Cytotoxic Styryl-Lactones from the Leaves and Twigs of *Polyalthia crassa*

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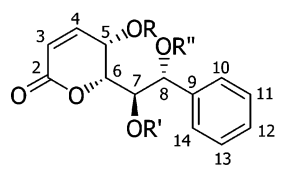
Four new styryl-lactones, crassalactones A−D (**1**−**4**), were isolated from a cytotoxic ethyl acetate-soluble extract of the leaves and twigs of *Polyalthia crassa*, together with seven known compounds, (+)-3-acetylaltholactone, (+)-altholactone, aristolactam AII, cinnamic acid, (+)-goniofufurone, (+)-goniopypyrone, and (+)-howiinol A. Their structures were determined on the basis of spectroscopic methods. The absolute configuration of **1**−**3** was established by chemical conversions. Single-crystal X-ray analysis and the Mosher ester method were used to confirm the absolute stereochemistry of **4**. Cytotoxic evaluation against several mammalian cancer cell lines was performed on all new isolates, aristolactam AII, and the modified (+)-tricinnamate derivative **11** obtained from **1**.

Several members of the genus *Polyalthia* have been widely used in traditional medicine in Asia. In Thailand, water decoctions from the roots of *P. evecta* and *P. debilis* are used as a galactagogue[1a,b] and for the treatment of abdominal pain,[1a] respectively. *Polyalthia bullata* is used in Malaysia as an aphrodisiac.[2] From various *Polyalthia* species, many constituents showing cytotoxic,[3] antimicrobial,[4] antimalarial,[5] and anti-HIV[6] activities have been reported previously. Earlier chemical examinations of plants in the genus *Polyalthia* yielded diterpenes,[3,7] triterpenes,[6a,8] benzopyrans,[9] 2-substituted furans,[1b,6b,10] and various types of alkaloids.[3d,5,11] Our previous work on *P. suberosa* has resulted in the isolation of an azaanthrazene alkaloid, kalasinamide,[12] 2-substituted furans,[6b] and carboxamides.[12a]

As part of our ongoing project on the discovery of new cytotoxic agents from plants, we describe herein the chromatographic separation of a cytotoxic ethyl acetate extract of the leaves and twigs of *P. crassa*, leading to the isolation of four new styryl-lactones, crassalactones A−D (**1**−**4**), along with the known (+)-howiinol A (**5**),[13] (+)-goniofufurone (**6**),[14] (+)-altholactone (**7**),[15] (+)-3-acetylaltholactone (**8**),[16] (+)-goniopypyrone (**9**),[14a] aristolactam AII (**10**),[17] and cinnamic acid. For further structural and stereochemical studies of compound **1**, (+)-tricinnamate **11** was prepared from both (+)-**1** and (+)-**5**, whereas the known (+)-**6** could be converted to (+)-**2** and (+)-(**3**). The modified compounds were characterized on the basis of spectroscopic methods. The four styryl-lactones **1**−**4**, aristolactam AII (**10**), and the semisynthetic (+)-tricinnamate **11** were evaluated for their cytotoxic activities against a panel of five mammalian cancer cell lines.



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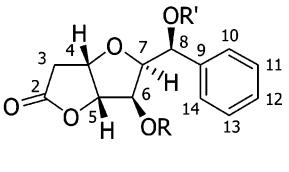
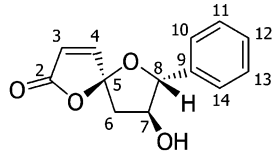
5 R = R' = H, R" = cinnamoyl
11 R = R' = R" = cinnamoyl



2 R = cinnamoyl, R' = H
3 R = H, R' = cinnamoyl
6 R = R' = H



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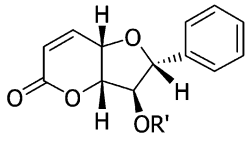
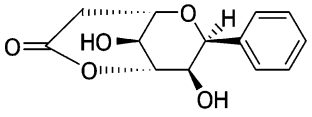


7 R = H
8 R = Ac



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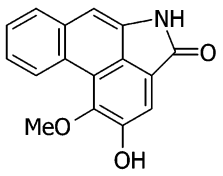

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Results and Discussion

(+)-Crassalactone A (**1**) displayed a molecular ion peak at *m*/*z* 380 in the EIMS, corresponding to the molecular formula $C_{22}H_{20}O_6$ and confirmed by elemental analysis. The presence of two free hydroxyls and a cinnamate group in **1** were indicated by the ion peaks at *m*/*z* 362 ($M^+ - H_2O$) and 344 ($M^+ - 2H_2O$), 148 [$(PhCH{=}CHCO_2H)^+$, base peak], and 131 $(PhCH{=}CHCO)^+$. The IR spectrum of **1** showed an OH stretch at 3456 cm^{-1} and two C=O absorptions at 1721 and 1711 cm^{-1}. The α,β-unsaturated δ-lactone character of **1** was confirmed by the presence of a small peak at δ_C 162.3 in its ^{13}C NMR spectrum. The ^1H NMR spectroscopic data of **1** (Table 1) were closely comparable to those observed for (+)-howiinol A (**5**) obtained from the same extract (for spectroscopic data of **5**, see Supporting Information) and previously reported from *Goniothalamus howii*.[13] The olefinic signals at δ 6.21 (d, J = 9.7 Hz, H-3) and 6.99 (dd, J = 9.7 and 5.9 Hz, H-4) were typical of an α,β-unsaturated δ-lactone, while a broad singlet at δ 2.75 (2H) confirmed the presence of two hydroxyl protons in **1**. The cinnamate group in **1** was characterized by a pair of doublets (J = 16 Hz each) at δ 6.32 (H-2′) and 7.63 (H-3′), together with the aromatic signals at δ 7.50 (2H, H-5′ and H-9′) and 7.37−7.41 (3H, H-6′, H-7′, H-8′). By analyses of its COSY spectrum, the four oxygen-linked methine signals at δ 5.30 (dd, J = 5.9 and 2.7 Hz), 4.79 (dd, J = 5.6 and 2.7 Hz), 4.29 (t, J = 5.6 Hz), and 4.91 (d, J = 5.6 Hz) were assigned to H-5, H-6, H-7, and H-8, respectively. The ^{13}C NMR spectrum of **1** (Table 1) displayed 18 signals for 22 carbons, of which the carbon types were determined by DEPT experiments. The connectivity of the cin-

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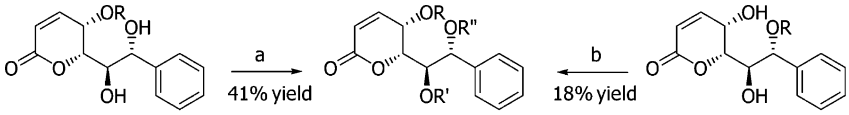
Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **1**−**4** (in $CDCl_3$)

	1		**2**		**3**		**4**	
C/H	δ_H mult. (*J*)[a]	δ_C mult.[b]	δ_H mult. (*J*)[a]	δ_C mult.[b]	δ_H mult. (*J*)[a]	δ_C mult.[b]	δ_H mult. (*J*)[a]	δ_C mult.[b]
2		162.3 s		174.7 s		175.3 s		169.0 s
3	6.21 d (9.7)	124.6 d	2.72 dd (19.0, 5.8) 2.60 d (19.0)	35.6 t	2.67 dd (18.8, 5.6) 2.56 d (18.8)	35.8 t	6.28 d (5.6)	124.9 d
4	6.99 dd (9.7, 5.9)	140.8 d	5.02−5.05 m	77.1 d	4.99−5.03 m	77.1 d	7.29 d (5.6)	151.0 d
5	5.30 dd (5.9, 2.7)	62.8 d	5.02−5.05 m	85.3 d	4.99−5.03 m	87.0 d		114.3 s
6	4.79 dd (5.6, 2.7)	77.6 d	5.75 d (2.6)	75.5 d	4.42 br s	73.2 d	2.57 dd (14.3, 6.4) 2.31 dd (14.3, 1.9)	42.4 t
7	4.29 t (5.6)	73.4 d	4.27 dd (8.6, 2.6)	83.1 d	4.25 dd (9.2, 2.2)	82.5 d	4.42 ddd (6.4, 1.9, 1.9)	78.2 d
8	4.91 d (5.6)	73.5 d	4.68 d (8.6)	70.9 d	6.00 d (9.2)	72.9 d	5.39 d (1.9)	91.4 d
9		139.9 s		140.4 s		136.8 s		138.4 s
10	7.43 d (7.4)	126.6 d	7.31−7.46 m	126.8 d	7.48 dd (8.1, 1.5)	127.7 d	7.29−7.34 m	125.0 d
11	7.34 t (7.4)	128.7 d	7.31−7.46 m	128.6 d	7.36−7.44 m	128.7 d	7.37−7.41 m	128.7 d
12	7.28 t (7.4)	128.3 d	7.31−7.46 m	128.4 d	7.36−7.44 m	128.9 d	7.29−7.34 m	128.2 d
13	7.34 t (7.4)	128.7 d	7.31−7.46 m	128.6 d	7.36−7.44 m	128.7 d	7.37−7.41 m	128.7 d
14	7.43 d (7.4)	126.6 d	7.31−7.46 m	126.8 d	7.48 dd (8.1, 1.5)	127.7 d	7.29−7.34 m	125.0 d
1′		165.5 s		166.4 s		167.5 s		
2′	6.32 d (16.0)	116.3 d	6.55 d (16.0)	116.1 d	6.46 d (15.9)	116.7 d		
3′	7.63 d (16.0)	146.6 d	7.84 d (16.0)	147.6 d	7.76 d (15.9)	147.3 d		
4′		133.8 s		133.8 s		133.8 s		
5′	7.50 dd (7.4, 2.0)	128.2 d	7.59 dd (7.4, 2.2)	128.4 d	7.53 dd (7.5, 2.1)	128.4 d		
6′	7.37−7.41 m	128.9 d	7.31−7.46 m	129.1 d	7.36−7.44 m	129.0 d		
7′	7.37−7.41 m	130.8 d	7.31−7.46 m	131.1 d	7.36−7.44 m	131.0 d		
8′	7.37−7.41 m	128.9 d	7.31−7.46 m	129.1 d	7.36−7.44 m	129.0 d		
9′	7.50 dd (7.4, 2.0)	128.2 d	7.59 dd (7.4, 2.2)	128.4 d	7.53 dd (7.5, 2.1)	128.4 d		
OH-6					4.18 br s			
OH-7	2.75 br s						2.43 (br s)	
OH-8	2.75 br s		2.91 br s					

[a] Spectra recorded at 500 MHz in $CDCl_3$, using TMS as an internal reference; *J* values (in Hz) in parentheses. [b] Spectra recorded at 125 MHz in $CDCl_3$, using $CDCl_3$ signal at δ_C 77.0 as reference; multiplicites determined by DEPT experiments.

Scheme 1. Chemical Conversions of (+)-Crassalactone A (**1**) and (+)-Howiinol A (**5**) to the (+)-Tricinnamate **11**



1 R = cinnamoyl **11** R = R' = R" = cinnamoyl **5** R = cinnamoyl

(a) Cinnamic acid (2.2 equiv), DCC (2.3 equiv), DMAP (4 equiv), Dry CH_2Cl_2, rt, 3 h; (b) cinnamic acid (2.2 equiv), DCC (2.3 equiv), DMAP (4 equiv), dry CH_2Cl_2, rt, 14 h.

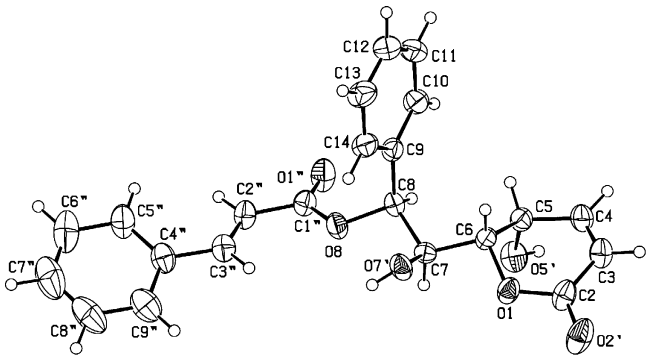
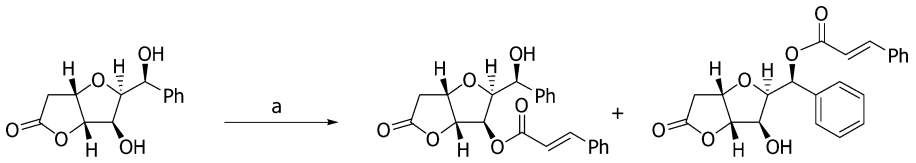


Figure 1. X-ray ORTEP diagram of (+)-howiinol A (**5**).

namate group to C-5 was indicated by the HMBC correlations of the H-5 signal to the C-2, C-3, C-4, C-6, C-7, C-1′, and C-2′ signals (Table S1, Supporting Information). As both (+)-**1** and (+)-**5** could be converted into the (+)-tricinnamate **11** (Scheme 1) and the products obtained from both reactions (41 and 18% yields, respectively) were found to be identical (mp, optical rotation, ^{1}H and ^{13}C NMR data), the relative configuration of both compounds was proved to be the same. The isolated (+)-howiinol A (**5**) and the compound reported previously by Chen et al.[13b] were confirmed by single-crystal X-ray diffraction analysis (see Figure 1). Since the absolute stereochemistry of natural (+)-howiinol A (**5**) has been proposed previously through synthesis,[13a] the absolute configuration of (+)-crassalactone A (**1**) was determined therefore as 5*S*, 6*R*, 7*R*, and 8*R*.

The molecular formula of (+)-crassalactone B (**2**) was determined as $C_{22}H_{20}O_6$ by HRTOFMS (ESI positive) measurement for $C_{22}H_{20}O_6Na$ at *m*/*z* 403.1158 (calcd 403.1158). The weak molecular ion $[M]^+$ at *m*/*z* 380, together with the fragment ions at *m*/*z* 362 ($M^+ - H_2O$), 148 [$(PhCH{=}CHCOOH)^+$, base peak], and 131 $(PhCH{=}CHCO)^+$ in the EIMS, suggested the presence of a hydroxy and a cinnamate group in **2**, which was confirmed by the IR absorption bands at 3518 (OH) and 1719 cm^{-1} (C=O of an α,β-unsaturated ester). Another carbonyl band at 1763 cm^{-1} indicated that **2** is also a saturated γ-lactone. Similar to **1**, the ^{1}H NMR spectrum of **2** (Table 1) exhibited a set of signals corresponding to a cinnamate group at δ 6.55 (H-2′), 7.84 (H-3′), 7.31−7.46 (H-6′, H-7′, and H-8′), and 7.59 (H-5′ and H-9′). The protons of another phenyl group (H-10 through H-14) were observed in the same range as H-6′, H-7′, and H-8′. Due to the proximity to the C-2 carbonyl in the γ-lactone ring, the downfield shifts of the H-3a and H-3b signals were observed at δ 2.72 (dd, J = 19.0 and 5.8 Hz) and 2.60 (d, J = 19.0 Hz), respectively. The COSY spectrum supported the assignments of the overlapping signals at δ 5.02−5.05 (2H) as H-4 and H-5, including the separated signals at δ 5.75 (d, J = 2.6 Hz), 4.27 (dd, J = 8.6 and 2.6 Hz), and 4.68 (d, J = 8.6 Hz) as H-6, H-7, and H-8, respectively. The COSY correlation between the H-8 signal and a broad singlet at δ 2.91 confirmed the location of the hydroxy group at position C-8. Eighteen carbon signals for 22 carbons were found present in the ^{13}C NMR spectrum of **2** (Table 1). The carbon types and assignments were analyzed by DEPT and 2D-NMR data. The presence of the cinnamate group at C-6 was established through the long-range HMBC correlations of H-6 to

Scheme 2. Preparation of (+)-Crassalactone B (**2**) and (+)-Crassalactone C (**3**) from (+)-Goniofufurone (**6**)[a]



[a] (a) Cinnamoyl chloride (1 equiv), Et_3N (2.5 equiv), and DMAP (4 equiv), rt, 4 h.

C-4, C-5, C-7, and C-1′ (Table S1, Supporting Information). Compound **2** was further proved to possess the same stereochemistry as (+)-goniofufurone (**6**) isolated from the same extract (for spectroscopic data of **6**, see the Supporting Information) and reported earlier from the stem bark of *Goniothalamus giganteus*.[14a] By the reaction of (+)-**6** with cinnamic acid (1 equiv) under the conditions shown in Scheme 2, (+)-**2** and (+)-**3** were obtained in 13 and 12% yields, respectively. The physical and spectroscopic data of the natural (+)-**2** were identical (mp, optical rotation, IR, UV, MS, ^{1}H and ^{13}C NMR) to the semisynthetic product. Hence, (+)-crassalactone (**2**) was suggested to possess the same relative configuration as its precursor (+)-goniofufurone (**6**). Since the absolute configuration of (+)-goniofufurone (**6**) has been determined previously through synthetic work,[14b−d] the absolute configuration of (+)-crassalactone B (**2**) was established as 4*R*, 5*S*, 6*R*, 7*R*, and 8*R*.

(+)-Crassalactone C (**3**) was found to possess the molecular formula $C_{22}H_{20}O_6$ from the $[M]^+$ peak at *m*/*z* 380 in the EIMS and confirmed by elemental analysis. The fragment ions at *m*/*z* 362 ($M^+ - H_2O$), 148 $(PhCH{=}CHCOOH)^+$, and 131 $[(PhCH{=}CHCO)^+$, base peak], as well as the IR absorptions at 3460 (OH stretch) and 1716 cm^{-1} (C=O stretch of cinnamate), indicated the presence of hydroxy and cinnamate groups in **3**. The absorption band at 1775 cm^{-1} was assigned to the C=O stretch of a saturated γ-lactone moiety. By comparison of its ^{1}H NMR data (Table 1) with those of **2**, the structure of **3** was closely related to **2**. The locations of the hydroxyl group at C-6 and cinnamate group at C-8 in **3** were suggested by an upfield shift of the broad singlet at δ 4.42 (H-6) and a downfield shift of doublets at δ 6.00 (H-8, $J_{7,8}$ = 9.2 Hz). Further confirmation was obtained through the observed HMBC correlations of OH-6 to C-5 and C-6, and H-8 to C-6, C-7, C-9, C-10, C-14, and C-1′ (Table S1, Supporting Information). Other proton signals were found in accordance with those of **2**, whereas the ^{13}C NMR signals (Table 1) showed 18 signals for 22 carbons, of which the carbon types were analyzed by DEPT experiments. The two downfield peaks at δ_C 175.3 and 167.5 were attributable to the carbonyls of saturated γ-lactone and cinnamate groups, respectively. As mentioned earlier, compound **3** was also obtained from the reaction of the isolated (+)-goniofufurone (**6**) with cinnamoyl chloride under the conditions shown in Scheme 2. The absolute configuration of (+)-crassalactone C (**3**) was thus proved to be 4*R*, 5*S*, 6*R*, 7*R*, and 8*R*.

Elemental analysis of (+)-crassalactone D (**4**) established its molecular formula as $C_{13}H_{12}O_4$. In its EIMS, the prominent peaks at *m*/*z* 214 $[M^+ - H_2O]$ suggested that **4** was a monohydroxy alcohol. The OH stretch band at 3469 cm^{-1} in the IR spectrum confirmed the alcoholic character of **4**. The presence of an α,β-unsaturated γ-lactone unit in **4** was indicated by the two carbonyl absorbances at 1758 and 1750 cm^{-1} and confirmed by the peak at δ_C 169.0 (C-2) in its ^{13}C NMR spectrum (Table 1). The ^{1}H NMR spectrum of **4** (Table 1) showed signals for a phenyl group, consisting of two sets of multiplets at δ 7.37−7.41 (2H) and 7.29−7.34 (3H). The olefinic protons (H-3 and H-4) in the unsaturated γ-lactone ring resonated as a pair of doublets (J = 5.6 Hz each) at δ 6.28 and 7.29, respectively. The assignments of the other protons were made from its COSY spectrum. The signal of H-7 at δ 4.42 (ddd, J = 6.4, 1.9, and 1.9 Hz) showed strong cross-peaks with the signal of H-6a at δ 2.57 (dd, J = 14.3 and 6.4 Hz) and the signal of H-8 at δ 5.39 (d, J = 1.9 Hz), but very weak cross-peaks with the signal of H-6b at δ 2.31 (dd, J = 14.3 and 1.9 Hz). The hydroxyl group in **4** was suggested to be located at C-7 because a rather broad signal was observed for H-7. The ^{13}C NMR and DEPT spectroscopic data of **4** (Table 1) indicated 11 signals for 13 carbons, consisting of one carbonyl, two quarternary carbons, nine methines, and one methylene. The signal of a quaternary carbon in the low-field region (δ_C 114.3) was assigned to the spiroketal carbon (C-5), since HMBC correlations of this signal to the H-3, H-4, H-6a, H-6b, H-7, and H-8 signals were observed (Table S1, Supporting Information). The complete structure and relative configuration of **4** were established by X-ray diffraction analysis. A view of the structure is provided in Figure 2.

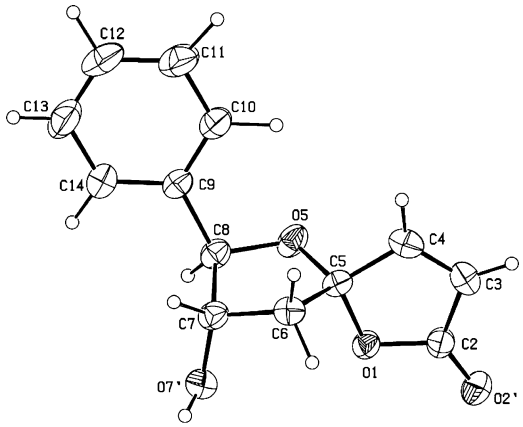


Figure 2. X-ray ORTEP diagram of crassalactone D (**4**).

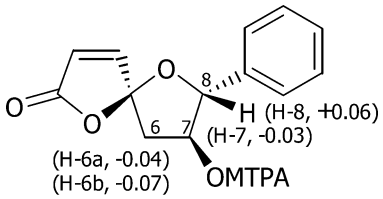


Figure 3. Distribution of $\Delta\delta_{S-R}$ values of the (*S*)- and (*R*)-MTPA esters of (+)-crassalactone D (**4**).

The absolute stereochemistry of **4** was determined by the Mosher ester method.[18] Crassalactone D (**4**) was reacted with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride at room temperature to give (*R*)- and (*S*)-MTPA esters [(*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid esters] of **4** in 21 and 19% yields, respectively. The observed chemical shift differences ($\Delta\delta_{S-R}$), shown in Figure 3, unambiguously established the absolute configuration at C-7 as *S*. Thus, the configuration in **4** was assigned as 5*S*, 7*S*, and 8*R*.

Pure isolated compounds (+)-**1**−(+)-**4**, the alkaloid aristolactam AII (**10**), and the (+)-tricinnamate **11** were evaluated for cytotoxic effects against a panel of cultured mammalian cell lines. The results are shown in Table 2. (+)-Crassalactone A (**1**) and (+)-crassalactone D (**4**) showed broad cytotoxic activity for all cell lines tested, while (+)-crassalactone B (**2**), **10**, and **11** exhibited moderate cytotoxic effects only for the P-388 cell line.

Table 2. Cytotoxicity of Compounds **1**−**4**, **10**, and **11** against Human and Rat Cancer Cell Lines

	cell line (ED_{50} μg/mL)[a]					
compound	P-388	KB	Col-2	BCA-1	Lu-1	ASK
1	0.18	1.7	1.9	0.92	1.9	1.6
2	3.8	>5	>5	>5	>5	>5
3	>5	>5	>5	>5	>5	>5
4	1.1	3.3	4.0	3.2	>5	3.1
10	2.7	>5	>5	>5	>5	>5
11	3.1	>5	>5	>5	>5	>5
ellipticine	0.52	0.65	0.53	0.65	0.56	0.60

[a] Cytotoxic assay: $ED_{50} \leq 5$ μg/mL is considered active; P-388: murine lymphocytic leukemia, KB: human nasopharyngeal carcinoma, Col-2: human colon cancer, BCA-1: human breast cancer, Lu-1: human lung cancer, ASK: rat glioma.

Although the presence of styryl-lactones in the genus *Polyalthia* is unusual, altholactone has been reported previously from an unnamed species of this genus.[15] Our work represents the second report on the discovery of styryl-lactones from the genus *Polyalthia*.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were recorded on a digital Electrothermal melting apparatus. Optical rotations were determined on a JASCO DIP 370 digital polarimeter using a 50 mm microcell (1 mL). UV spectra were measured in ethanol on a JASCO 530 spectrometer, and IR spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 500 spectrometer in $CDCl_3$, using TMS as internal standard. EIMS were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 eV (probe). The HRMS were recorded on a Micromass model VQ-TOF spectrometer. Solvents for extraction, chromatography, and recrystallization were distilled prior to use. Silica gel 60 (Merck, 70−230 mesh) and silica gel plates (Merck, Kieselgel 60F_{254}, 0.5 mm) were used for column chromatography and preparative TLC, respectively.

Plant Material. The leaves and twigs of *P. crassa* Parker (Annonaceae) were collected from Kangkrachan District, Petchburi Province, in October 2001 and identified by one of us (T.S.). A voucher specimen (BKF no. 109931) of *P. crassa* has been deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

Extraction and Isolation. The air-dried and finely powdered mixed leaves and twigs of *P. crassa* (2.6 kg) were sequentially percolated with hexane (5 × 4 L), EtOAc (5 × 3.5 L), and MeOH (5 × 4 L), at room temperature. Removal of solvents yielded the hexane, EtOAc, and MeOH extracts in 91, 184, and 203 g quantities, respectively.

The cytotoxic EtOAc extract (183 g) was subjected to silica gel column chromatography (1.6 kg), eluting with EtOAc−hexane (0−100%), followed by MeOH−EtOAc (0−100%) to give fractions A1−A11 after combination and removal of solvents. Fraction A5 (4.92 g, eluted with 8−10% EtOAc−hexane) was further separated by column chromatography (silica gel, 20% acetone−hexane as eluent), followed by recrystallization with EtOAc−hexane to give cinnamic acid (185.5 mg). Fraction A7 (7.57 g, eluted with 20−30% EtOAc−hexane) was purified by column chromatography (silica gel, 4% EtOAc−CH_2Cl_2 as eluent) to yield fractions B1−B6. Fraction B4 (100.6 mg) afforded (+)-3-acetylaltholactone (**8**) (31.4 mg) after separation by preparative TLC (20% acetone−hexane as eluent), followed by recrystallization from EtOH−CH_2Cl_2. Fraction B5 (3.01 g) yielded (+)-altholactone (**7**) (2.53 g) as a pure yellow oil after column chromatography (60% EtOAc−hexane as eluent). Fraction A8 (8.09 g, eluted with 30−35% EtOAc−hexane) was further separated using a silica gel column (MeOH−EtOAc−CH_2Cl_2, 1:1:48, as eluent) to provide an additional amount of pure **7** (4.56 g). Fraction A9 (16.6 g, eluted with 40−50% EtOAc−hexane) was rechromatographed on a Sephadex LH-20 column (MeOH as eluent) to give fractions C1−C4. Further separation of fraction C4 (7.77 g) on a silica gel column (MeOH−CH_2Cl_2−hexane, 1:12:7, as eluent) yielded fractions D1−D5. Fraction D3 (882.5 mg) was further separated by column chromatography (2% acetone−CH_2Cl_2 as eluent) to give fractions E1−E7. Fraction E1 (50.2 mg) afforded aristolactam AII (**10**) (33.6 mg) upon recrystallization with MeOH−C_6H_6. Fraction E2 yielded (+)-crassalactone B (**2**) (22.7 mg) after recrystallization with EtOH. Fraction E4 (882.5 mg), after preparative TLC (5% MeOH−CH_2Cl_2 as eluent) and recrystallization with EtOH, gave (+)-crassalactone C (**3**) (147.5 mg). Fraction E5 was further purified by column chromatography (40% EtOAc−hexane as eluent) to provide fractions F1−F4. Fraction F3 (252.9 mg) yielded (+)-crassalactone A (**1**) (67.7 mg) upon recrystallization with EtOAc−hexane. Fraction F4 gave (+)-goniopypyrone (**9**) (62.9 mg) after column chromatography (10% EtOAc−hexane as eluent) and recrystallization from EtOAc−hexane. Fraction E6 (2.38 g) afforded (+)-howiinol A (**5**) (390.9 mg) upon recrystallization with EtOH−CH_2Cl_2. An additional quantitiy of (+)-**5** (304.9 mg) was obtained after column chromatography (30% acetone−hexane as eluent) and recrystallization. Fraction D4 (941.6 mg) was further separated by passage on a silica gel column (10% EtOAc−CH_2Cl_2 as eluent) to yield fractions G1−G6. Fraction G1 yielded (+)-crassalactone D (**4**) (30.2 mg) after preparative TLC (5% EtOAc−CH_2Cl_2) and recrystallization with EtOAc−hexane. Fraction G3 (299.7 mg) provided (+)-goniofufurone (**6**) (87.8 mg) after column chromatography (50% EtOAc−hexane as eluent) and recrystallization with EtOAc−hexane. Fraction A10 (27.9 g, eluted with 50−80% EtOAc−hexane) was rechromatographed on a silica gel column (MeOH−CH_2Cl_2−hexane, 1:12:7, as eluent) to give fractions H1−H5. Fraction H3 (1.74 g) yielded an additional amount of (+)-**6** (128.3 mg) after separation on two consecutive silica gel columns (10% acetone−hexane and 2% MeOH−CH_2Cl_2 as eluents, respectively), followed by recrystallization with EtOAc−hexane.

(+)-Crassalactone A (1): white powder, mp 133−134 °C; $[\alpha]_D^{28}$ +326.0 (*c* 0.1, $CHCl_3$); $[\alpha]_D^{30}$ +329.6 (*c* 0.5, EtOH); UV (EtOH) λ_{max} (log ϵ) 224 (sh) (4.88), 277 (4.58) nm; IR (KBr) υ_{max} 3456, 1721, 1711, 1636, 1450, 1338, 1253, 1164, 1099, 1069, 985 cm^{-1}; ^{1}H and ^{13}C NMR, Table 1; EIMS *m*/*z* 380 $[M]^+$ (0.7), 362 $[M^+ - H_2O]$ (0.6), 344 $[M^+ - 2H_2O]$ (0.4), 273 (10), 178 (15), 148 (89), 131 (100); *anal.* C 69.44%, H 5.78%, calcd for $C_{22}H_{20}O_6$, C 69.47%, H 5.30%.

Preparation of (+)-Tricinnamate 11 from (+)-Crassalactone A (1). A mixture of (+)-crassalactone A (**1**) (38 mg, 0.1 mmol), cinnamic acid (32.6 mg, 0.22 mmol), DCC (47.4 mg, 0.23 mmol), and DMAP (48.9 mg, 0.40 mmol) in dry CH_2Cl_2 (6 mL) was stirred at room temperature for 3 h. The solution was quenched with water (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed successively with brine and water and dried over anhydrous $MgSO_4$. After solvent removal, the crude product (122.8 mg) was further purified by preparative TLC (silica gel, 40% EtOAc−hexane) to yield the (+)-tricinnamate **11** (26.4 mg, 41% yield).

Preparation of (+)-Tricinnamate 11 from (+)-Howiinol A (5). A mixture of (+)-howiinol A (**5**) (38 mg, 0.1 mmol), cinnamic acid (32.6 mg, 0.22 mmol), DCC (47.4 mg, 0.23 mmol), and DMAP (48.9 mg, 0.40 mmol) in dry CH_2Cl_2 (6 mL) was stirred at room temperature overnight (14 h). The solution was quenched with water (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed successively with brine and water and dried over anhydrous $MgSO_4$. After solvent removal, the crude product (125.9 mg) was purified by preparative TLC (silica gel, 2% MeOH−CH_2Cl_2) to afford the (+)-tricinnamate **11** (11.3 mg, 18% yield). Pure (+)-**11** was obtained as a white powder by recrystallization from EtOH−CH_2Cl_2, mp 195−197 °C; $[\alpha]_D^{29}$ +175.8 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 221 (sh) (2.79), 274 (2.89) nm; IR (KBr) ν_{max} 1731, 1721, 1710, 1636, 1577, 1496, 1450, 1308, 1164, 1039, 979 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.76 (1H, d, J = 16.0 Hz, H-3‴), 7.62 (1H, d, J = 16.0 Hz, H-3′), 7.57 (1H, d, J = 16.0 Hz, H-3″), 7.56−7.53 (2H, m, ArH), 7.49 (2H, dd, J = 7.9, 1.2 Hz, H-10, H-14), 7.43−7.30 (13H, m, Ar−H), 7.27−7.19 (3H, m, Ar−H), 7.05 (1H, dd, J = 9.7, 5.5 Hz, H-4), 6.49 (1H, d, J = 16.0 Hz, H-2‴), 6.37 (1H, d, J = 16.0 Hz, H-2″), 6.28 (1H, d, J = 6.7 Hz, H-8), 6.27 (1H, d, J = 16.0 Hz, H-2′), 6.23 (1H, d, J = 9.7 Hz, H-3), 5.94 (1H, dd, J = 6.7, 4.1 Hz, H-7), 5.52 (1H, dd, J = 5.5, 3.1 Hz, H-5), 4.93 (1H, dd, J = 4.1, 3.1 Hz, H-6); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 165.5 (s, C-1′), 165.2 (s, C-1″), 165.1 (s, C-1‴), 161.8 (s, C-2), 146.7 (d, C-3′), 146.33 (d, C-3‴), 146.27 (d, C-3″), 140.5 (d, C-4), 135.7 (s, C-9), 134.1 (s, C-4‴), 134.0 (s, C-4″), 133.7 (s, C-4′), 130.64, 130.60, 130.57, 128.91, 128.85, 128.82, 128.78, 128.6, 128.3 (d each, ArCH), 127.3 (d each, C-10 and C-14), 124.6 (d, C-3), 117.1 (d, C-2‴), 116.9 (d, C-2″), 116.1 (d, C-2′), 75.5 (d, C-6), 73.4 (d, C-8), 71.4 (d, C-7), 63.0 (d, C-5); EIMS *m*/*z* 640 $[M]^+$ (0.06), 147 (7), 131 (100), 103 (31), 77 (9).

(+)-Crassalactone B (2): white powder, mp 171−173 °C; $[\alpha]^{28}{}_D$ +8.0 (*c* 0.5, EtOH); UV (EtOH) λ_{max} (log ϵ) 224 (sh) (4.88), 279 (4.74) nm; IR (KBr) ν_{max} 3518, 1763, 1719, 1635, 1578, 1497, 1449, 1398,

1333, 1315, 1163, 1045, 901 cm^{-1}; ^1H and ^{13}C NMR, Table 1; EIMS *m/z* 380 $[M]^+$ (0.6), 362 $[M - H_2O]^+$ (2), 232 (20), 173 (20), 160 (78), 148 (100), 131 (99), 126 (26); HRTOFMS (ESI positive) *m/z* 403.1158 (calcd for $C_{22}H_{20}O_6Na$, 403.1158).

(+)-Crassalactone C (3): white powder, mp 147−150 °C; $[\alpha]^{30}_D$ +98.4 (*c* 0.5, EtOH); UV (EtOH) λ_{max} (log ϵ) 223 (sh) (4.65), 277 (4.46) nm; IR (KBr) ν_{max} 3460, 1775, 1716, 1682, 1638, 1578, 1498, 1450, 1333, 1167, 1068, 1047, 1004, 981 cm^{-1}; ^1H and ^{13}C NMR, Table 1; EIMS *m/z* 380 $[M]^+$ (1), 362 $[M - H_2O]^+$ (2), 335 (6), 275 (14), 232 (23), 173 (22), 148 (32), 131 (100); *anal.* C 69.19%, H 5.36%, calcd for $C_{22}H_{20}O_6$, C 69.47%, H 5.30%.

(+)-Crassalactone D (4): colorless needles, mp 138−139 °C; $[\alpha]^{30}_D$ +7.0 (*c* 0.2, EtOH); UV (EtOH) λ_{max} (log ϵ) 252 (2.89), 258 (2.90), 264 (2.80), 268 (sh) (2.64) nm; IR (KBr) ν_{max} 3469, 1758, 1750, 1608, 1500, 1455, 1413, 1347, 1312, 1191, 1129, 1097, 1056, 982, 925 cm^{-1}; ^1H and ^{13}C NMR, Table 1; EIMS *m/z* 233 $[M + H]^+$ (1), 214 $[M^+ - H_2O]$ (3), 126 (22), 107 (100), 97 (27), 79 (42); *anal.* C 67.53%, H, 5.70%, calcd for $C_{13}H_{12}O_4$, C 67.24%, H 5.21%.

Preparation of the (*R*)-MTPA Ester of (+)-4.[18] (*S*)-(+)-MTPA Cl (25.3 mg, 0.100 mmol) was added to a CH_2Cl_2 (1 mL) solution of crassalactone D (**4**) (11.9 mg, 0.051 mmol), DMAP (25.0 mg, 0.205 mmol), and Et_3N (0.32 mL, 0.24 M solution in CH_2Cl_2) at 0 °C. The reaction mixture was stirred at room temperature for 6 h. The solution was quenched with water (10 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed successively with brine and water and dried over anhydrous $MgSO_4$. After solvent removal, the crude product (18.6 mg) was purified by preparative TLC (silica gel, 5% MeOH−CH_2Cl_2) to give the (*R*)-MTPA ester of **4** (4.9 mg, 21% yield).

Preparation of the (*S*)-MTPA Ester of (+)-4.[18] (*R*)-(−)-MTPA Cl (22.9 mg, 0.091 mmol) was added to a CH_2Cl_2 (1 mL) solution of crassalactone D (**4**) (10.5 mg, 0.045 mmol), DMAP (22.1 mg, 0.181 mmol), and Et_3N (0.28 mL, 0.24 M solution in CH_2Cl_2) at 0 °C. The reaction mixture was stirred at room temperature for 4 h. The solution was quenched with water (10 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed successively with brine and water and dried over anhydrous $MgSO_4$. After solvent removal, the crude product (20.9 mg) was purified by preparative TLC (silica gel, 5% MeOH−CH_2Cl_2) to give the (*S*)-MTPA ester of **4** (4.4 mg, 19% yield).

X-ray Structure Determination of (+)-Crassalactone D (4). $C_{13}H_{12}O_4$, MW 232.24, monoclinic, $P2_1$, a = 9.7649(9) Å, b = 4.8852(3) Å, c = 11.8369(11) Å, β = 99.310 (3)°, V = 557.22 (8) Å^3, D_x = 1.384 g/cm^3, Z = 2, $F(000)$ = 244. A total of 6287 reflections, of which 1994 were unique reflections (1784 observed, $|F_o| > 4\sigma|F_o|$), were measured at room temperature from a 0.25 × 0.15 × 0.10 mm^3 colorless crystal using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a Bruker-Nonius kappaCCD diffractometer. The crystal structure was solved by direct methods using SIR-97, and then all atoms except hydrogen atoms were refined anisotropically by full-matrix least-squares methods on F^2 using SHELXL-97 to give a final *R*-factor of 0.0375 (R_w = 0.0986 for all data).

X-ray Structure Determination of (+)-Howiinol A (5). $C_{22}H_{20}O_6$, MW 380.396, orthorhombic, $P2_12_12_1$, a = 6.0723(4) Å, b = 12.0285(6) Å, c = 25.741(2) Å, V = 1880.1(2) Å^3, D_x = 1.344 g/cm^3, Z = 4, $F(000)$ = 800. A total of 6605 reflections, of which 2632 were unique reflections (1996 observed, $|F_o| > 4\sigma|F_o|$), were measured at room temperature from a 0.20 × 0.05 × 0.05 colorless crystal using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a Bruker-Nonius kappaCCD diffractometer. The crystal structure was solved by direct methods using SIR-97, and then all atoms except hydrogen atoms were refined anisotropically by full-matrix least-squares methods on F^2 using SHELXL-97 to give a final *R*-factor of 0.0505 (R_w = 0.1175 for all data).

Crystallographic data for structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition numbers CCDC 612409 for compound **4** and CCDC 612408 for compound **5**. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccds.cam.ac.uk).

Cytotoxicity Testing. Cytotoxicity assays of compounds **1**−**4**, **10**, and **11** were performed employing the colorimetric method as described by Skehan et al.,[19] and ellipticine was used as a positive control.

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Supporting Information Available: HMBC correlations observed for (+)-crassalactones A−D (**1**−**4**) and physical and spectroscopic properties of (+)-howiinol A (**5**) and (+)-goniofufurone (**6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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