HETEROCYCLES, Vol. 57, No. 2, 2002, pp. 337-340, Received, 19th November, 2001

THE ABSOLUTE CONFIGURATION OF LEIOCARPIN B

Qing Mu,[++] Weidong Tang,[+] Chaoming Li, Yongping Xu,[+] Handong Sun, Liguang Lou,[+] Xiaojiang Hao,* Bin Xu,[+] and Chang Qi Hu[++]

Phytochemistry Laboratory, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming 650204, China [+]Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Science, CAS, Shanghai 200032, China [++] School of Pharmacy, Fudan University, Shanghai 200032, China

Abstract - Leiocarpin B, a natural compound isolated from the plant, *Goniothalamus leiocarpus*, is a styryllactone showing anticancer activity *in vitro* test. Its chemical structure and relative configuration were determined by means of spectral methods and X-Ray crystallographic analysis. In the present investigation, its absolute configuration was determined using the Mosher's method and its anticancer activities were tested *in vivo*.

Leiocarpin B is an anticancer styryllactone isolated from a tropical plant of *Goniothalamus leiocarpus* (Annonaceae family).[1] There are four stereogenic carbon atoms in the compound **(1)**, and its chemical structure including relative configuration has been previously determined by spectroscopy and X-Ray crystallography.[2] However, the absolute configuration of **1** has remained undetermined. We report here the absolute configuration of **1** as determined by the Mosher's method[3-5] using the ^{1}H NMR anisotropy effect of MTPA esters (Figure 1).

Leiocarpin B (**1**) was obtained as colorless needles, mp 189-191 ^{o}C, $[\alpha]_D^{24}$ +28.8 ^{o} (c 0.5 in $CHCl_3$). To elucidate the absolute configuration of C-7, the hydroxyl group at C-7 was esterified with (*S*) - MTPA (α-methoxy-α-(trifluoromethyl)phenylacetic acid) and (*R*) - MTPA, respectively to yield diastereomeric

esters **(2a** and **2b)** (Figure 2 and Experimental section). The Mosher's rule suggests that these esters **(2a** and **2b)** take preferred conformations as shown in Figure 2, where the hydrogens on the same side with

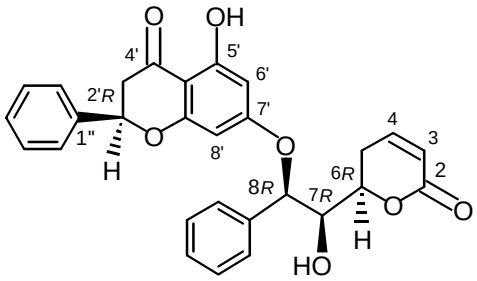
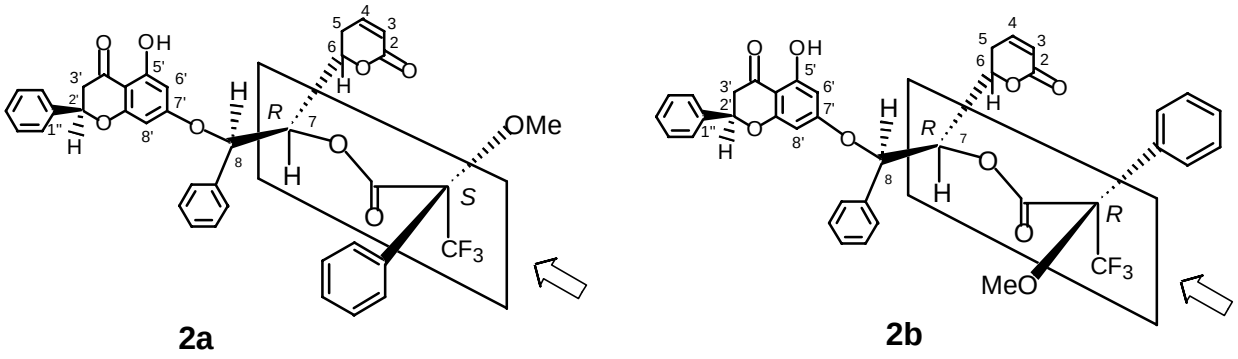


Figure 1. Structure of leiocarpin B (**1**)

the phenyl group of MTPA feel the diamagnetic anisotropy effect generated by the phenyl group leading to high field shifts. All proton signals of **2a** and **2b** were fully assigned by ^{1}H NMR and ^{1}H -^{1}H COSY spectra (EXPERIMENTAL). The Mosher's parameter $\Delta\delta$ (= δ_S-δ_R) was calculated for each proton as shown in Table 1. Since H-3 ~ H-6 protons have positive $\Delta\delta$ values, the rule indicates that those hydrogens are located on the right side of the MTPA plane. Namely in ester **(2b)**, those hydrogens are on the same side with phenyl group feeling the diamagnetic anisotropy effect and giving smaller δ values in ester **(2b)** and larger δ values in **2a**. Therefore the $\Delta\delta$ values become positive. On the other hand, H-8, H-2' ~ H-8' protons show negative $\Delta\delta$ values, and therefore those hydrogens are on the left side of the MTPA plane leading to the 7*R* configuration. Since the relative configuration of leiocarpin B (**1**) had been established by X-Ray crystallography, the absolute configuration of 1 was determined as 6*R*, 7*R*, 8*R* and 2'*R*.



2a **2b**

Figure 2. (*S*)- and (*R*)-MPTA esters of leiocarpin B (**1**)

Leiocarpin B showed selective activities against HL-60 and U937 (Leukemia) *in vitro* anticancer test.[2] *In vivo* test, **1** against H22 tumor showed inhibitory rate as 14.2 % (17 mg/kg) and 41.0 % (24 mg/kg), while cytoxan as the positive control was 65 % (30 mg/kg).

Table 1 ^{1}H NMR Data of the (*S*)- and (*R*)-MTPA Ester Derivatives of Leiocarpin B (**1**)

Proton	**2a**	**2b**	$\Delta\delta$ (= δ_S-δ_R)
H-3	6.03	5.94	+0.09
H-4	6.86	6.74	+0.12
H-5	2.35	2.21	+0.14
H-6	4.69	4.47	+0.21
H-7	5.83	5.89	-0.06 *R**
H-8	5.52	5.58	-0.06
H-8'	5.99	6.03	-0.04
H-6'	5.97	6.00	-0.03
H-2'	5.38	5.39	-0.01
Ha-3'	3.07	3.08	-0.01
Hb-3'	2.80	2.88	-0.08

* Absolute configuration of carbinol center.

EXPERIMENTAL

MS were measured on an Autospec-3000 Spectrometer and EIMS under 70 ev. ^{1}H NMR spectra were recorded at 400 MHz with a Brucker AM-400 Spectrometer. 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. (*S*)- and (*R*)-MTPA were purchased from Sigma Co. Ltd.

Anticancer activity tests were carried out by the Tumor Research Laboratory, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Science, CAS,

***In vivo* bioassay**: Female mice (weighing 18-22 g, certification number: CAS animal administration 005) were purchased from Shanghai Center of Experimental Animal. Tumor was H22 (mice liver cancer). A suspension of tumor in 0.2 mL of 0.9% NaCl solution was inoculated subcutaneously into right flank of mice for the tumor assay. Leiocarpin B (**1**) was dissolved in NaCl solution and adminisered intraperitoneally once daily during 7 consecutive days. Control animals were given a 0.9% solution by i.p. injection. Tumor weights were measured and the effect was represented by inhibitory rate (i.r. = 1−mean value of treated group/mean value of control group) × 100. The significance of differences between the experimental groups was calculated by Dunnett's test and $p< 0.05$ was considered significance.

Preparation of (*S*)-MTPA ester (**2a**): A mixture of **1** (20 mg, 0.042 mmol), DCC (14 mg, 0.068 mmol), DMAP (3 mg, 0.024 mmol), (*S*)-MTPA (10 mg, 0.042 mmol), and anhydrous CH_2Cl_2 (8 mL), was stirred for 18 h at rt. After removal of the solvent, the residue was subjected to silica gel chromatography eluting

with petrol-EtOAc (6:4) giving **2a** (8 mg, 0.012 mmol, 28%). FABMS m/z: 688 $[M-H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.03 (H-3, dd, *J* = 9.5, 1.8 Hz), 6.86 (H-4, m), 2.35 (H-5, m), 4.69 (H-6, m), 5.83 (H-7, t, *J* = 4.9 Hz), 5.52 (H-8, d, *J* = 4.9 Hz), 5.38 (H-2', dd, *J* = 2.9, 13.0 Hz), 3.07 (Ha-3', dd, *J* =13.0, 17.2 Hz), 2.80 (Hb-3', dd, *J* = 2.9, 17.2 Hz), 5.97 (H-6', d, *J* = 2.3 Hz), 5.99 (H-8', d, *J* = 2.3 Hz).

Preparation of (*R*)-MTPA ester (**2b**): A mixture of **1** (20 mg, 0.042 mmol), DCC (14 mg, 0.068 mmol), DMAP (3 mg, 0.024 mmol), (*R*)-MTPA (10 mg, 0.042 mmol), and anhydrous CH_2Cl_2 (8 mL) was stirred for 18 h at rt. After removal of the solvent, the residue was subjected to silica gel chromatography eluting with petrol-EtOAc (6:4) giving **2b** (3 mg, 0.04 mmol, 3.5%). FABMS m/z: 689 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 5.94 (H-3, dd, *J* = 9.6, 1.7 Hz), 6.74 (H-4, m), 2.21 (H-5, m), 4.47 (H-6, m), 5.89 (H-7, t, J = 5.2 Hz), 5.58 (H-8, d, *J* = 5.2 Hz), 5.39 (H-2', dd, *J* = 2.9, 13.0 Hz), 3.08 (Ha-3', dd, *J* =13.0, 17.2 Hz), 2.88 (Hb-3', dd, *J* = 2.9, 17.2 Hz), 6.00 (H-6', d, *J* = 2.3 Hz), 6.03 (H-8', d, *J* = 2.3 Hz).

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation of China (Grant No. 39770089) and the Applied and Basic Research Foundation of Yunnan province (Grant No. 97B038q). Thanks for the support of Chinese Postdoctoral Foundation (2000).

REFERENCES

1. Q. Mu, C. M. Li, and H. D. Sun, *Chin. Chem. Lett.*, 1999, **10**, 135.

2. Q. Mu, W. D. Tang, and Y. Lu, *Heterocycles*, 1999, **51**, 2969.

3. J. A. Dale and S. H. Mosher, *J. Am. Chem. Soc.*, 1973, **95**, 512.

4. G. R. Sullivan, J. A. Dale, and H. S. Mosher, *J. Org. Chem.*, 1973, **38**, 2143.

5. I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.

6. M. J. Rieser, Y. H. Hui, and J. K. Rupprechet, *J. Am. Chem. Soc.*, 1992, **114**, 10203.

7. X. P. Pan and D. Q. Yu, *Acta Pharm. Sinica*, 1999, **32**, 286.