CYTOTOXIC 6-SUBSTITUTED 5, 6-DIHYDRO-2*H*-PYRAN-2-ONES FROM A BRAZILIAN MEDICINAL PLANT, *CHORISIA CRISPIFLORA*

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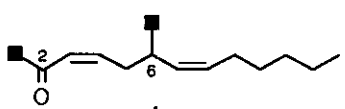
Abstract --- Three cytotoxic 6-substituted 5, 6-dihydro-2*H*-pyran-2-ones (**1** - **3**) were isolated from a Brazilian medicinal plant, *Chorisia crispiflora*. **1** was identified as argentilactone, and the structures of two new compounds (**2** and **3**) were determined on the basis of the physicochemical properties.

In the course of our program to isolate new biologically active compounds from Brazilian medicinal plants,[1] the methanolic extract of *Chorisia crispiflora* (Bombacaceae) ("Invira-tanha" or "Paineira" in Brazil), one of the folk medicines used for rheumatism and menorrhalgia, was found to show a strong cytotoxic activity against P-388 mouse leukemia cells. The investigation on the active principles led to the isolation of three cytotoxic compounds. In this paper, we report the isolation and structure determination of these compounds.

The methanolic extract of the leaves of *C. crispiflora* was extracted with EtOAc and *n*-BuOH. A large portion of the cytotoxic activity passed into the EtOAc fraction, which was subjected to column chromatography on silica gel repeatedly to afford compounds I, II, and III.

Compound I (**1**), $[\alpha]_D$ -18.0°(*c* 0.82, $CHCl_3$), was isolated as colorless oil, and the molecular formula $C_{12}H_{18}O_2$ was established by the HREI ms (*m/z* 194.1314, M^+) and ^{13}C nmr spectrum.[2] The ^1H nmr spectrum of **1** (Table)[3] exhibited signals due to a primary methyl group (δ 0.89), five methylene groups (δ 1.20 - 2.40), a methine group (δ 5.22), a *cis* double bond (δ 5.56 and δ 5.66), and an enone group (δ 6.05 and δ 6.90). Analysis of ^1H-^1H and ^1H-^{13}C COSY spectra of **1** revealed the connection of all signals to give the partial structure **A**.

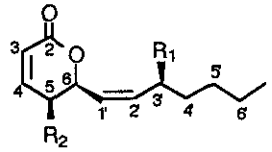


A

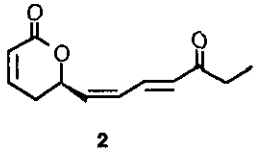
Considering the molecular formula, C-6 and C-2 should be connected through an oxygen atom, and the α, β-unsaturated δ-lactone structure (**1**) was given for compound I, which was supported by the ir (1720 cm^{-1}) and uv (211.2 nm, log ε 3.69) spectra. This is the same structure with that of argentilactone isolated from *Aristrochia argentina*.[4] The identification was made by comparison of both spectral data including the optical rotation ($[\alpha]_D$ -21.1°(c 2.25, EtOH)). Thus, compound I was concluded as (*R*)-6-[(*Z*)-1-heptenyl]-5, 6-dihydro-2*H*-pyran-2-one (**1**).

Compound II (**2**), $[\alpha]_D$ -50.8°(*c* 0.18, $CHCl_3$), colorless oil, had the molecular formula $C_{12}H_{14}O_3$ as determined by HREI ms (*m/z* 206.0953, M^+). The presence of 6-substituted 5, 6-dihydro-2*H*-pyran-2-one in **2** was deduced by the ir (1725 cm^{-1}), uv (206.0 nm, log ε 4.07) and ^1H nmr (δ 2.46, δ 5.49, δ 6.10, and δ 6.92) (Table) spectra. The existence of a dienone system was also deduced by the ^1H nmr (δ 5.97, 1H, dd, *J*=11.0 and 10.0 Hz; δ 6.29, 1H, t, *J*=11.0 Hz; δ 7.38, 1H, dd, *J*=15.0 and 11.0 Hz; δ 6.30, 1H, d, *J*=15.0 Hz) and uv (264.6 nm, log ε 4.14) spectra together with the ^{13}C nmr spectrum (a carbonyl carbon signal at δ 201.0).[3] Analysis of the ^1H nmr spectrum with the aid of decoupling technique demonstrated the dienone group to be located at 6-position of the pyranone. Thus, the remaining ethyl group (δ 1.12, 3H, t, *J*=7.3 Hz; δ 2.61, 2H, q, *J*=7.3 Hz) should be connected to the carbonyl group of the dienone, and the structure of **2** was given for compound II. The configurations of the dienone group were concluded as 1'*Z* and 3'*E* by considering the coupling constants ($J_{1'-2'}$=11.0, $J_{3'-4'}$=15.0 Hz) in the ^1H nmr spectrum. The cd spectrum of **2** exhibited split Cotton effects ($\Delta\varepsilon_{264}$-4.7 and $\Delta\varepsilon_{209}$+9.0) due to the interaction of π–π* transitions of the enone and the dienone, indicating the configuration at C-6 is *R* .[5] Compound II, therefore, was concluded to be (*R*)-6-[(1*Z*, 3*E*)-5-oxo-1,3-heptadienyl]-5, 6-dihydro-2*H*-pyran-2-one (**2**).

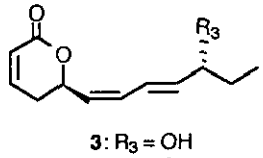
Compound III (**3**), $[\alpha]_D$ -117.0° (*c* 0.99, $CHCl_3$), was isolated as colorless oil. The molecular formula $C_{12}H_{16}O_3$ of **3** was deduced from the ^{13}C nmr spectrum of **3** and the HREI ms of the acetate **4** (*m/z* 250.1224, M^+, $C_{14}H_{18}O_4$). The ^1H nmr, ^{13}C nmr,[3] ir (1730 cm^{-1}) and uv (228.0 nm, log ε 4.45) spectra of **3** quite resembled to those of **2**, and the differences are as follows: In the ^1H nmr spectrum of **3**, the signals due to H-1', H-3' and H-4' showed upfield shifts compared to those of **2**, and a methine signal (H-5') was observed at δ 4.15, which



1: $R_1 = R_2 = H$
5: $R_1 = R_2 = OAc$



2



3: R_3 = OH
4: R_3 = OAc

Table. ^{1}H Nmr spectral data of compound I (**1**), II (**2**) and III (**3**)[3]

	1	**2**	**3**
H-3	6.05 (1H, ddd, J=11 0, 2.5, 1.0)	6.10 (1H, dd,J=10.0, 2 3)	6.07 (1H, dt, J=10.0, 2.0)
H-4	6.90 (1H, ddd, J=11.0, 6.0, 3.0)	6.92 (1H, dt, J=10.0, 2 0)	6.91 (1H, m)
H-5	2.40 (2H, m)	2.46 (2H, m)	2.43 (2H, m)
H-6	5.22 (1H, ddd, J=11.0, 8.3, 4.4)	5.49 (1H, m)	5.39 (1H, dt, J=8.8, 5.0)
H-1'	5.56 (1H, t, J=11.0)	5.97 (1H, dd, J=11 0, 10 0)	5.56 (1H, dd, J=11.0, 8 8)
H-2'	5.66 (1H, dt, J=11.0, 7.3)	6.29 (1H, t, J=11 0)	6.20 (1H, t, J=11.0)
H-3'	2.10 (2H, m)	7.38 (1H, dd, J=15.0, 11.0)	6.48 (1H, dd, J=15 0, 11.0)
H-4'	1.40 (2H, m)	6.30 (1H, d, J=15.0)	5.85 (1H, dd, J=15.0, 7.3)
H-5'	1.20 (2H, m)	—	4.15 (1H, q, J=7.3)
H-6'	1.20 (2H, m)	2.61 (2H, q, J=7.3)	1.57 (2H, quin, J=7.3)
H-7'	0.89 (3H, t, J=7.3)	1.12 (3H, t, J=7.3)	0.94 (3H, t, J=7.3)

exhibited a downfield shift (δ 4.80) on acetylation; The carbonyl carbon signal of **2** (δ 201.0) disappeared in the ^{13}C nmr spectrum of **3**; The uv absorption of **2** due to the dienone moiety disappeared in **3**. These facts suggested that **3** has a secondary hydroxy group at C-5'. Thus, compound III was oxidized by CrO_3 to the enone, which was identical with **2** in all respects including cd spectrum. The configuration at C-5' was determined by modified Mosher's method.[6] The Δ values of *S*-(-)- and *R*-(+)-MTPA esters of **3**[7] indicated the absolute configuration at C-5' to be *R*. In conclusion, compound III was elucidated as (6*R*, 5'*R*)- 6-[(1*Z*, 3*E*)-5-hydroxy-1,3-heptadienyl]-5, 6-dihydro-2*H*-pyran-2-one (**3**).

Compounds I, II, and III showed a cytotoxic activity against P-388 mouse leukemia cells with IC_{50} values of 4.15, 0.53 and 1.52 μg/ml, respectively. There are a group of cytotoxic 6-substituted 5, 6-dihydro-2*H*-pypan-2-ones such as pectinolides A (**5**),[8] and it is thought that the cytotoxic activity of these compounds is due to the α, β-unsaturated δ-lactone nucleus. Compounds II and III have another dienone or diene system, whose effects on the cytotoxicity are being studied.

ACKNOWLEDGMENT

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2. ^{13}C Nmr data: **1** (125 MHz, $CDCl_3$) δ 13.9 (q), 22.4 (t), 27.8 (t), 29.0 (t), 29.9 (t), 31.4 (t), 73.9 (d), 121.6 (d), 126.4 (d), 135.7 (d), 144.9 (d), 164.2 (s). **2** (75 MHz, $CDCl_3$) δ 8.4 (q), 30.2 (t), 35.3 (t), 74.1 (d), 122.1 (d), 130.4 (d), 132.5 (d), 134.6 (d), 135.6 (d), 144.8 (d), 163.7 (s), 201.0 (s). **3** (125 MHz, $CDCl_3$) δ 10.1 (q), 29.4 (t), 29.5 (t), 73.2 (d), 74.5 (d), 122.3 (d), 125.1 (d), 127.1 (d), 133.8 (d), 141.2 (d), 146.0 (d), 164.1 (s).

3. ^1H Nmr spectra were recorded as $CDCl_3$ solution at 500 or 300 MHz with TMS as internal standard.

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7. Δ values = δ [*S*-(-)-MTPA ester] - δ [*R*-(+)-MTPA ester]: H-3 (-0.004), H-4 (-0.005), H-5 (-0.026), H-6 (-0.007), H-1' (-0.032), H-2' (-0.051), H-3' (-0.150), H-4' (-0.089), H-5' (-0.120), H-6' (+0.032), H-7' (+0.093)

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