Novel Bioactive Styryl-lactones: Goniofufurone, Goniopypyrone, and 8-Acetylgoniotriol from *Goniothalamus giganteus* (Annonaceae). *X*-Ray Molecular Structure of Goniofufurone and of Goniopypyrone

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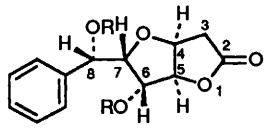
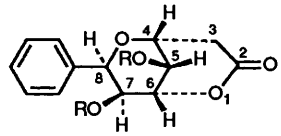
By use of brine shrimp lethality for activity-directed fractionation, three novel styryl-lactones which are cytotoxic to human tumour cells, goniofufurone, goniopypyrone, and 8-acetylgoniotriol, have been isolated from ethanolic extracts of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae). The structures were elucidated by IR, mass, ^{1}H NMR, ^{13}C NMR, and 2D NMR (^{1}H–^{1}H COSY, and ^{1}H–^{13}C HETCOR) spectra. The structures of goniofufurone and goniopypyrone, which represent unusual natural skeletons, were confirmed by X-ray crystallographic analysis, to help establish the relative configurations. Goniopypyrone was the most bioactive, showing nonselective ED_{50} values of $\sim 6.7 \times 10^{-1}$ μg/ml in each of our three human tumour cell lines.

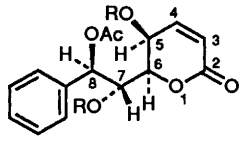
The ethanol extract of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae), obtained from Thailand, showed significant murine toxicity in the 3PS lymphocytic leukaemia system.[1] Our previous bioactivity-directed studies of this material have yielded the bioactive compounds altholactone (syn: goniothalenol, a furano-2-pyrone),[2] goniothalamin (a styrylpyrone),[2] pinocembrin (5,7-dihydroxyflavone),[2] goniotriol (a 5,6-dihydroxystyryl-2-pyrone),[3] and goniothalamicin, annonacin, and gigantecin (three acetogenins).[4,5] A number of 5,6-dihydro-2-pyrones[6] and alkaloids[7] were previously reported from other species of the genus *Goniothalamus*. During our continuing investigation of bioactive constituents from the stem bark of *G. giganteus*, three novel cytotoxic styryl-lactones, goniofufurone (**1**), goniopypyrone (**2**), and 8-acetylgoniotriol (**3**), were isolated, and their structures were determined by IR, mass, ^{1}H NMR, ^{13}C NMR, and 2D NMR (^{1}H–^{1}H COSY and ^{1}H–^{13}C HETCOR) spectra. The structure of compounds (**1**) and (**2**), which are new natural skeletons, was confirmed by X-ray crystallography, and the relative configurations were established. All of the separation steps were monitored with a convenient bioassay, brine shrimp lethality,[8] and by TLC.



(**1**) R = H
(**4**) R = Ac



(**2**) R = H
(**5**) R = Ac



(**3**) R = H
(**6**) R = Ac

Results and Discussion

The CIMS (isobutane) of goniofufurone (**1**) gave a prominent peak at *m/z* 251 (MH^+), and the EIMS of compound (**1**) showed a small peak at *m/z* 250 (M^+). The CIMS gave peaks at *m/z* 233 ($MH^+ - H_2O$), and 215 ($MH^+ - 2H_2O$), indicating the presence of two hydroxy group moieties. Both the isobutane and methane CIMS of the diacetyl derivative (**4**) gave prominent peaks at *m/z* 335 (MH^+), 275 (MH^+ – HOAc), and 215 (MH^+ – 2HOAc), and the ^{1}H NMR spectrum of compound (**4**) showed two peaks, at δ 2.15 (3 H, OAc) and 2.00 (3 H, OAc), which also indicated the presence of two hydroxy groups. Thus, a molecular weight of 250 and the presence of two hydroxy groups were confirmed in the structural analysis of goniofufurone (**1**).

The IR spectrum of goniofufurone (**1**) presented a hydroxy band at 3 401 cm^{-1} and the carbonyl peak of a saturated γ-lactone at 1 755 cm^{-1}. The ^{13}C NMR spectrum showed the presence of 13 carbons (Table 1), and a small downfield peak at δ_C 176.07 was assigned to the carbonyl carbon; four peaks, at δ_C 143.62 (C-1′), 128.81 (C-3′, -5′), 128.14 (C-4′), and 127.71 (C-2′, -6′),[2] indicated a monosubstituted phenyl group; also, five carbons linked to oxygen, and one methylene carbon, were observed and were confirmed in the ^{1}H–^{13}C HETCOR spectrum. The ^{1}H NMR spectrum of compound (**1**) (Table 2)

Table 1. ^{13}C NMR Chemical shifts and assignments for compounds (**1**)–(**3**).

Carbon	(1)	(2)	(3)
C-2	176.07	168.11	163.49
C-3	36.52	35.29	123.01
C-4	78.07	71.49	145.08
C-5	88.57	64.83	74.71
C-6	74.80	73.96	78.34
C-7	85.03	70.39	63.20
C-8	72.30	71.09	73.67
C-1′	143.62	139.11	139.22
C-2′, 6′	127.71	127.33	128.78
C-3′, 5′	128.81	128.24	129.02
C-4′	128.14	127.69	131.34
C=O (Ac)			169.69
CH_3 (Ac)			21.01

Table 2. ^{1}H NMR data [δ (*J*/Hz); 500 MHz] of compounds (**1**)–(**3**).

Proton	(1) ($CDCl_3$)	(2) [$(CD_3)_2CO$]	(3) [$(CD_3)_2CO$ + D_2O]
3-H_a	2.74 dd (18.9, 5.9)	3.16 dd (19.4, 5.3)	6.00 d (9.7)
3-H_b	2.66 br d (18.9, 1.0)	2.98 dd (19.4, 1.5)	
4-H	5.10 m (5.9, 4.2, 1.0)	4.42 m (5.3, 2.4, 1.5)	7.01 dd (9.7, 5.7)
5-H	4.85 br d (4.2, ~0.4)	4.21 m ($w_{\frac{1}{2}}$ 8.4)	4.57 dd (5.7, 2.9)
6-H	4.38 br t (2.7, ~0.4)	4.65 dt (3.9, 2.4)	4.49 dd (3.5, 2.9)
7-H	4.08 dd (4.8, 2.7)	4.02 br s ($w_{\frac{1}{2}}$ 7.8)	4.45 dd (7.5, 3.5)
8-H	5.19 dd (4.8, 2.8)	4.97 d (1.2)	5.86 d (7.5)
5-OH		4.76 d (8.0)	
6-OH	4.15 d (2.7)		
7-OH		5.22 d (7.4)	
8-OH	2.78 d (2.8)		
Ph	7.32–7.43 m	7.26–7.46 m	7.29–7.48 m
Ac			2.85 s

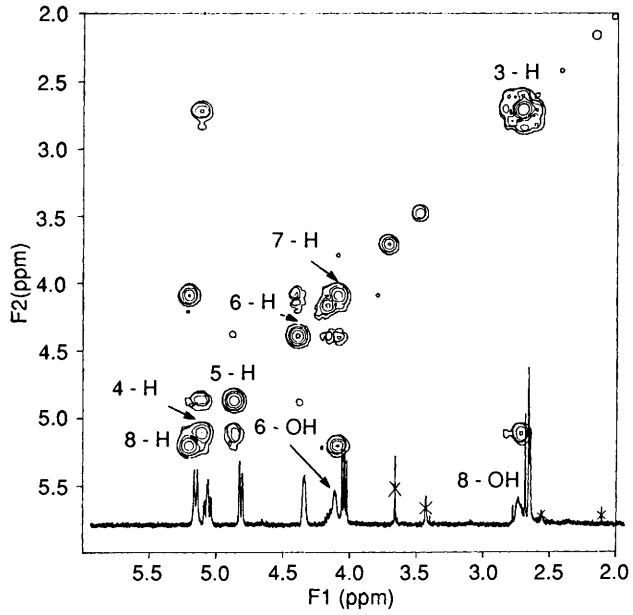
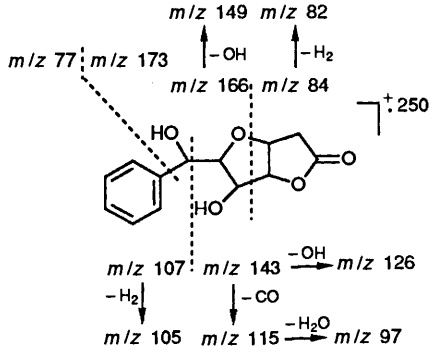


Figure 1. ^{1}H–^{1}H COSY spectrum of goniofufurone (**1**).

showed the presence of five phenyl protons at δ 7.32–7.43 (5 H), five oxygen-linked methine protons at δ 5.19–4.08, two hydroxy protons, at δ 4.15 and 2.78 (exchangeable with D_2O), and two methylene protons, at δ 2.74 and 2.66, for a total of 14 protons in compound (**1**). The γ-lactone carbonyl and two hydroxy groups had been previously determined, indicating that there were, at least, four oxygens. The existence of a fifth oxygen was suspected because there were five oxygen-linked carbons. The above analysis of the spectral data suggested the molecular formula $C_{13}H_{14}O_5$, which exactly matched with the molecular weight of 250 as determined by mass spectrometry.

The ^{1}H NMR spectrum of diacetylgoniofufurone (**4**) showed that upon acetylation the chemical shifts of 6-H and 8-H† obviously moved downfield by 1.37 and 0.66 ppm, respectively; therefore, the two hydroxy groups were linked to C-6 and C-8. The coupling constants of 7-H and 8-H also showed a significant change after acetylation of goniofufurone, increasing from $J_{7/8}$ 4.8 Hz to $J_{7/8}$ 9.1 Hz; this indicated that C-8 was out of the perhydrofurofuranone ring system, and the dihedral angle between 7-H and 8-H could easily change when a hydroxy group at C-8 was replaced by an acetoxy group which increased steric hindrance. The ^{1}H NMR system (Table 2) and ^{1}H–^{1}H COSY spectrum (Figure 1) of compound (**1**) showed that the two methylene protons (3-H_a and 3-H_b) and 4-H formed an ABX system; examination of the molecular model and the coupling constant $J_{4/5}$ 4.2 Hz indicated that 4-H and 5-H had to be in the *cis* configuration;[9–13] the dihedral angle between 4-H and 5-H is close to 180°, which suggests that the $J_{4/5}$-value should be greater, and there would be considerable ring strain in the *trans* configuration. The observed $J_{5/6}$-value was smaller than 0.4 Hz, which indicated that 5-H and 6-H should be in the *trans* configuration,[11–13] as their dihedral angle in the *trans* configuration is close to 90° in the stable conformation as shown by molecular models. The $J_{6/7}$-value (2.7 Hz) indicates that 6-H and 7-H were in the *cis* configuration.[10–12] The small long-range coupling constants $^4J_{3b/5}$ and $^4J_{4/6}$ made the corresponding peaks broad. The formation of an intramolecular hydrogen bond between C^8–OH and C^6–OH resulted in a relative *cis*-like configuration for 7-H and 8-H [either 7*S*/8*S* or 7*R*/8*R*], which was consistent with the coupling constant $J_{7/8}$ 4.8 Hz. (This was substantiated by the X-ray crystallographic data which showed that the positions of the two hydroxy groups are favourable to the formation of an intramolecular hydrogen bond.) If the relative configuration were 7*S*, 8*R* [or 7*R*, and 8*S*], the $J_{7/8}$-value should be greater than 5 Hz when the intramolecular hydrogen bond is formed. Thus, the structure of goniofufurone (**1**) was determined as 6-hydroxy-5-(α-hydroxybenzyl)-3a,5,6,-6a-tetrahydrofuro[3,2-*b*]furan-2(3*H*)-one, and the relative configuration of compound (**1**) should be 4*S**, 5*R**, 6*S**, 7*S**, 8*S**. To substantiate the structural elucidation, a scheme was proposed (Scheme 1) for the mass fragments proposed in the



Scheme 1. The EIMS fragments of goniofufurone (**1**).

EIMS scheme were confirmed by high-resolution EIMS. Finally, the above structural elucidation was confirmed by X-ray crystallographic data (Figure 2, and Tables 3–5), which showed the existence of an intramolecular hydrogen bond

† Throughout the paper, the numbering scheme used to describe the NMR spectral data is that (non-systematic) one shown in structures (**1**)–(**6**).

Table 3. Atomic fractional co-ordinates with esds in parentheses.

Compound	(1)			(2)		
Atom	*x*	*y*	*z*	*x*	*y*	*z*
O(1)	0.396 5(3)	1.139 1(2)	0.572 84(7)	0.067 1(1)	0.366 0(1)	0.463 3(3)
O(2)	0.127 4(4)	1.308 4(2)	0.547 17(8)	0.162 6(2)	0.564 7(2)	0.485 1(4)
O(6 or 5)	0.633 4(3)	0.736 7(3)	0.601 68(8)	0.054 0(1)	0.160 6(1)	0.034 7(2)
O(8 or 7)	0.319 3(4)	0.515 4(2)	0.627 08(7)	0.110 3(1)	0.105 3(1)	0.411 6(2)
O(9)	0.195 2(3)	0.777 5(2)	0.553 86(7)	0.292 3(1)	0.337 4(1)	0.212 00
C(2)	0.220 8(5)	1.176 5(3)	0.542 0(1)	0.151 5(2)	0.478 0(2)	0.391 3(5)
C(3)	0.171 1(5)	1.038 7(3)	0.503 2(1)	0.221 1(2)	0.487 4(2)	0.193 0(5)
C(4)	0.314 6(5)	0.895 0(3)	0.521 5(1)	0.211 9(2)	0.370 7(2)	0.110 2(4)
C(5)	0.482 7(4)	0.974 6(3)	0.559 9(1)	0.080 4(2)	0.271 5(2)	0.134 6(3)
C(6)	0.478 1(4)	0.866 8(3)	0.610 3(1)	0.054 9(2)	0.255 8(2)	0.370 5(3)
C(7)	0.239 9(4)	0.808 2(3)	0.609 8(1)	0.141 0(2)	0.223 5(2)	0.484 5(3)
C(8)	0 182 0(5)	0.652 6(3)	0.642 3(1)	0.274 4(2)	0.317 3(2)	0.437 4(3)
C(1′)	0.204 8(5)	0.686 2(3)	0.702 4(1)	0.359 3(2)	0.275 4(2)	0.516 3(3)
C(2′)	0.400 1(5)	0.652 7(4)	0.729 6(1)	0.388 3(2)	0.202 2(2)	0.394 6(4)
C(3′)	0.420 1(1)	0.690 0(5)	0.784 3(1)	0.462 2(2)	0.160 4(2)	0.473 1(4)
C(4′)	0.246 8(7)	0.756 1(4)	0.811 8(1)	0.507 7(2)	0.189 8(2)	0.676 9(5)
C(5′)	0 054 9(7)	0.789 1(5)	0.785 8(1)	0.480 2(2)	0.262 8(2)	0.799 6(4)
C(6′)	0.032 7(6)	0.755 7(4)	0.730 7(1)	0.407 7(2)	0.305 9(2)	0.721 2(4)
H(O6 or 5)	0.5820	0.6250	0.6094	0.0820	0.1250	0.0820
H(O8 or 7)	0.2930	0.4590	0.5957	0.1250	0.0625	0.5404

Table 4. Bond lengths (Å) with esds in parentheses.

Compound (1)				Compound (2)			
O(1)–C(2)	1.341(3)	C(5)–C(6)	1.513(4)	O(1)–C(2)	1.356(3)	C(5)–C(6)	1.510(3)
O(1)–C(5)	1.455(3)	C(6)–C(7)	1.515(4)	O(1)–C(6)	1.446(2)	C(6)–C(7)	1.520(3)
O(2)–C(2)	1.206(3)	C(7)–C(8)	1.525(4)	O(2)–C(2)	1.189(3)	C(7)–C(8)	1.529(3)
O(6)–C(6)	1.420(3)	C(8)–C(1′)	1.511(4)	O(5)–C(5)	1.415(2)	C(8)–C(1′)	1.500(3)
O(8)–C(8)	1.429(3)	C(1′)–C(2′)	1.384(4)	O(7)–C(7)	1.419(2)	C(1′)–C(2′)	1.384(3)
O(9)–C(4)	1.430(3)	C(1′)–C(6′)	1.370(4)	O(9)–C(4)	1.431(2)	C(1′)–C(6′)	1.396(3)
O(9)–C(7)	1.424(3)	C(2′)–C(3′)	1.386(4)	O(9)–C(8)	1.438(2)	C(2′)–C(3′)	1.375(3)
C(2)–C(3)	1.493(4)	C(3′)–C(4′)	1.356(5)	C(2)–C(3)	1.497(4)	C(3′)–C(4′)	1.378(4)
C(3)–C(4)	1.513(4)	C(4′)–C(5′)	1.352(5)	C(3)–C(4)	1.513(3)	C(4′)–C(5′)	1.373(3)
C(4)–C(5)	1.528(4)	C(5′)–C(6′)	1.390(4)	C(4)–C(5)	1.509(3)	C(5′)–C(6′)	1.372(3)
O(6)–H(O6)	0.9676	O(2)–H(O8)**	1.9727	O(5)–H(O5)	0.760	O(5)–H(O7)**	1.7047
O(8)–H(O6)*	1.8678	O(8)–H(O8)	0.9095	O(7)–H(O5)*	2.1394	O(7)–H(O7)	1.0415

* Intramolecular hydrogen bond; ** intermolecular hydrogen bond.

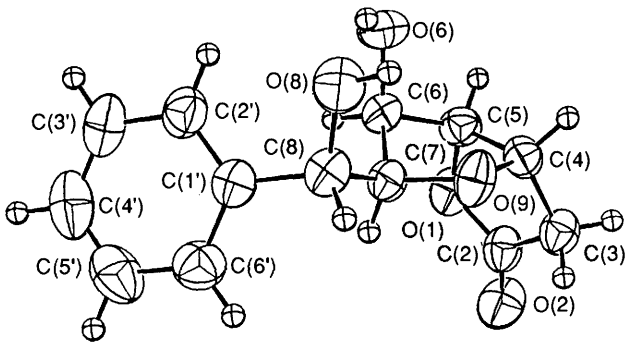


Figure 2. ORTEP plot of goniofufurone (**1**).

between 6-OH and the oxygen of 8-OH, and an intermolecular hydrogen bond between 8-OH and the carbonyl group.

The CIMS (isobutane) of goniopypyrone (**2**) gave a prominent peak at *m/z* 251 (MH^+), and the EIMS of compound (**2**) showed a peak at *m/z* 250 (M^+). Again, two hydroxy groups were indicated by two successive losses of water from the molecular ion in the CIMS. The molecular weight and the presence of the two hydroxy groups were confirmed by peaks at *m/z* 334 [M^+ for diacetyl goniopypyrone (**5**)], 291 (M^+ − Ac), 274 (M^+ − HOAc), and 214 (M^+ − 2HOAc) in the EIMS, and two singlet peaks, at δ 2.20 (3 H, OAc) and 1.79 (3 H, OAc), in the ^{1}H NMR spectrum of compound (**5**). The measurement of a high-resolution CIMS at *m/z* 251.0914 (MH^+) gave the molecular formula as $C_{13}H_{15}O_5$ (*calc: M*H, 251.0919). Hydroxy-group bands at 3 405 and 3 280 cm^{-1}, and a saturated δ-lactone carbonyl band at 1 743 cm^{-1}, were present in the IR spectrum, and the carbonyl group was confirmed by a small peak at δ_C 168.11 in the ^{13}C NMR spectrum.

The molecular structure of goniopypyrone (**2**) was suggested to be 8,9-dihydroxy-7-phenyl-2,6-dioxabicyclo[3.3.1]nonan-3-one by comparison of the ^{1}H NMR spectrum of compound (**2**) with those of altholactone (syn: goniothalenol)[2,9] (**7**), the synthetic epimers of altholacetone,[10,11] and goniofufurone (**1**), and by careful examination of the ^{1}H–^{1}H COSY spectrum of goniopypyrone (**2**) (Figure 3); the latter was especially useful in assigning the ^{1}H NMR peaks. The ^{1}H–^{1}H COSY spectrum showed that both 5-H and 7-H coupled with four protons [two ^3J with vicinal protons, a W-configuration long-range coupling (W-^4J) with each other, and a ^3J with a hydroxy-group proton, respectively], and 4-H and 6-H also had a W-^4J coupling. The positions of the two hydroxy groups were determined by the changes of the chemical shifts of 5-H and 7-H which moved

Table 5. Bond angles (°) with esds in parentheses.

Compound (1)			
C(2)–O(1)–C(5)	111.3(2)	O(9)–C(7)–C(6)	104.0(2)
C(4)–O(9)–C(7)	109.2(2)	O(9)–C(7)–C(8)	108.8(2)
O(1)–C(2)–O(2)	120.5(3)	C(6)–C(7)–C(8)	117.9(2)
O(1)–C(2)–C(3)	110.9(2)	O(8)–C(8)–C(7)	111.1(2)
O(2)–C(2)–C(3)	128.6(3)	O(8)–C(8)–C(1′)	110.0(2)
C(2)–C(3)–C(4)	105.0(2)	C(7)–C(8)–C(1′)	110.3(2)
O(9)–C(4)–C(3)	112.3(2)	C(8)–C(1′)–C(2′)	121.1(3)
O(9)–C(4)–C(5)	105.5(2)	C(8)–C(1′)–C(6′)	120.1(3)
C(3)–C(4)–C(5)	104.3(2)	C(2′)–C(1′)–C(6′)	118.8(3)
O(1)–C(5)–C(4)	106.1(2)	C(1′)–C(2′)–C(3′)	120.2(3)
O(1)–C(5)–C(6)	109.4(2)	C(2′)–C(3′)–C(4′)	120.2(3)
C(4)–C(5)–C(6)	104.8(2)	C(3′)–C(4′)–C(5′)	120.2(3)
O(6)–C(6)–C(5)	106.6(2)	C(4′)–C(5′)–C(6′)	120.5(3)
O(6)–C(6)–C(7)	113.5(2)	C(1′)–C(6′)–C(5′)	120.1(3)
C(5)–C(6)–C(7)	100.8(2)	O(6)–H(O6)–O(8)*	139.04
		O(2)–H(O8)–O(8)**	155.28
Compound (2)			
C(2)–O(1)–C(6)	121.4(2)	O(7)–C(7)–C(6)	105.9(1)
C(4)–O(9)–C(8)	114.9(1)	O(7)–C(7)–C(8)	111.7(1)
O(1)–C(2)–O(2)	117.8(3)	C(6)–C(7)–C(8)	111.1(2)
O(1)–C(2)–C(3)	119.0(2)	O(9)–C(8)–C(7)	110.1(1)
O(2)–C(2)–C(3)	123.1(3)	O(9)–C(8)–C(1′)	108.1(1)
C(2)–C(3)–C(4)	117.0(2)	C(7)–C(8)–C(1′)	111.6(2)
O(9)–C(4)–C(3)	114.4(2)	C(8)–C(1′)–C(2′)	121.9(2)
O(9)–C(4)–C(5)	111.0(2)	C(8)–C(1′)–C(6′)	120.0(2)
C(3)–C(4)–C(5)	106.9(2)	C(2′)–C(1′)–C(6′)	118.0(2)
O(5)–C(5)–C(4)	113.3(2)	C(1′)–C(2′)–C(3′)	121.1(2)
O(5)–C(5)–C(6)	112.2(2)	C(2′)–C(3′)–C(4′)	120.1(2)
C(4)–C(5)–C(6)	106.5(2)	C(3′)–C(4′)–C(5′)	119.5(2)
O(1)–C(6)–C(5)	110.9(2)	C(4′)–C(5′)–C(6′)	120.6(2)
O(1)–C(6)–C(7)	108.8(2)	C(1′)–C(6′)–C(5′)	120.7(2)
C(5)–C(6)–C(7)	111.4(2)	O(5)–H(O5)–O(7)*	127.12
		O(5)–H(O7)–O(7)**	158.20

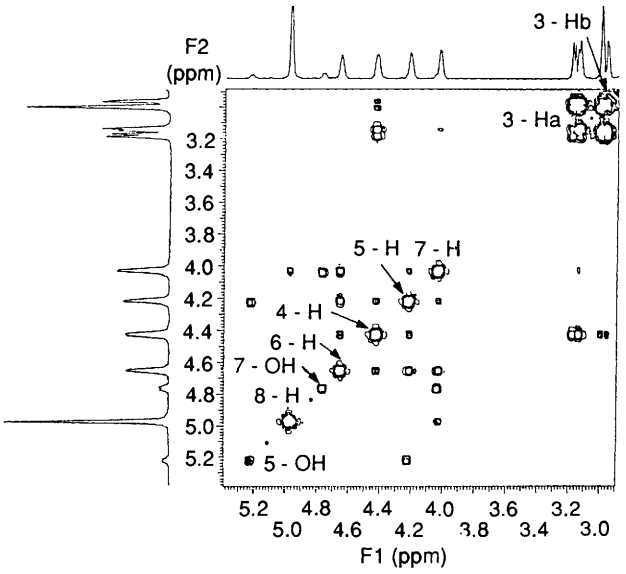


Figure 3. ^1H–^1H COSY spectrum of goniopypyrone (2).

downfield by 0.95 and 1.12 ppm, respectively, after acetylation of compound (2) to give diacetylgoniopypyrone (5), and by the coupling relationship shown by the ^1H–^1H COSY spectrum. The two upfield methylene protons, 3-$H_{a,b}$, with 4-H formed a typical ABX system, and molecular models revealed that 4-H and 6-H had to be in a *cis* configuration, which was confirmed by the observed W-^4J value between 4-H and 6-H. By the same

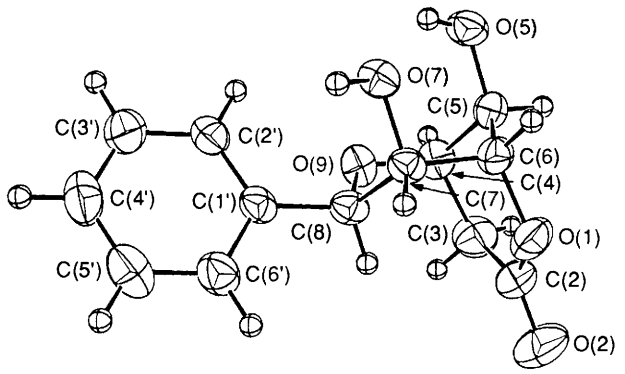
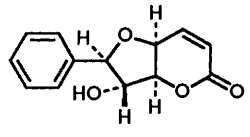


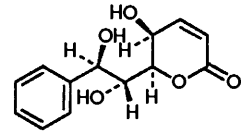
Figure 4. ORTEP plot of goniopypyrone (2).

reasoning, the relative configurations of 4-H, 5-H, 6-H, 7-H, and 8-H were assigned as 4*R**, 5*R**, 6*S**, 7*S**, and 8*R** by the presence of the W-^4J coupling between 5-H and 7-H, and the absence of the W-^4J coupling between 3-H and 5-H, and between 6-H and 8-H. A small $J_{7/8}$-value (1.2 Hz) also indicated a *cis* configuration between 7-H and 8-H. Different W-^4J coupling relations would be observed if the relative configurations were different from the above assignments, according to the molecular model which was fixed by the 2′,4′-4,6 chair fusion and semichair conformational 6-membered rings (a tetrahydropyran and a tetrahydropyranone) in its stable conformation.

The above structural elucidation of goniopypyrone (2) was confirmed by X-ray crystallographic data (Figure 4, and Tables 3–5), which also indicated the existence, by their steric positions and bond lengths, of an intramolecular hydrogen bond between 5-OH and the oxygen of 7-OH and an intermolecular hydrogen bond between 7-OH and the oxygen of 5-OH. The ^{13}C NMR spectrum of goniopypyrone (2) was also assigned (Table 1) by the use of ^1H–^{13}C HETCOR. The main EIMS fragments of compound (2) were similar to those of compound (1).



(7)



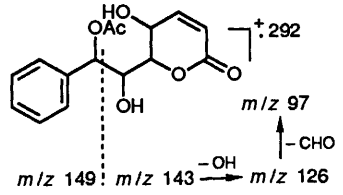
(8)

The molecular weight (292) of 8-acetylgoniotriol (3) was indicated by a prominent peak at *m/z* 293 (MH^+) in the CIMS (isobutane). The presence of an acetoxy and two free hydroxy groups in compound (3) were indicated by peaks at *m/z* 275 ($MH^+ - H_2O$), 233 (MH^+ – HOAc), and 215 ($MH^+ - H_2O$ – HOAc) in the CIMS; a singlet peak at δ 2.12 (3 H in OAc) in the ^1H NMR spectrum (in $CDCl_3$) of compound (3), and three singlet peaks, at δ 2.12 (3 H), 2.08 (3 H), and 2.03 (3 H), for three acetyl groups in the ^1H NMR spectrum (in $CDCl_3$) of the diacetyl derivative (6). The hydroxy-group bands at 3 532 and 3 414 cm^{-1}, and α,β-unsaturated δ-lactone bands at 1 688 and 1 628 cm^{-1}, were present in the IR spectrum. The acetyl and α,β-unsaturated δ-lactone were confirmed by two small peaks, at δ_C 169.69 (C-9) and 163.49 (C-2), in the ^{13}C NMR spectrum. The position of the acetyl group was indicated by the peak at δ_H 5.86 (d, 8-H) in the ^1H NMR spectrum of compound (3), and the positions of the two hydroxy groups were determined by the downfield shifts of 5-H and 7-H (from δ 4.37 to 5.29, and 4.43 to 5.75, respectively) in the ^1H NMR spectrum (in $CDCl_3$) after acetylation of compound (3), which gave triacetylgoniotriol (6).

Comparison of the ^1H NMR and ^{13}C NMR of companion (3) with those of goniotriol[3,6] (8) suggested that the structure

Table 6. Bioactivities of compounds (1)–(3) and their acetyl derivatives (4)–(6).

Compound	BS LC_{50} (ppm)	PD Inhibition	A-549 ED_{50} (μg/ml)	MCF-7 ED_{50} (μg/ml)	HT-29 ED_{50} (μg/ml)
(1)	123	14%	4.76	>10	>10
(2)	13	67%	6.60×10^{-1}	6.72×10^{-1}	6.78×10^{-1}
(3)	99	56%	9.26	>10	4.70
(4)			33.60	29.55	36.98
(5)			23.05	15.30	21.65
(6)			>10	>10	4.50



Scheme 2. The EIMS fragments of 8-acetylgoniotriol (3).

might be 6-(β-acetoxy-α-hydroxyphenethyl)-5-hydroxy-5,6-dihydro-2-pyrone (or 8-acetylgoniotriol). The relative configuration of 7-H and 8-H could be determined by the $J_{7/8}$-value (7.5 Hz), which indicated a 7,8-*erythro* configuration, and the $J_{5/6}$-value (2.9 Hz) suggested a *cis* configuration between 5-H and 6-H.[6] The final relative configuration of compound (3) was determined as 5*S**, 6*R**, 7*R**, and 8*R** by comparison of the ^1H NMR spectrum of compound (6) with that of the triacetyl derivative of authentic goniotriol whose structure was previously determined by X-ray crystallography;[3] the two spectra were completely identical. The ^{13}C NMR spectrum of compound (3) was assigned by comparison with those of similar compounds[3,6] (Table 1). The EIMS of compound (3) was basically similar to that of goniofufurone (1); however, there were two significant and prominent peaks [higher relative intensity than those of compound (1)] at *m/z* 149 and 97 which further supported the proposed structure (Scheme 2).

The biological activities of goniofufurone (1), goniopypyrone (2), 8-acetylgoniotriol (3), and their acetyl derivatives (4)–(6) are summarized in Table 6. Compound (2) was the most bioactive, showing nonselective ED_{50}-values in cytotoxicity of $\sim 6.7 \times 10^{-1}$ μg/ml in the A-549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), and HT-29 (human colon adenocarcinoma), high toxicity to the brine shrimp (BS), and significant inhibition of the formation of crown gall tumours on potato discs (PD); this plant tumour system[14] has a positive correlation with the 3PS *in vivo* murine lymphocytic leukaemia bioassay.[1] The activities of the diacetyl derivative (5) of goniopypyrone (2) were reduced three-fold in the three human tumour cell lines. Compound (1) showed selective but moderate cytotoxic activity in A-549 and moderate toxicity to the brine shrimp, and its diacetyl derivative (4), likewise, showed weaker activities in the human tumour cell lines. Compound (3) was moderately and selectively cytotoxic to HT-29 and A-549, moderately toxic to the brine shrimp, but quite active in the potato disc test; its diacetyl derivative (6) showed nearly the same activities as its parent (3) in the three human tumour cell lines. The above data again show that there are useful correlations among brine shrimp (BS) lethality,[8] the potato disc (PD) test,[14] and cytotoxicities to the human tumour cell lines.

Experimental

General Experimental Procedures.—M.p.s were determined on a Mettler FP 5 hot-stage apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were taken in EtOH on a Beckman DU-7 spectrometer. IR spectra were obtained in KBr pellets on a Perkin-Elmer 1600 FTIR spectrometer. Low-resolution mass spectra were recorded on a Finnigan 4000 mass spectrometer. Exact mass measurements were determined on a Kratos MS 50 spectrometer through peak matching. ^1H NMR spectra were recorded on a Chemagnetics A-200 or a Varian VXR-500S spectrometer. ^{13}C NMR spectra were recorded on a Chemagnetics A-200 spectrometer. 2D NMR spectra were recorded on a Varian XL-200 or a Varian VXR-500S spectrometer.

Extracts were chromatographed on columns packed with silica gel 60–200 or 260–400 mesh (Merck). TLC was performed on pre-coated silica gel 60 F-254 TLC plates (Merck), and spots were detected under UV light (254 and 366 nm) or visualized by spraying with 5% phosphomolybdic acid and heating.

Plant Material.—The stem bark[2–5] of the plant *Goniothalamus giganteus* Hook. f., Thomas was provided under the identification number B-826538 as collected in Thailand in September 1978 by the USDA for the National Cancer Institute (NCI), National Institute of Health (NIH).

Bioassays.—Extracts, fractions, and isolated compounds were routinely evaluated for brine shrimp lethality (BS),[8] and the purified compounds were tested in the potato disc assay[14] in our laboratory. The cytotoxic tests of A-549 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), and HT-29 (human colon adenocarcinoma) were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, following standard protocols established by the NCI/NIH.

Extraction and Isolation.—The residue of the crude ethanol extract (F001) of the stem bark (4 kg) was partitioned between water and $CHCl_3$ to give residues F002 (water layer), F003 (chloroform layer), and F004 (interface residue). F003 was partitioned between hexane and 10% water in MeOH to give F005 (methanol layer, ~100 g dry residue), and F006 (hexane layer). The brine shrimp lethality bioassay demonstrated that F005 (BS LC_{50} 15.1 μg/ml, 95% confidence intervals 8.7–23.7 μg/ml) was the most active fraction. F005 was chromatographed over a silica gel (2 000 g) column with gradient elution with benzene–EtOAc–MeOH. Fractions were combined into pools according to their similar appearance after analytical TLC. Pool 2 (fractions 5–9; BS LC_{50} 1.6 μg/ml, 95% confidence intervals 0.0008–7.8 μg/ml), which contained mainly altholacetone and similar compounds, was repeatedly chromatographed over silica gel columns (gradients of hexane–EtOAc or $CHCl_3$–EtOAc) and monitored *via* the BS test; active fractions gave crude crystals of three compounds, which were recrystallized from EtOAc–hexane, to give crystals of compounds (1), (2), and (3).

Goniofufurone (1).—Crystals (50 mg) from EtOAc–hexane, m.p. 152–154 °C; $[\alpha]_D^{22}$ +9° (*c* 0.5 in EtOH); λ_{max}(EtOH) 207

nm (log ε 3.83); ν_{max}(KBr) 3 401 (OH), 1 755 (γ-lactone), 1 635, 1 185, 1 062, and 1 040 cm^{-1}; CIMS (isobutane) *m/z* (%) 251 (MH^+, 74), 233 ($MH^+ - H_2O$, 65), and 215 ($MH^+ - 2H_2O$, 8); EIMS (Scheme 1); HR EIMS of fragments: *m/z* 126.0317 ($C_6H_6O_3$. Calc. *m/z* 126.0317), 107.0491 (C_7H_7O. Calc. *m/z*, 107.0497), 97.0287 ($C_5H_5O_2$. Calc. *m/z*, 97.0289), and 91.0543 (C_7H_7. Calc. *m/z*, 91.0548); δ_H(Table 2); δ_C(Table 1); ^1H–^1H COSY (Figure 1); X-ray data (Figure 2, and Tables 3–5).

Diacetylgoniofufurone (**4**).—Goniofufurone (**1**) (4 mg) was acetylated (Ac_2O–pyridine; 24 h; room temp.), and the mixture was partitioned between water and $CHCl_3$. The $CHCl_3$ extract on concentration and chromatography afforded compound (**4**), m.p. 130–132 °C; CIMS (methane) *m/z* (%) 335 (MH^+, 35), 275 (MH^+ – HOAc, 30), 233 (MH^+ – HOAc – C_2H_2O, 22), and 215 (MH^+ – 2HOAc, 24); δ_H(200 MHz; $CDCl_3$) 7.36 (5 H, m, Ph), 5.85 (d, *J* 9.1 Hz, 8-H), 5.75 (d, *J* 3.1 Hz, 6-H), 4.95 (ddd, *J* 4.4, 5.2, and 1.5 Hz, 4-H), 4.87 (d, *J* 4.4 Hz, 5-H), 4.47 (dd, *J* 9.1 and 3.1 Hz, 7-H), 2.70 (dd, *J* 17.7 and 5.2 Hz, 3-H_a), 2.55 (dd, *J* 17.7 and 1.5 Hz, 3-H_b), 2.15 (3 H, s, Ac), and 2.00 (3 H, s, Ac).

Goniopypyrone (**2**).—Needles (120 mg) from EtOAc–hexane, m.p. 182–184 °C; $[\alpha]_D^{22}$ +54° (*c* 0.4 in EtOH); λ_{max}(EtOH) 208 nm (3.35); ν_{max}(KBr) 3 405 (OH), 3 280 (OH), 1 743 (δ-lactone), 1 635, 1 220, 1 159, 1 072, and 1 056 cm^{-1}; CIMS (isobutane) *m/z* (%) 251 (MH^+, 100), 233 (MH^+ – H_2O, 23), and 215 (MH^+ – $2H_2O$, 11); HR CIMS 251.0914 ($C_{13}H_{15}O_5$. Calc. *m/z*, 251.0919); EIMS (%) 250 (M^+, 5), 233 (13), 215 (2.3), 173 (1.3), 149 (6), 144 (6), 126 (3), 107 (100), 105 (42), 97 (17), 91 (79), and 77 (72); δ_H(Table 2); δ_C(Table 1); ^1H–^1H COSY (Figure 3); X-ray data (Figure 4, and Tables 3–5).

Diacetylgoniopypyrone (**5**).—Acetylation of goniopypyrone (**2**) by the same procedure as with compound (**1**) gave the title compound (**5**), m.p. 155–157 °C; EIMS *m/z* (%) 334 (M^+, 3), 291 (M^+ – Ac, 2), 274 (M^+ – HOAc, 3), 249 (MH^+ – 2Ac, 5), and 214 (M^+ – 2HOAc, 2); δ_H(200 MHz; $CDCl_3$) 7.35 (5 H, m, Ph), 5.26 (dd, *J* 3.1 and 2.4 Hz, 7-H), 5.09 (d, *J* 2.4 Hz, 8-H), 5.02 (dd, *J* 3.2 and 1.2 Hz, 5-H), 4.85 (q, *J* 3.2, 3.1, and 3.0 Hz, 6-H), 4.59 (br, *J* 3.5, 3.0, and 1.2 Hz, 4-H), 3.14 (2 H, d, *J* 3.5 Hz, 3-H_2), 2.20 (3 H, s, Ac), and 1.79 (3 H, s, Ac).

8-*Acetylgoniotriol* (**3**).—Crystals (25 mg) from EtOAc–hexane, m.p. 158–159 °C; $[\alpha]_D^{22}$ +30° (*c* 0.4 in EtOH); λ_{max}(EtOH) 206 nm (3.92); ν_{max}(KBr) 3 532 (OH), 3 414 (OH), 1 724 (Ac), 1 688 and 1 628 (α,β-unsaturated lactone), 1 278, 1 237, 1 050, and 1 019 cm^{-1}; CIMS (isobutane) *m/z* (%) 293 (MH^+, 7), 275 (MH^+ – H^2O, 42), 233 (MH^+ – HOAc, 100), and 215 (275 – HOAc, 26); EIMS (Scheme 2); HR EIMS of fragments: *m/z* 143.0345 ($C_6H_7O_4$. Calc. *m/z*, 143.0344), 126.0314 ($C_6H_6O_3$. Calc. *m/z*, 126.0317), 107.0493 (C_7H_7O. Calc. *m/z*, 107.0497), 97.0292 ($C_5H_5O_2$. Calc. *m/z*, 97.0289), and 91.0545 (C_7H_7. Calc. *m/z*, 91.0548); δ_H(Table 2); δ_C(Table 1).

Triacetylgoniotriol (**6**).—Acetylation of compound (**3**) by the same procedure as for compound (**1**) gave the title compound (**6**), m.p. 90–93 °C; CIMS (isobutane) *m/z* (%) 377 (MH^+, 1), 317 (MH^+ – HOAc, 100), 257 (MH^+ – 2HOAc, 15), and 199 (M^+ – 3OAc, 58); δ_H(200 MHz; $CDCl_3$) 7.35–7.43 (5 H, m, Ph), 6.94 (dd, *J* 9.8 and 5.9 Hz, 4-H), 6.18 (d, *J* 9.8 Hz, 3-H), 5.97 (d, *J* 4.9 Hz, 8-H), 5.75 (dd, *J* 4.9 and 6.8 Hz, 7-H), 5.29 (dd, *J* 5.9 and 3.2 Hz, 5-H), 4.54 (dd, *J* 6.8 and 3.2 Hz, 6-H), 2.12 (3 H, s, Ac), 2.08 (3 H, s, Ac), and 2.03 (3 H, s, Ac).

X-Ray Crystallographic Analysis of Goniofufurone (**1**).—Crystals were obtained as transparent prisms by recrystallization from EtOAc–hexane.

Crystal Data.—$C_{13}H_{14}O_5$, M = 250.25. Orthorhombic, a = 6.047(1), b = 8.027(1), c = 24.625(2) Å, V = 1 195.2 $Å^3$ (by least-squares refinement, using the setting angles of 25 reflections in the range 16 < θ < 22°, measured by the computer-controlled diagonal slit method of centering), λ(Mo-K_α) = 0.710 73 Å, space group $P2_12_12_1$ (No. 19), Z = 4, D_x = 1.39 g cm^{-3}. Crystal dimensions 0.47 × 0.38 × 0.13 mm, μ(Mo-K_α) = 1.0 cm^{-1}.

Data Collection and Processing.—Enraf-Nonius CAD4 diffractometer, ω/2θ mode with ω scan width = 0.76 + 0.35 tan θ, 2θ range 4.00–50.00°, take-off angle 3.15°, ω scan rate 2–20 deg min^{-1}, graphite-monochromated Mo-K_α radiation; 1 295 reflections measured (*h,k,l* limits: 0–7, 0–9, 0–29), 1 295 unique, giving 1 034 with $I > 3.0\sigma(I)$. Corrections were applied for Lorentz and polarization factors, but not for absorption.

Structure Analysis and Refinement.—The structure was solved by direct methods using SHELX-86. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were located and added to the structure factor calculations but their positions were not refined. The structure was refined in full-matrix least-squares where the function minimized was $\Sigma w(|F_o| - |F_c|)^2$ and the weight w is defined as per the Killean and Lawrence method with terms of 0.020 and 1.0.[15] Scattering factors were taken from Cromer and Waber.[16] Anomalous dispersion effects were included in F_c;[17] the values for $\delta f'$ and $\delta f''$ were those of Cromer.[16] Only the 1 034 reflections having intensities greater than 3.0 times their standard deviation were used in the refinements. The final cycle of refinement included 163 variable parameters and converged with unweighted and weighted agreement factors R = 0.033, and R_w = 0.042. The standard deviation of an observation of unit weight was 1.19. There were no correlation coefficients >0.50. The factor for the determination of the enantiomorph refined to 1.00.[18] Plots of $\Sigma w(|F_o| - |F_c|)^2$ *versus* $|F_o|$, reflection order in data collection, sin θ/λ, and various classes of indices showed no unusual trends. All calculations were performed on a VAX computer using SDP/VAX.*

X-Ray Crystallographic Analysis of Goniopypyrone (**2**).—Crystals were obtained from EtOAc–hexane as transparent needles.

Crystal Data.—$C_{13}H_{14}O_5$, M = 250.25. Trigonal, a = 12.642(1), c = 6.291(1) Å (by least-squares refinement, using the setting angles of 25 reflections in the range 19 < θ < 23°, measured by the computer-controlled diagonal slit method of centring), λ (Mo-K_α) = 0.710 73 Å, space group $P3_1$ (No. 144), Z = 3, D_x = 1.432 g cm^{-1}. Crystal dimensions: 0.73 × 0.20 × 0.15 mm, μ(Mo-K_α) = 1.03 cm^{-1}.

Data Collection and Processing.—Enraf-Nonius CAD4 diffractometer, ω/2θ mode with ω scan width = 0.55 + 0.35 tan θ, ω scan rate 2–20 deg min^{-1}, graphite-monochromated Mo-K_α radiation; 2 046 reflections measured (*h,k,l* limits: −13 to 0, 0–13, −6 to 6), 1 912 unique, giving 1 744 with $I > 3.0\sigma(I)$. Lorentz and polarization corrections were applied, but no absorption correction was made.

Structure Analysis and Refinement.—Similar to that described above for goniofufurone (**1**). The final cycle of refinement included 163 variable parameters and converged with unweighted and weighted agreement factors R = 0.033 and R_w = 0.043. The standard deviation of an observation of unit weight was 1.28. There were 51 correlation coefficients >0.50. The

highest correlation coefficient was 0.68 between parameters 50 and 53.*

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* *Supplementary data:* (see Instructions for Authors, section 5.6.3, in the January issue). Tables of thermal parameters and H-atom co-ordinates have been deposited at the Cambridge Crystallographic Data Centre.

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