

## A New Iridoid Alkaloid from the Flowers of *Plumeria rubra* L. cv. *acutifolia*

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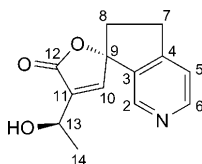
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A new iridoid alkaloid containing a spiro lactone unit, plumericidine (**1**), was isolated from the flowers of *Plumeria rubra* L. cv. *Acutifolia*. Its structure was elucidated by spectroscopic evidence and confirmed by X-ray diffraction crystallography. Its anticancer and antiviral activities were evaluated, but found to be insignificant.

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**Introduction.** – The genus *Plumeria* (Apocynaceae family) originated from Central America and consists of about eight species of which many are widely distributed in tropical countries. Phytochemical studies on this genus demonstrated that it is a source of the rather rare lactone-containing iridoids of the plumieride-type [1–4] which possess anticancer activities [5].

*P. rubra* L. cv. *acutifolia*, one of the two species of the genus occurring in China, is a small tree of which the flowers are used to treat cold and fever, whooping-cough, tracheitis, infective hepatitis, diarrhea, calculus of urethra, and mastitis [6]. Recently, we have isolated two new iridoids from these flowers [7]. In this article, we report on the isolation and structure elucidation of a new alkaloid (*Fig. 1*) from the title plant and on its related bioactivity assay.



**1**

Fig. 1. Structure of **1**<sup>1)</sup>

**Results and Discussion.** – *Structure Elucidation.* Plumericidine (**1**) was obtained as a cubic crystalline compound (MeOH). The molecular formula was established as C<sub>13</sub>H<sub>13</sub>NO<sub>3</sub> by an M<sup>+</sup> ion peak at m/z 231.0890 (calc. 231.0895) in the HR-EI-MS

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<sup>1)</sup> Arbitrary atom numbering. For the systematic name, see *Exper. Part*.

spectrum. In the  $^{13}\text{C}$ -NMR spectrum, the C-atoms which resonated at  $\delta(\text{C})$  149.1, 138.3, and 170.9 could be ascribed to an  $\alpha,\beta$ -unsaturated CO structure. Additionally, an HMBC interaction between  $\text{H}-\text{C}(10)^1$  with C(12), in addition to the strong absorption band at  $1761\text{ cm}^{-1}$  in the IR spectrum, was also supportive for the presence of an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone unit in compound **1**. In the  $^1\text{H}$ -NMR spectrum, the signal at  $\delta(\text{H})$  7.54 for  $\text{H}-\text{C}(10)$  was coupled with a CH H-atom bearing a OH group as indicated by the chemical shift  $\delta(\text{H})$  4.47, which also coupled with a Me group ( $\delta(\text{H})$  1.37). Further HMBC correlations between Me(14) with C(11),  $\text{H}-\text{C}(13)$  with C(10), and  $\text{H}-\text{C}(10)$  with C(13) indicated that the 1-hydroxyeth-1-yl fragment was the  $\alpha$ -side chain of the five-membered  $\alpha,\beta$ -unsaturated lactone.

The downfield signals at  $\delta(\text{H})$  7.43, 8.25, and 8.51 in the  $^1\text{H}$ -NMR spectrum of **1** represent an *ABX* coupling system. Together with a strong absorption band ( $1604\text{ cm}^{-1}$ ) in the IR spectrum, it suggested the presence of a pyridine ring in compound **1**. This assumption was verified by the three CH C-atoms ( $\delta(\text{C})$  120.9, 145.0, 150.2) and two quaternary C-atoms at  $\delta(\text{C})$  136.0 and 154.2, observed in the  $^{13}\text{C}$ -NMR spectrum.

Hitherto, two cyclic fragments of the molecule, the five-membered unsaturated lactone, and the disubstituted pyridine were deduced and left two  $\text{CH}_2$  groups to be assigned. On considering the degree of unsaturation, compound **1** should be endowed with a third ring. Thus, the three *multiplets* of H-atom signals at  $\delta(\text{H})$  3.01–3.15 (2 H),  $\delta(\text{H})$  2.23–2.32, and  $\delta(\text{H})$  2.52–2.60 (1 H each) were assignable to two  $\text{CH}_2$  groups of a cyclopentene ring, which was confirmed by the HMBC experiment. HMBC correlations observed between  $\text{CH}_2(7)$  and C(3), C(4), C(5), C(8), and C(9), and between  $\text{CH}_2(8)$  and C(3), C(4), C(9), and C(10) established the presence of the cyclopentene moiety in **1**. All the HMBC correlations are listed in the *Table*.

Table.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data of **1**<sup>1</sup>) (in  $(\text{D}_6)$ DMSO;  $\delta$  in ppm, *J* in Hz)

	$\delta(\text{H})$	$\delta(\text{C})$	H $\rightarrow$ C correlations
H–C(2)	8.25 (br. s)	145.0 ( <i>d</i> )	C(2), C(4), C(5), C(6), C(9)
C(3)		136.0 ( <i>s</i> )	
C(4)		154.2 ( <i>s</i> )	
H–C(5)	7.43 ( <i>dd</i> , <i>J</i> = 5.1, 0.9)	120.9 ( <i>d</i> )	C(2), C(6), C(7)
H–C(6)	8.51 ( <i>d</i> , <i>J</i> = 5.1)	150.2 ( <i>d</i> )	C(3), C(4), C(5), C(7)
$\text{CH}_2(7)$	3.01–3.15 ( <i>m</i> )	29.8 ( <i>t</i> )	C(3), C(4), C(5), C(6), C(8), C(9)
$\text{CH}_2(8)$	2.23–2.32 ( <i>m</i> ), 2.52–2.60 ( <i>m</i> )	35.3 ( <i>t</i> )	C(3), C(4), C(7), C(9), C(10)
C(9)		93.1 ( <i>s</i> )	
H–C(10)	7.54 ( <i>d</i> , <i>J</i> = 1.2)	149.1 ( <i>d</i> )	C(8), C(9), C(11), C(12), C(13), C(14)
C(11)		138.3 ( <i>s</i> )	
C(12)		170.9 ( <i>s</i> )	
H–C(13)	4.47 ( <i>dq</i> , <i>J</i> = 6.6, 1.5)	61.6 ( <i>d</i> )	C(10), C(11), C(12), C(14)
Me(14)	1.37 ( <i>d</i> , <i>J</i> = 6.6)	22.4 ( <i>q</i> )	C(11), C(13)
HO–C(13)	5.38 ( <i>d</i> , <i>J</i> = 4.5)		C(11), C(13), C(14)

A single-crystal X-ray diffraction analysis (*Fig. 2*) of plumericidine (**1**) revealed a unique system with a five-membered ring fused with a pyridine ring, being spiro-linked to an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone. Intermolecular H-bonds were observed between the HO–C(13) and N(1). On the basis of the configuration of the plumieride-type iridoids

of this genus, the absolute configuration at C(9) and C(13) of the alkaloid was assigned as (*S*) and (*R*), respectively, as those of plumeride by biogenetic basis and the literature data [8][9]. To the best of our knowledge, only three alkaloids identified from *Plumeria* genus [10][11], the skeletons of the isolated alkaloids comprise indole, indolizidine, and quinolizidine types. This iridoid alkaloid could contribute to chemotaxonomic significance within this species.

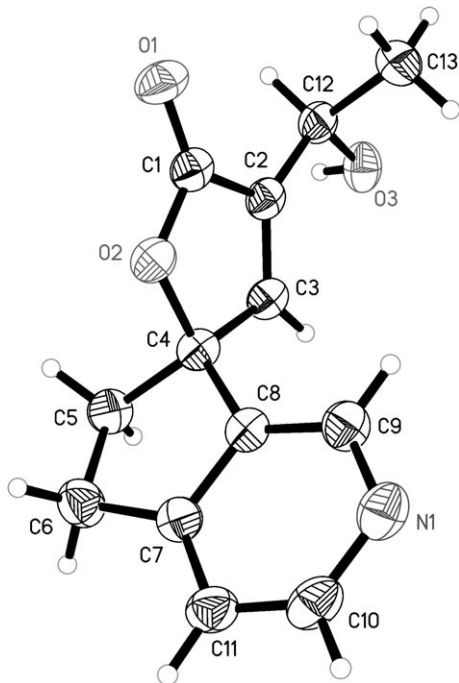


Fig. 2. Single-crystal X-ray structure of **1**)

### Experimental Part

**General.** All solvents used were of anal. grade (*Shanghai Chemical Plant*). Column chromatography (CC): silica gel *H* ( $\text{SiO}_2$ ; 200–300 mesh; *Qingdao Marine Chemical Ltd.*), *Sephadex LH-20* (25–100  $\mu\text{m}$ ; *Pharmacia*). TLC: silica gel *GF254* (*Yantai Huiyou*). M.p.: *Leica Galen III* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer 577* spectrometer with KBr disk. NMR Spectra: *Varian INOVA-500* spectrometer operating at 500 and 125 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , resp.; the detections were carried out at r.t., about 5 mg of samples were dissolved in 0.5 ml DMSO to record the NMR spectra; chemical shifts are given in ppm with TMS as internal reference and coupling constants (*J*) in Hz. LR- and HR-EI-MS: *Finnigan MAT-95* spectrometer.

**Plant Material.** The flowers of *P. rubra* L. *cv. acutifolia* were collected in March 2007 in Guangdong Province, P. R. China. Authenticated by Prof. *Jin-Gui Shen* of Shanghai Institute of Materia Medica, a voucher specimen (No. 20070310) was deposited in the herbarium of Shanghai Institute of Materia Medica, Shanghai, P. R. China.

**Extraction and Isolation.** Dried flowers of *P. rubra* L. *cv. acutifolia* (2.9 kg) were extracted with 95% EtOH for three times (1.5 h each). The extracts were concentrated, suspended in  $\text{H}_2\text{O}$ , and then partitioned with petroleum ether, AcOEt, and BuOH sequentially three times each. The AcOEt soluble fraction (2.5 g) was chromatographed on a  $\text{SiO}_2$  column eluted with a gradient of  $\text{CHCl}_3/\text{MeOH}$  to afford

three fractions (*Fr. 1–3*). *Fr. 2* was rechromatographed on SiO<sub>2</sub>, and then chromatographed on *Sephadex LH-20*, yielding compound **1** (20 mg).

*Plumericidine* (= (7*S*)-5,6-Dihydro-4-[(1*R*)-1-hydroxyethyl]-5'H-spiro[cyclopenta[*c*]pyridine-7,2'-furan]-5'-one; **1**). M.p. 214–216°. [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +17 (*c* = 0.12, MeOH). IR (KBr): 2989, 1761, 1604, 1425, 1356, 790. <sup>1</sup>H- and <sup>13</sup>C-NMR data: *Table*. EI-MS: 231. HR-EI-MS: 231.0890 (*M*<sup>+</sup>, C<sub>13</sub>H<sub>13</sub>NO<sub>3</sub><sup>+</sup>; calc. 231.0895).

*X-Ray Crystal-Structure Analysis of 1*<sup>2</sup>). Cubic crystals of **1** were obtained by recrystallization in MeOH. The crystal (0.490 × 0.451 × 0.397 mm) belongs to the orthorhombic system, with the formula C<sub>13</sub>H<sub>13</sub>NO<sub>3</sub> (*M*<sub>w</sub> 231.24), space group *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with *a* = 8.4549(9), *b* = 8.9753(9), *c* = 15.6471(16) Å;  $\alpha = \beta = \gamma = 90.0^\circ$ ; *V* = 1187.4(2) Å<sup>3</sup>; *Z* = 4; and  $\rho_{\text{calc}} = 1.294 \text{ Mg m}^{-3}$ . A total of 6183 reflections were collected to a maximum  $2\theta$  value of 53.98° by using the  $\Phi/\omega$  scan technique at 293(2) K. The structure was solved by direct methods and was refined by means of the full-matrix least-squares procedure. The collection data were reduced by the Saint program [12] and the empirical absorption correction was performed with the Sadabs program [13]. All non-H-atoms were given anisotropic thermal parameters. The H-atom positions were geometrically idealized and allowed to ride on their parent atoms. The refinement converged to the final *R* = 0.0423, *wR* = 0.1069 for 1290 observed reflections (*I* > 2 $\sigma$ (*I*)) and 160 variable parameters.

*Cytotoxicity Assay*. Cells were cultured in 96-well microtiter plates for the assay. After incubation for 24 h and treatment with 10<sup>-2</sup> to 10<sup>2</sup> μM of the test compound for 72 h, growth inhibition of the cancer was investigated. Cells were evaluated by the SRB method (adherent cells: HepG2, KB, and LoVo) or WST-1 method (suspended cell: K562), as described in the literature [14][15]. Adriamycin and taxotere were used as positive controls. However, no activity was detected in these assays (highest concentration tested: 400 μg ml<sup>-1</sup>).

*Antiviral Assay*. HBsAg and HBeAg in the HepG2 2215 cell line supernatant were assayed with commercial enzyme-linked immunosorbent assay (ELISA) kits. The results revealed that **1** inhibited the production of HBsAg (49.6%) at 400 μg ml<sup>-1</sup>.

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#### REFERENCES

- [1] J. J. W. Coppen, A. L. Cobb, *Phytochemistry* **1983**, 22, 125.
- [2] B. S. Siddiqui, A. Naeed, S. Begum, S. Siddiqui, *Phytochemistry* **1994**, 37, 769.
- [3] F. Abe, R.-F. Chen, T. Yamauchi, *Chem. Pharm. Bull.* **1988**, 36, 2784.
- [4] L. B. S. Kardono, S. Tsauri, K. Padmawinata, J. M. Pezzuto, A. D. Kinghorn, *J. Nat. Prod.* **1990**, 53, 1447.
- [5] M. P. Dobhal, G. Li, A. Gryshuk, A. Graham, A. K. Bhatnager, S. D. Khaja, Y. C. Joshi, M. C. Sharma, A. Oseroff, R. K. Pandey, *J. Org. Chem.* **2004**, 69, 6165.
- [6] Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 'Chinese Materia Medica', Shanghai Science & Technology Press, Shanghai, 1999, Vol. 6, p. 5620.
- [7] G. Ye, Y. L. Yang, G. X. Xia, M. S. Fan, C. G. Huang, *Magn. Reson. Chem.* **2008**, 46, 1195.
- [8] P. J. Stephens, J. J. Pan, F. J. Devlin, K. Krohn, T. Kurtán, *J. Org. Chem.* **2007**, 72, 3521.
- [9] F. Abe, T. Mori, T. Yamauchi, *Chem. Pharm. Bull.* **1984**, 32, 2947.
- [10] O. O. França, R. T. Brown, C. A. M. Santos, *Fitoterapia* **2000**, 71, 208.
- [11] S. N. H. Kazmi, Z. Ahmed, W. Ahmed, A. Malik, *Heterocycles* **1989**, 29, 1901.
- [12] Saint, Program to integrate and reduce raw crystallographic area detector data, *Bruker AXS Inc.*, Madison USA, 1996.

<sup>2</sup>) CCDC-cd27438 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre* via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).

- [13] G. M. Sheldrick, Sadabs, Program to empirical absorption correction of area detector data, University of Göttingen, Göttingen, 1996.
- [14] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, 82, 1107.
- [15] Y. Zhou, W. Zhu, Q.-X. Zhang, *J. Trop. Med.* **2005**, 5, 580.

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