FOUR NEW STYRYLLACTONES FROM *GONIOTHALAMUS LEIOCARPUS*

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Abstract – Four new styryllactones, leiocarpin A (**1**), 7-*epi*-goniodiol (**2**), leiocarpin B (**3**) and leiocarpin C (**4**), respectively, were isolated from the stem bark of *Goniothalamus leiocarpus*. Their structures were elucidated by means of spectral method. The relative configurations of **1**, **2** and **3** were determined by X-Ray crystallographic analysis. Compounds **2** and **3** possess antitumor activities.

Goniothalamus leiocarpus (Annonaceae family) is a tropical plant distributed in south of Yunnan province in China. We have isolated four known annonaceous acetogenins[1] from the seeds of *Goniothalamus leiocarpus*. In this paper, we report four styryllactones, named leiocarpin A (**1**), 7-*epi*-goniodiol (**2**), leiocarpin B (**3**) and leiocarpin C (**4**), respectively, isolated from the ethanolic extract of stem barks of the plant by repeat chromatography over silica gel. Their structures were elucidated by means of spectral. The relative configurations of **1**, **2** and **3** were determined by X-Ray crystallographic analysis. Compounds (**2**) and (**3**) showed activities in *vitro* anticancer test.

Leiocarpin A (**1**) was isolated as crystal, mp 132-134°C, $[\alpha]_D^{24}$ -98.4° (c 0.60 in $CHCl_3$). IR spectrum indicated the presence of a δ-lactone (1720 cm^{-1}) and a hydroxyl (3360 cm^{-1}) groups. ^1H and ^{13}C NMR spectra[2] showed that **1** had a phenyl (δ 7.25-7.43 ppm, 5H, m), four oxymethines (δ 65.62, 72.27, 73.92, and 76.38 ppm) and two methylenes (δ 29.58 and 36.35 ppm). These spectral data suggested that **1** was a styryllactone. The same molecular formula $C_{13}H_{14}O_4$ as 9-deoxygoniopypyrone (**5**)[3] was given by

measurements of EIMS at m/z 234 and HREIMS at 234.0890 (calcd 234.0892). The structure of **1** was established as 8-hydroxy-7-phenyl-2,6-dioxabicyclo[3,3,1]nonan-3-one, which was the same as that of **5**,

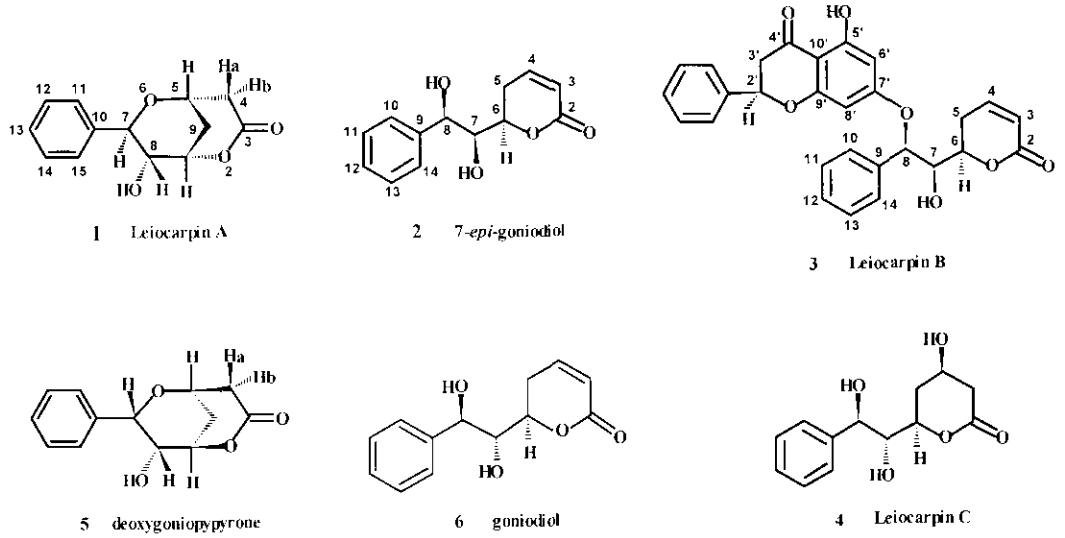

Figure 1 Structures of **1** - **6**

by analysis the ^{1}H, ^{13}C NMR, COLOC and MS spectra of **1**. Meanwhile, the spectra of ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY supported the above structure. However, the obvious distinction in $[\alpha]_D$ values (-98° for **1** and +12° for **5**), and difference of $J_{8/7}$ value and $J_{8/1}$ value between **1** and **5** revealed that there was distinction in configuration between the two compounds. Finally, the relative configuration of **1** was determined as 1*S**, 5*S**, 7*R** and 8*R** (Figures 1 and 2) by analysis of both NOESY spectrum and the X-Ray crystallographic data,[7] while, those of **5** were 1*R**, 5*R**, 7*S** and 8*R** (Figure 1).

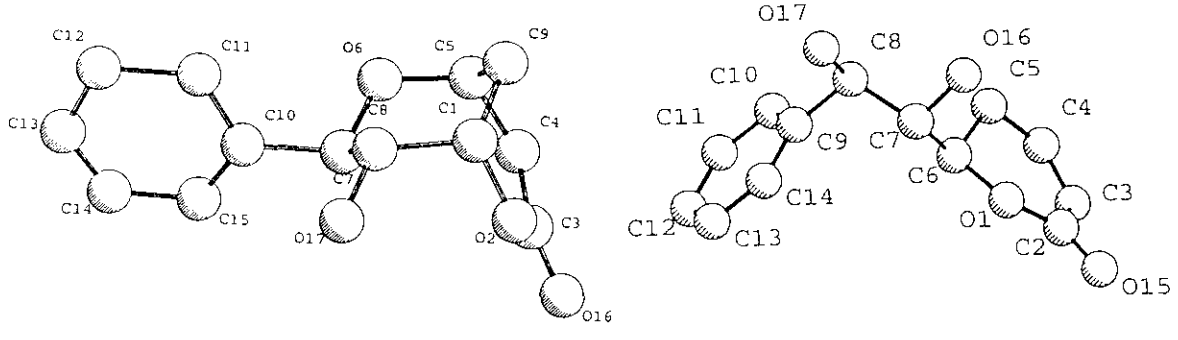

Figure 2 X-Ray Plot of **1** and **2**

Table 1. NMR Data of Compound (**2**) and (**4**)

(^{1}H NMR 400 MHz and ^{13}C NMR 100 MHz, δ, ppm; J, Hz, in C_5D_5N,)

Compound	**2**		**4**	
Position	H	C	H	C
2	----	164.16	----	174.34
3	6.95 ddd, 9.8,1.6, 0.8	121.13	3.48 dd, 15.4, 8.9; 3.13 dd, 15.4, 5.4	41.20
4	6.82 ddd, 9.8, 5.8, 2.6	146.55	4.79 m*	69.17
5	2.78 ddt, 18.7, 10.9, 2.6; 2.71 ddd, 18.7, 5.8, 4.8	25.93	2.43 ddd, 13.2, 6.8, 6.6; 2.17 dd, 13.2, 4.2	35.54
6	4.90 ddd, 10.9, 5.8, 4.8	78.62	4.39 dt, 7.5, 3.6	67.64
7	4.30 dd, 5.8, 3.7	72.81	4.68 dd, 5.8, 2.9	71.77
8	5.39 d, 3.7	76.95	5.51 d, 5.8	74.96
9	----	144.25	----	141.15
10, 14	7.74 d, 7.3	127.49	7.79 d, 7.6	127.95
11, 13	7.39 t, 7.3	128.62	7.35 t, 7.6	128.64
12	7.29 t, 7.3	127.57	7.25 t, 7.6	127.54

* Only J=5.4 Hz could be measured in the ^{1}H NMR spectrum

Compound (**2**), $[\alpha]_D^{20}$ +85.4°(c 0.3, in MeOH), had the molecular weight suggested by a prominent peak at m/z 235 $[MH]^+$ in the FABMS spectrum. The presence of two hydroxyl groups was indicated by peaks at m/z 217 $[MH\text{-}H_2O]^+$ and 199$[MH\text{-}2H_2O]^+$ in the FABMS spectrum as well. The hydroxyl group at 3422 cm^{-1} and α, β-unsaturated δ-lactone band at 1704 cm^{-1} were present in the IR spectrum. The same molecular formula $C_{13}H_{14}O_4$ and planar structure as a known styryllactone compound, goniodiol (**6**),[4] was given by the data of ^{1}H and ^{13}C NMR spectra of **2**. Whereas the careful examination of the ^{1}H NMR gave distinguished differences of H-6, H-7 and H-8 either in chemical shifts or in coupling constants between **2** and goniodiol. The coupling constants of H-6/H-7 and H-7/H-8 in **2** were 5.8 and 3.7 Hz, while those in **6** were reported to be 2.2 and 7.0 Hz (6,7-*threo* and 7,8-*erythro*), respectively. This means that there were distinction of configuration in H-6, H-7 and H-8 between **2** and goniodiol. Finally, the relative configuration of **2** was established as 6,7-*erythro* and 7,8-*threo* or 6*R**, 7*S** and 8*R** by comparison of coupling constants and crystallographic analysis of X-ray,[7] and **2** was therefore determined as 7-*epi*-goniodiol (Figures 1 and 2).

Leiocarpin B (**3**) was colorless needle, mp 189-191°C, $[\alpha]_D^{24}$ +28.8° (c 0.5 in $CHCl_3$). The IR spectrum of **3** presented a hydroxyl band at 3500 cm^{-1} and the carbonyl peak of a unsaturated δ-lactone at 1700 cm^{-1}. The ^{13}C NMR showed the existence of 28 carbons (Table 2), which were respectively attributed to two structural units: 7-*epi*-goniodiol (**2**) and pinocembrin (5,7-dihydroxydihydroflavone); two mono-substituted phenyls and the other 12 protons were also respectively corresponding to the above two structures in the ^{1}H and ^{13}C NMR. The molecular weight of **3** was indicated by a prominent peak at m/z

Table 2. NMR Spectral Data of Leiocrpin B (**3**)

(^{1}H NMR 400 MHz and ^{13}C NMR 100 MHz, δ, ppm; J, Hz, in C_5D_5N)

No.	H	C	No.	H	C
2	----	163.65	3'	3.12 dd, 17.1, 13.2; 2.78 dd, 17.1, 3.0	43.42
3	6.10 dd, 9.8, 1.8	121.17	4'	----	196.41
4	6.93 ddd, 9.8, 2.6, 1.8	146.17	5'	----	164.47
5	2.17 dt, 13.2, 6.7; 2.77 ddd, 18.9, 13.2, 2.6	26.21	6'	4.39 d, 2.3	97.62
6	4.96 ddd, 6.8, 4.6, 4.4	77.94	7'	----	167.04
7	4.38 dd, 6.8, 3.2	75.76	8'	4.48 d, 2.3	95.91
8	6.01 d, 3.2	80.01	9	----	163.42
9	----	138.82	10'	----	103.85
10, 14	7.25-7.70 m	127.64	1"	----	139.30
11, 13	7.25-7.70 m	129.10	2", 6"	7.25-7.70 m	126.83
12	7.25-7.70 m	128.49	3", 5"	7.25-7.70 m	129.10
2'	5.34 dd, 13.2, 3.0	79.52	4"	7.25-7.70 m	129.04

472 in the EIMS spectrum, and the molecular formula $C_{28}H_{24}O_7$ was determined by the peak at m/z 472.1536 (calcd 472.1552) in the HREIMS. The structure of **3** was established as Figure 1 by the spectra of ^{1}H-^{1}H COSY, HECTOR and NOESY. 5'-Hydroxy-7'-*O*-dihydroflavone group positioned at C-8, that was suggested by the long-range coupling signal between H-8 and C-7' in the COLOC spectrum. The structure of **3** (Figure 3) was conformed by X-Ray crystallographic analysis.[7]

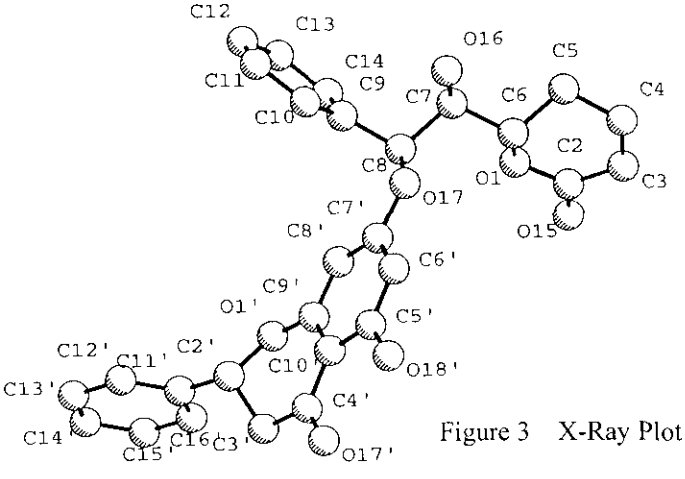

Figure 3 X-Ray Plot of **3**

Compound (**2**) and (**3**) showed selective activities in test of trypan blue dye exclusion method. Under different concentration of 100, 10, 1, 0.1, 0.01 μ g/mL, the inhibition against HL-60 cells of compound (**2**) were 100, 100, 41, 21, 27%; and those of **3** were 100, 100, 55, 20, 4%. IC_{50} against Bel7404 (Hepatocarcinoma), Bcap32 (Breast Cancer), Hela of **2** were 0.96, 12.8, 3 μg/mL respectively; while those of **3** were 0.79, >100, 30 μg/mL against HL-60, K-562, U937 (Leukemia).

Leiocarpin C (**4**) was isolated as needle, mp 131-132 °C, $[\alpha]_D^{24}$ -63.9° (c 0.46, $CHCl_3$). The molecular weight of **4** was indicated by a prominent peak at m/z 253$[MH]^+$ in the FABMS and a peak at m/z 252 $[M]^+$ in the EIMS. The HRFABMS gave m/z 253.0984 (calcd 253.1076) for MH^+ of **4**, corresponding to the molecular $C_{13}H_{16}O_5$. The presence of three hydroxyl groups was indicated by peaks at m/z 235 $[MH-H_2O]^+$, 217 $[MH-2H_2O]^+$, 199 $[MH-3H_2O]^+$ in the EIMS and 234 $[M-H_2O]^+$, 216 $[M-2H_2O]^+$, 198 $[M-3H_2O]^+$, and by two absorption bands at 3400 and 3260 cm^{-1} in the IR spectrum. The existence of a saturated δ-lactone was supported by carbonyl group absorption bands at 1710 and 1690 cm^{-1} in the IR spectrum. By analysis of the ^1H NMR spectral data (Table 1), the molecular structure of **4** was established as 6-(7,8-dihydro-7,8-dihydroxystyryl)-3,4,5,6-tetrahydro-4-hydroxy-2-pyrone (Figure 1).

The relative configuration C-6, C-7 and C-8 of **4** could be determined by careful examination of coupling constants between H-6 and H-7, H-7 and H-8.[4,5] The coupling constants H-6/H-7 and H-7/H-8 in goniodiol (**6**) were reported to be 2.2 and 7.0 Hz (6,7-*threo*-7,8-*erythro*).[4,6] In compound (**4**), the constants H-6/H-7 and H-7/H-8 were observed to be 2.9 and 5.8 Hz respectively. So, the relative configuration of H-6/H-7 and H-7/H-8 in **4** agreed with that of **6**, and was determined as 6,7-*threo* and 7,8-*erythro* (Figure 1).

In the NOE spectrum of **4**, the presence of correlation of Ph-H/H_2-3, Ph-H/H_2-5, and the absence of correlation of Ph-H/H-6, Ph-H/H-4 suggested that H-6 and H-4 positioned on the same plane. Since H-6 was arranged in α-orientation in the determined configuration (6,7-*threo* -7,8-*erythro*), H-4 was therefore assigned on α-orientation. That was to say, the hydroxyl group at C-4 was identical as 4β-OH. Thus, the relative configuration of leiocarpin C (**4**) was determined as 6,7-*threo* -7,8-*erythro* and 4β-OH (Figure 1).

EXPERIMENTAL

General Experimental Procedures -- Melting points were taken on a Koffler melting point apparatus and uncorrected. The UV spectra were obtained using a UV-210A Spectrophotometer. The IR spectra were measured on a Perkin-Elmer-577 Spectrophotometer. MS were performed on a Autospec-3000 Spectrometer and EIMS under 70ev. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, with a Brucker AM-400 Spectrometer. Elemental analyses were carried out on an EA-MOD1106 instrument. Silica gel-H (made in Qingdao Marine Chemical and Industrial Factory, China) was used for column chromatography and pre-coated Silica-G plates were employed for analytical TLC.

Plant Material -- The stem bark of *Goniothalamus leiocarpus* used in this investigation was collected in south of Yunnan province, China. A voucher specimen of this plant was deposited in Kunming Institute of Botany, Kunming, China.

Extraction and Isolation -- The powdered the stem bark (5 kg) was extracted with EtOH (10 L×3) for 72 h at rt. The alcohol was concentrated and then dried *in vacuo* to give 830 g of the dark brown resin. 200 g of EtOH extract was separated into three fractions by silica gel column (500 g) chromatography with $CHCl_3$, EtOAc and MeOH, repeatedly. The Fr. 1 (88 g) was carried out silica gel chromatography with gradient mixture of $CHCl_3$ and MeOH, and gave the crude crystals of **2** (800 mg, $CHCl_3$-MeOH 99:1), **1** (3.2 g, $CHCl_3$-MeOH 98:2), **3** (1.2 g, $CHCl_3$-MeOH 95:5) and **4** (510 mg, $CHCl_3$-MeOH 90:10). These crude crystals were recrystallized in the different mixture of solvents to yield colorless needles of **1** (2 g, Me_2CO-Petrol), **2** (500 mg, EtOAc-Ben), **3** (800 mg, $CHCl_3$-MeOH) and **4** (470 mg, MeOH).

Bioassays -- Activity test were performed according to MTT method. Cancer cells with concentration of $1.2 10^5$ cells/mL were inoculated into every cell of 96-well microculture. The cells were acted with different concentration of the compounds, and OD (optical density) values were taken on with microdlisa reder.

Leiocarpin A(**1**) mp 132-134 °C (Me_2CO-Petrol Ether); $[\alpha]_D^{24}$ -98.42° (c 0.6, $CHCl_3$); UV(MeOH)λ_{max}: 207 (log ε 3.29) nm; IR (KBr)ν_{max} 3360 (hydroxyl), 1720 (δ-lactone), 1660, 1180 cm^{-1}; EIMS m/z(%): 234(60) $[M]^+$, 216(7) $[M-H_2O]^+$, 188 (10), 177(17), 144(15), 128(35), 107(100), 91(40), 77(43), 69(80); HREIMS m/z 234.0890 for $C_{13}H_{14}O_4$ (calcd 234.0892); ^1H and ^{13}C NMR spectral data see References and Note.[2] Anal. Calcd for $C_{13}H_{14}O_4$: C, 66.67; H, 5.98. Found: C, 67.00; H, 6.01.

7-*epi*-goniodiol (**2**) ($CHCl_3$-MeOH) $[\alpha]_D^{23}$ +85.42° (c 0.6, MeOH); UV(MeOH)λ_{max}: 206 (log ε 3.20); IR (KBr)ν_{max}: 3422, 2930, 1704, 1386, 1261, 1082, 1020 cm^{-1}; EIMS m/z(%): 216(33) $[M-H_2O]^+$, 200(13), 170(7), 155(13), 128(78), 110(50), 105(67), 91(100), 77(84); FABMS m/z: 235 $[MH]^+$, 217 $[MH-H_2O]^+$; ^1H and ^{13}C NMR (in C_5D_5N) see Table 1. Anal. Calcd for $C_{13}H_{14}O_4$: C, 66.67; H, 5.98. Found: C, 66.84; H, 6.09.

Leiocarpin B (**3**) mp 189-191°C (EtOAc-Benzene); $[\alpha]_D^{24}$ +28.79° (c 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 210(3.65), 289(3.33), 318(2.59) nm; IR(KBr)ν_{max}: 3500, 3040, 2910, 1700, 1620, 1560, 1240, 1160 cm^{-1}; EIMS m/z(%): 472(45) $[M]^+$, 345(48), 303(19), 256(78), 241(27), 179(66), 152(54), 131(60), 97(100), 69(94); HREIMS m/z 472.1536 for $C_{28}H_{24}O_7$ (calcd 472.1552); ^1H and ^{13}C NMR (in C_5D_5N) see Table 2. Anal. Calcd for $C_{28}H_{24}O_7$: C, 71.18; H, 5.08. Found: C, 71.07; H, 5.10.

Leiocarpin C (**4**) mp 131-132 °C (MeOH); $[\alpha]_D^{24}$ -63.5° (c 0.5, MeOH); UV(MeOH)λ_{max}: 207(log ε 2.90); IR (KBr)ν_{max}: 3400, 3200, 2920, 1710, 1690, 1485, 1440, 1200, 1100 cm^{-1}; EIMS m/z(%): 252(1) $[M]^+$, 234(60) $[M-H_2O]^+$, 216(10) $[M-2H_2O]^+$, 198(3) $[M-3H_2O]^+$, 188(5), 157(9), 128(47), 107(100), 91(80), 77(74), 60(90); HRFABMS m/z 253.0984 (calcd 253.1076) for MH^+, ^1H and ^{13}C NMR see Table 1. Anal. Calcd for $C_{13}H_{16}O_5$: C, 61.90; H, 6.35. Found: C, 61.66; H, 6.37.

X-Ray Crystallographic Analysis of **1** Data collection -- A colorless rod of $C_{13}H_{14}O_4$, monoclinic. The intensity data collection were performed on a MAC DIP-2030K Probing Apparatus with MoKα radiation and monochromator; the distance between the crystal and IP plate was 120mm(d=120mm); ω scan with 0-180°; oscillation angles Δϕ=3°. The crystal data and data collection parameters were given in Table 3.

Table 3. Crystal Data and Data Collection Parameters of Compounds (**1**, **2** and **3**)

Data and parameters	**1**	**2**	**3**
Formula	$C_{13}H_{14}O_4$	$C_{13}H_{14}O_4$	$C_{28}H_{24}O_7$
Molecular weight	234	234	472
Space group	$P2_1$	P1	$P2_1$
a, Å	7.137(1)	8.668(1)	9.782(1)
b, Å	35.495(3)	8.686(1)	11.457(4)
c, Å	9.312(1)	9.576(1)	11.610(1)
α, °	----	109.20(1)	----
β, °	91.14(1)	116.89(1)	114.80(8)
γ, °	----	90.03(1)	----
V, Å^3	2358.5(5)	597.6(1)	1183.11(49)
Z	2	2	2
D_c, g·cm^{-3}	1.314	1.302	1.329
Crystal dimensions, mm	0.4×0.5×0.6	----	0.4×0.7×0.3
2θ rang, °	0-180	0-50	0-50
Data collected	3618	2092	2171
Unique data	3273	1844	1692
Rf	0.133	0.043	0.041
Rw (w=1/1/σ^2|F|)	0.128	0.050	0.046
(Δ/σ)max	----	0.035	0.265
(Δρ)max e/Å^3	----	0.260	0.250
(Δρ)min e/Å^3	----	-0.210	-0.200

Structure solution and refinement -- The structure was solved using structure direct methods (SHELEXS-97). Initial carbon and oxygen atom coordinates were obtained from an E map. Using a series of difference Fourier syntheses and leas-squares, 18 non-hydrogen atoms were located and their position were corrected, and the kind of atoms were determined. Hydrogen atoms except for which belonging hydroxyl groups were obtained in geometrical add-hydrogen method. R_f = 0.133, Rw = 0.128(w=1/σ^2|F|), GoF = 17.921. The crystal data and data collection parameters were given in Table 3.

X-Ray Crystallographic Analysis of **2**. Data Collection -- A colorless piece of $C_{13}H_{14}O_4$, triclinic. The intensity collection were performed using MoKα radiation and graphite monochromater on an NoniousCAD-4 four-circle diffractometer, with the ω scans, 0°<2θ<50°. 2092 reflection spots were collected and 1844 unique reflections were considered.

Structure Solution and Refinement -- The crystal structure was solved using direct method (SHELEXS-86). 34 non-hydrogen atoms were obtained from E map. Hydrogen atoms were obtained in succeeding difference Fourier syntheses and the structural parameters were refined in full-matrix least-squares, R_f= 0.043, R_w = 0.050 ($w=1/\sigma^2|F|$), $(\Delta/\sigma)_{max}$ = 0.035, $(\Delta\rho)_{max}$=0.260 e/Å^3, $(\Delta\rho)_{min}$=-0.210 e/Å^3, s=1.052. The crystal data and data collection parameters were given in Table 3.

X-Ray Crystallographic Analysis of **3**. Procedures were essentially the same as those followed for the X-Ray crystallographic analysis of **2**, except the hydrogen positions and isotropic thermal parameters of **3** were refined, and the crystal data and collection were given in Table 3.

ACKNOWLEDGEMENTS

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2. NMR Data of Leiocarpin A (**1**). ^{1}H NMR (400 MHz in C_5D_5N) δ: 4.80 (br s, 1H, H-1), 2.81 (dd, J = 5.0, 19.5 Hz, 1H, H-4a), 2.90 (d, J = 19.5 Hz, 1H, H-4b), 4.35 (br s, H-5), 4.41 (d, J = 8.8 Hz, 1H, H-7), 3.45 (d, J = 8.8 Hz, 1H, H-8), 2.11 (br s, 2H, H_2-9), 7.25 - 7.43 (m, 5H, Ph); ^{13}C NMR data of **1** δ (ppm, in $CDCl_3$, 100 MHz): 76.88 (C-1), 169.21 (C-3), 36.35 (C-4), 65.62 (C-5), 73.92 (C-7), 72.27 (C-8), 29.58 (C-9), 138.21 (C-10), 127.41 (C-11, 15), 128.27 (C-12,14), 128.27 (C-13).
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