values. A significant cross-peak between H-6 and H-7 in the NOESY spectrum indicated that the two protons were located at the same side of ring E, and a cross-peak of H-4 with H-5 revealed that rings D and E were fused in a *cis* form. In the ^{1}H NMR spectrum, coupling between H-5 and H-6 was not observed, suggesting the two protons possess a dihedral angle close to 90°.[2] The $J_{7/8}$ value of 8.5 Hz was close to the value observed for 8-acetyl-*epi*-goniofufurone ($J_{7/8}$ = 9.5 Hz), but was different from that of goniofufurone ($J_{7/8}$ = 4.8 Hz).[2,15] This indicated that H-7 was *trans* to H-8. Finally, from a biogenetic viewpoint, the chiral centers of the 8-*epi*-goniofufurone moiety in **5** should have the same absolute configuration as 8-acetyl-*epi*-goniofufurone[15,16] and so were assigned in **5** as 4*R*, 5*S*, 6*R*, 7*R*, 8*S*, and 2′*S*.



5 R= H

6 R= $COCH_3$

Goniolactone F (**6**) was obtained as a white powder. The molecular formula was determined to be $C_{30}H_{26}O_9$ by HREIMS (*m*/*z* 530.1577, $[M]^+$). The similarity between **5** and **6** in their UV spectra indicated that both compounds have the same chromophore. Comparison of their ^{1}H and ^{13}C NMR data revealed that an acetoxyl group was present

in **6** in place of a hydroxyl at C-6 in **5**. In **6**, the resonance for the protons at δ 1.93 (3H, s) and the carbons at δ 168.9 and 20.5 showed the presence of an acetyl group, and H-6 was shifted downfield by 1.09 ppm. The ^{1}H NMR data and the TLC R_f value of the hydrolysate of **6** were identical to **5** (see Experimental Section), which provided further support as **6** being an acetyl derivative of **5**. Thus, **6** has the same stereochemistry as **5**, namely, 4*R*, 5*S*, 6*R*, 7*R*, 8*S*, and 2′*S*.[15,16]

The results of the present investigation suggest that goniolactone-type compounds are comprised of two structural moieties: either a dihydroflavone unit and a styrylpyrone unit or two styrylpyrone units. The dihydroflavone moieties showed fewer variations in structure and had three linkage positions (C-6′, C-7′, and C-8′). In contrast, the styrylpyrone units exhibited a greater variation in structure (8-*epi*-goniofufrone, goniodiol, 8-deoxygoniodiol, and 8-deoxygoniotiol), but only C-8 was found as the linkage position. On the basis of biogenetic considerations, it may be assumed that these types of compounds are derived from dihydroflavone or styrylpyrone condensing with styrylpyrone and are linked through a carbon−carbon bond or an ether bond formed via dehydration in enzymatic processes. Furthermore, the configurations of most chiral centers in these compounds remain constant during condensation or dehydration.

Goniolactone B (**2**) exhibited significant inhibitory activities toward A2780, HCT-8, and KB cells with IC_{50} values of 7.40, 4.43, and 7.23 μM, respectively. Goniolactones A (**1**), D (**4**), and F (**6**) showed no significant inhibitory activities toward the tested cell lines, while goniolactones C (**3**) and E (**5**) were not evaluated biologically due to insufficient supplies of these compounds.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 683 FT infrared spectrometer. UV spectra were obtained on a Shimadzu UV-240 instrument. NMR spectra were run on a Varian INOVA-500 NMR spectrometer with TMS as internal standard. EIMS were obtained on a VG ZAB-2F mass spectrometer, and HREIMS were performed on an Autospec-Utima ETOF Spec mass spectrometer. X-ray diffraction intensity data for **1** were collected on a MAC DIP-2030K diffractometer with graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) by the $\omega-2\theta$ scan technique ($2\theta = 0-50°$) and were corrected by Lorentz and polarization. Altogether 1966 reflections were collected, of which 1950 with $|F|^2 \geq 8\sigma|F|^2$ were observed. The structure was solved by direct methods (SHELXS)[17] and refined by a block-matrix least-squares procedure to $R = 0.051$ and $R_w = 0.048$ [$w = 1/\sigma(F)^2$]. Hydrogen positions were found from difference Fourier maps and generated in calculated positions.[18]

Plant Material. The roots of *G. cheliensis* Hu were collected in Jinghong County, Yunnan Province, People's Republic of China, in July 1996 and identified by Prof. Shao-Rong Guo. A voucher specimen (No. 00055) is deposited in the herbarium of the Institute of Medical Plants, Chinese Academy of Medical Sciences.

Extraction and Isolation. Dried roots (20.0 kg) were ground into a crude powder and extracted with 95% ethanol to afford 1.3 kg of a residue on removal of solvent under reduced pressure. The ethanol extract was partitioned between water and chloroform, giving a water-soluble fraction I (290 g) and a chloroform-soluble fraction II (530 g) as well as an insoluble fraction III (460 g). Fraction II was first dissolved in 90% methanol and then defatted with petroleum ether to give a methanol-soluble fraction IV (316 g). Fraction IV was repeatedly subjected to Si gel column chromatography and eluted with a gradient of petroleum ether/ethyl acetate, yielding 50 mg of **1** (80:20), 26 mg of **6** (83:17), 6 mg of **5** (75:25), 10 mg of **3** (72:28), 58 mg of **2** (68:32), and 42 mg of **4** (55:45).

Goniolactone A (1): white needles; mp 166−168 °C; $[\alpha]_D^{20}$ +83.6° (*c* 0.26, EtOH); IR (KBr) ν_{max} 3427 (OH), 2927, 1736 (C=O), 1687 (C=O), 1650, 1590, 1394, 1263, 1107, 1039, 819, 700 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 273 (3.14), 228 (3.93), 206 (4.62) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 449 ([M + H]$^+$, 3), 431 (0.9), 321 (90), 215 (22), 119 (51), 107 (28), 105 (35), 97 (80), 77 (18), 69 (18); HREIMS *m/z* 449.1596 ([M + H]$^+$, calcd for $C_{26}H_{25}O_7$ 449.1600), 321.1193 (calcd for $C_{20}H_{17}O_4$, 321.1126), 215.0346 (calcd for $C_{12}H_7O_4$, 215.0344), 119.0640 (calcd for $C_5H_{11}O_3$, 119.0708), 97.0282 (calcd for $C_5H_3O_2$, 97.0289).

Crystal Data for 1: $C_{26}H_{24}O_7$, $M_r = 448.47$, monoclinic, space group $P2_1$, $a = 9.2600(4)$ Å, $b = 9.280(2)$ Å, $c = 13.2200(2)$ Å, $\beta = 99.308°$, $V = 1121.07$ Å^3, $Z = 2$, $D_{calc} = 1.332$ g/cm^3; μ(Mo Kα) = 1.08 cm^{-1}, $F(000) = 483$, crystal dimensions 0.2 × 0.2 × 0.7 mm, $T_{calc} = 293 \pm 1$ K.

Preparation of (*R*)- and (*S*)-MTPA Esters of 1. To a solution of **1** (8 mg) in dry CH_2Cl_2 (4 mL) were added DCC (30 mg), (*R*)-MTPA (30 mg), and DMPA (1 mg), and the mixture was stirred overnight at room temperature. The reaction mixture was purified by preparative TLC with petroleum ether/ethyl acetate (7:3) to give (*R*)-MTPA ester **1r** (5.0 mg). By the same procedure, the (*S*)-MTPA ester **1s** (3.8 mg) was prepared. ^{1}H NMR data of **1r** and **1s**, see Table 2.

Goniolactone B (2): amorphous powder; mp 176−178 °C; $[\alpha]_D^{20}$ +33.7° (*c* 0.92, EtOH); IR (KBr) ν_{max} 3419 (OH), 3062, 3032, 1697 (C=O), 1633, 1495, 1450, 1381, 1338, 1317, 1302, 1259, 1184, 1151, 1088, 1032, 818, 764, 700 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 330 (3.84), 294 (3.81), 239 (4.40), 211 (4.63) nm; +NaOAc, 330, 250, 220 nm; +$AlCl_3$/HCl (>4 h), 385, 314, 280, 220 nm; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m/z* 472 ([M]$^+$, 58), 455 (23), 454 (20), 395 (10), 369 (15), 345 (100), 265 (15), 241 (80), 131 (22), 103 (5); HREIMS *m/z* 472.1519 ([M]$^+$, calcd for $C_{28}H_{24}O_7$, 472.1522), 345.1180 (calcd for $C_{22}H_{17}O_4$, 345.1127), 241.0556 (calcd for $C_{14}H_9O_4$, 241.0501), 131.0552 (calcd for C_9H_7O, 131.0497).

Preparation of (*R*)- and (*S*)-MTPA Esters of 2. The preparation procedure for the (*R*)- and (*S*)-MTPA esters **2r** and **2s** was analogous to that described for **1r** and **1s**. ^{1}H NMR spectral data of **2r** and **2s**, see Table 2.

Goniolactone C (3): colorless oil; $[\alpha]_D^{20}$ −53.9° (*c* 0.71, EtOH); IR (KBr) ν_{max} 3514 (OH), 2927, 1718 (C=O), 1703 (C=O), 1637, 1496, 1437, 1397, 1257, 1076, 818, 700 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 335 (3.74), 292 (3.92), 230 (4.42), 206 (4.59) nm; +NaOAc, 340, 250, 210 nm; +$AlCl_3$/HCl, 400, 320, 230, 206 nm; ^{1}H and ^{13}C NMR data, see Table 3 and 4; EIMS *m/z* 472 ([M]$^+$, 48), 454 (5), 345 (100), 241 (98), 131 (10), 103 (16), 91 (4), 77 (1), 69 (2); HREIMS *m/z* 472.1516 ([M]$^+$, calcd for $C_{28}H_{24}O_7$, 472.1522), 345.1157 (calcd for $C_{22}H_{17}O_4$, 345.1127), 241.0482 (calcd for $C_{14}H_9O_4$, 241.0501).

Goniolactone D (4): white needles; mp 158−160 °C; $[\alpha]_D^{20}$ +17.6° (*c* 0.42, EtOH); IR (KBr) ν_{max} 3419 (OH), 2925, 1711 (C=O), 1633, 1495, 1452, 1340, 1302, 1259, 1153, 1107, 818, 700 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 330 (3.54), 292 (4.22), 233 (4.36), 205 (4.57) nm; +NaOAc, 340, 252, 210 nm; +$AlCl_3$/HCl (>4 h), 380, 312, 233, 206 nm; ^{1}H and ^{13}C NMR data, see Table 3; EIMS *m/z* 488 ([M]$^+$, 3), 470 (19), 345 (100), 295 (2), 265 (11), 241 (51), 131 (29), 103 (12), 91 (4), 77 (5), 69 (16); HREIMS *m/z* 488.1470 ([M]$^+$, calcd for $C_{28}H_{24}O_8$, 488.1471), 470.1499 (calcd for $C_{28}H_{22}O_7$, 470.1366), 345.1167 (calcd for $C_{22}H_{17}O_4$, 345.1127), 241.0573 (calcd for $C_{14}H_9O_4$, 241.0501), 104.0593 (calcd for C_8H_8, 104.0626).

Goniolactone E (5): white powder; mp 238−240 °C; $[\alpha]_D^{20}$ 0° (*c* 0.10, EtOH); IR (KBr) ν_{max} 3375 (OH), 3064, 2875, 1778 (C=O), 1641, 1608, 1572, 1493, 1454, 1302, 1163, 1068, 1047, 702 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 330 (2.85), 285 (3.50), 225 (3.53), 210 (3.75) nm; +NaOAc, 350, 285, 225, 210 nm; +$AlCl_3$/HCl, 380, 307, 230, 210 nm; ^{1}H and ^{13}C NMR data, see Table 4; EIMS *m/z* 488 ([M]$^+$, 1), 470 (3, M − H_2O), 446 (4), 345 (10), 256 (38), 232 (30), 179 (40), 152 (14), 103 (20), 91 (70), 83 (10),

Table 3. ^{1}H and ^{13}C NMR Spectral Data for **3** and **4**[a]

position	**3** (δ_H)[b]	**4** (δ_H)[c]	**3** (δ_C)[b]	**4** (δ_C)[c]
2			164.0	165.3
3	5.97 dd (9.8, 2.5)	5.97 d (9.7)	120.9	123.0
4	6.89 ddd (9.8, 6.8, 1.5)	6.99 dd (9.7, 5.8)	145.7	145.4
5α	2.71 m	4.52 dd (5.8, 3.0)	26.3	79.5
5β	2. 17 m			
6	4.47 ddd (11.8, 3.2, 1.5)	4. 35 t (5.1, 3.0)	78.4	79.4
7	4.65 dd (8.2, 3.2)	5.28 dd(8.0, 5.1)	77.3	73.2
8	4.90 d (8.2)	5.04 d (8.0)	43.5	43.7
9			140.0	143.2
10(14)			126.9	127.3
11(13)			128.0	130.2
12			127.0	127.3
2′	5.34 dd (13.3, 2.9)	5.52 dd (13.3, 3.0)	79.6	79.8
3′α	3.04 dd (17.2, 13.3)	2.77 dd (17.1, 3.0)	43.5	43.6
3′β	2.75 dd (17.2, 2.9)	3.15 dd (17.1, 13.3)		
4′			195.8	197.1
5′			162.8	162.8
6′	6.10 s		98.1	108.6
7′		6.02 s	164.0	164.7
8′			108.5	96.5
9′			159.9	162.5
10′			102.9	102.6
1″			138.4	140.1
2″ (6″)	7.38.m	7.45 m	126.2	126.7
3″ (5″)	7.49 m	7.62 m	129.0	129.6
4″	7.18 m	7.24 m	129.0	129.6
PhOH	12.08 s	12.94 s		

[a] Chemical shift values are given in ppm, and *J* values in parentheses are given in Hz. Assignments were confirmed by ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] ^{1}H and ^{13}C NMR spectra were measured in CD_3COCD_3. [c] ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$.

Table 4. ^{1}H and ^{13}C NMR Spectral Data for **5** and **6** in $CDCl_3$[a]

position	**5** (δ_H)	**6** (δ_H)	**5** (δ_C)	**6** (δ_C)
2			175.0	174.3
3α	2.59 d (19.0)	2.58 d (19.0)	35.9	35.7
3β	2.68 dd (19.0, 5.0)	2.68 dd (19.0, 5.0)		
4	5.05 t (5.0)	5.00 t (5.0)	78.0	77.5
5	4.91 d (5.0)	4.91 d (5.0)	87.2	85.1
6	4.64 d (3.0)	5.73 d (3.5)	73.9	74.7
7	4.23 dd (8.5, 3.0)	4.42 dd (8.5, 3.5)	83.3	82.1
8	5.46 d (8.5)	5.24 d (8.5)	76.7	76.7
9			138.7	137.2
10(14)			126.2	126.7
11(13)			128.9	128.9
12			128.9	128.9
2′	5.34 dd (13.5, 3.0)	5.35 dd (13.5, 3.0)	79.2	79.1
3′α	2.77 dd (17.0, 3.0)	2.78 dd (17.0, 3.0)	43.4	43.3
3′β	3.03 dd (17.0, 13.5)	3.04 dd (17.0, 13.5)		
4′			195.9	195.8
5′			163.0	163.9
6′	6.05 d (2.0)	5.98 d (2.0)	97.1	96.4
7′			165.2	165.2
8′	6.02 d (2.0)	5.97 d (2.0)	95.5	95.6
9′			162.8	162.8
10′			103.6	103.4
1″			137.0	138.1
2″ (6″)	7.37 m	7.35 m	126.1	126.1
3″ (5″)	7.43 m	7.41 m	128.9	128.9
4″	7.34 m	7.33 m	128.9	128.9
OAc		1.93 s		168.9, 20.5
PhOH	11.85 s	11.91 s		

[a] Chemical shift values are given in ppm, and *J* values in parentheses are given in Hz. Assignments were confirmed by ^{1}H−^{1}H COSY, HSQC, and HMBC experiments.

69 (22); HREIMS *m/z* 489.1548 ([M + H]$^+$, calcd for $C_{28}H_{25}O_8$ 489.1549), 256.0535 (calcd for $C_{15}H_{12}O_4$, 256.0702), 232.0695 (calcd for $C_{13}H_{12}O_4$, 232.0736), 179.0297 (calcd for $C_9H_6O_4$, 179.0299), 91.0481 (calcd for C_7H_7, 91.0547).

Goniolactone F (6): white powder; mp 257−258 °C; $[\alpha]_D^{20}$ +17.6° (*c* 0.21, EtOH); IR (KBr) ν_{max} 3437 (OH), 1784 (C=O), 1739 (C=O), 1645, 1574, 1508, 1456, 1227, 1188, 1066, 702 cm^{-1}; UV (EtOH) λ_{max} (log ϵ) 330 (2.81), 285 (3.42), 226 (3.49), 210 (3.68) nm; +NaOAc, 350, 285, 226, 210 nm; +$AlCl_3$/HCl, 380, 309, 230, 210 nm; ^{1}H and ^{13}C NMR data, see Table 4; EIMS *m/z* 530 ([M]$^+$, 1), 470 (3), 452 (26), 256 (68), 232 (100),-179 (53),152 (45), 131 (43), 103 (34), 91 (77); HREIMS *m/z* 530.1591 ([M]$^+$, calcd for $C_{30}H_{26}O_9$, 530.1577), 345.1073 (calcd for $C_{22}H_{17}O_4$, 345.1127), 274.0823 (calcd for $C_{15}H_{24}O_5$, 274.0841), 233.0799 (calcd for $C_{13}H_{13}O_4$, 233.0813), 173.0714 (calcd for $C_8H_{13}O_4$, 173.0813), 91.0469 (calcd for C_7H_7, 91.0547).

Hydrolysis of 6. Compound **6** (8.0 mg) was dissolved in 5 mL of THF and 1 mL of 1% NaOH was added, with the mixture stirred at room temperature for 30 min and then neutralized with 1% HCl to pH 7.0. The reaction mixture was purified by preparative TLC with petroleum ether/ethyl acetate (8:2) to afford **6a** (4.0 mg), showing the same R_f value (0.52, petroleum ether/ethyl acetate, 1:1) as **5**. ^{1}H NMR of **6a** ($CDCl_3$, 500 MHz) δ 2.59 (H-3α, d, J = 19.0 Hz), 2.69 (H-3β, dd, J = 19.0, 5.0 Hz), 5.05 (H-4, t, J = 5.0 Hz), 4.92 (H-5, d, J = 5.0 Hz), 4.64 (H-6, d, J = 3.0 Hz), 4.22 (H-7, dd, J = 8.5, 3.0 Hz), 5.44 (H-8, d, J = 8.5 Hz), 5.33 (H-2′, dd, J = 13.5, 3.0 Hz), 2.76 (H-3α′, dd, J = 17.0, 3.0 Hz), 3.04 (H-3β′, dd, J = 17.0, 13.5 Hz), 6.05 (H-6′, d, J = 2.0 Hz), 6.02 (H-8′, d, J = 2.0 Hz), 7.34−7.43 (10 ArH, m).

Bioassays. Cytotoxicity against human cancer cell lines for **1**, **2**, **4**, and **6** was measured in a five-day MTT test at the Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, using A2780 human epithelial carcinoma cells, HCT-8 human ileocecal carcinoma cells, and KB human epidermoid cancer cells.[19,20] Cells were seeded in 96-well plates (1000 cells/well per 0.2 mL of growth medium 1640 containing 10% NCS (newborn calf serum), and fresh medium with or without compounds was added 24 h later. After culturing for 5 days, 0.2 mg/mL of MTT (0.2 mg/mL in medium) was then aspirated. The cells were dissolved in 0.2 mL of DMSO, and the absorbance at 540 nm was measured in a microplate reader.

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(18) Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, No. 170256, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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