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

by **Guan Ye**[a]), **Zhi-Xiong Li**[a]), **Guang-Xin Xia**[a]), **Hua Peng**[b]), **Zhao-Lin Sun**[a]), and **Cheng-Gang Huang***[a])

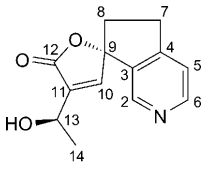
[a]) Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-tech Park, Shanghai, 201203, P. R. China (phone: +86-21-50806716; fax: +86-21-50806716; e-mail: ygsimm@sina.com.cn)

[b]) Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650204, P. R. China

A new iridoid alkaloid containing a spirolactone 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.

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**[1])

Results and Discussion. – *Structure Elucidation.* Plumericidine (**1**) was obtained as a cubic crystalline compound (MeOH). The molecular formula was established as $C_{13}H_{13}NO_3$ by an M^+ ion peak at m/z 231.0890 (calc. 231.0895) in the HR-EI-MS

[1]) Arbitrary atom numbering. For the systematic name, see *Exper. Part.*

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

The downfield signals at δ(H) 7.43, 8.25, and 8.51 in the ^{1}H-NMR spectrum of **1** represent an *ABX* coupling system. Together with a strong absorption band (1604 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 (δ(C) 120.9, 145.0, 150.2) and two quaternary C-atoms at δ(C) 136.0 and 154.2, observed in the ^{13}C-NMR spectrum.

Hitherto, two cyclic fragments of the molecule, the five-membered unsaturated lactone, and the disubstituted pyridine were deduced and left two CH_2 groups to be assigned. On considering the degree of unsaturation, compound **1** should be endowed with a third ring. Thus, the three *multiplet*s of H-atom signals at δ(H) 3.01–3.15 (2 H), δ(H) 2.23–2.32, and δ(H) 2.52–2.60 (1 H each) were assignable to two CH_2 groups of a cyclopentene ring, which was confirmed by the HMBC experiment. HMBC correlations observed between CH_2(7) and C(3), C(4), C(5), C(8), and C(9), and between 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}H- and ^{13}C-NMR Data of* **1**[1]) (in (D_6)DMSO; δ in ppm, *J* in Hz)

	δ(H)	δ(C)	H → C correlations
H–C(2)	8.25 (br. *s*)	145.0 (*d*)	C(2), C(4), C(5), C(6), C(9)
C(3)		136.0 (*s*)	
C(4)		154.2 (*s*)	
H–C(5)	7.43 (*dd*, *J* = 5.1, 0.9)	120.9 (*d*)	C(2), C(6), C(7)
H–C(6)	8.51 (*d*, *J* = 5.1)	150.2 (*d*)	C(3), C(4), C(5), C(7)
CH_2(7)	3.01–3.15 (*m*)	29.8 (*t*)	C(3), C(4), C(5), C(6), C(8), C(9)
CH_2(8)	2.23–2.32 (*m*), 2.52–2.60 (*m*)	35.3 (*t*)	C(3), C(4), C(7), C(9), C(10)
C(9)		93.1 (*s*)	
H–C(10)	7.54 (*d*, *J* = 1.2)	149.1 (*d*)	C(8), C(9), C(11), C(12), C(13), C(14)
C(11)		138.3 (*s*)	
C(12)		170.9 (*s*)	
H–C(13)	4.47 (*dq*, *J* = 6.6, 1.5)	61.6 (*d*)	C(10), C(11), C(12), C(14)
Me(14)	1.37 (*d*, *J* = 6.6)	22.4 (*q*)	C(11), C(13)
HO–C(13)	5.38 (*d*, *J* = 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 α,β-unsaturated γ-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 plumieride 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.

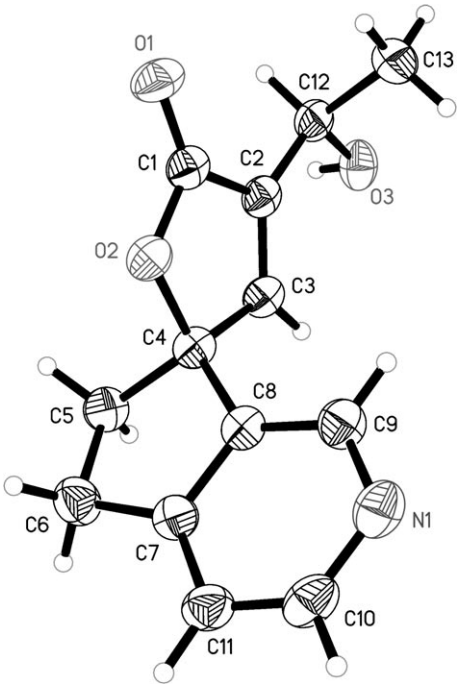


Fig. 2. *Single-crystal X-ray structure of* **1**[1])

Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*). Column chromatography (CC): silica gel *H* (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Ltd.*), *Sephadex LH-20* (25–100 μm; *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 ^1H and ^{13}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 H_2O, and then partitioned with petroleum ether, AcOEt, and BuOH sequentially three times each. The AcOEt soluble fraction (2.5 g) was chromatographed on a SiO_2 column eluted with a gradient of $CHCl_3$/MeOH to afford

three fractions (*Fr. 1–3*). *Fr. 2* was rechromatographed on SiO_2, and then chromatograhed 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°. $[\alpha]_D^{23} = +17$ ($c = 0.12$, MeOH). IR (KBr): 2989, 1761, 1604, 1425, 1356, 790. ^{1}H- and ^{13}C-NMR data: *Table*. EI-MS: 231. HR-EI-MS: 231.0890 (M^+, $C_{13}H_{13}NO_3^+$; calc. 231.0895).

X-Ray Crystal-Structure Analysis of **1**[2]). Cubic crystals of **1** were obtained by recrystallization in MeOH. The crystal ($0.490 \times 0.451 \times 0.397$ mm) belongs to the orthorhombic system, with the formula $C_{13}H_{13}NO_3$ (M_w 231.24), space group $P2_12_12_1$ with $a = 8.4549(9)$, $b = 8.9753(9)$, $c = 15.6471(16)$ Å; $\alpha = \beta = \gamma = 90.0°$; $V = 1187.4(2)$ Å^3; $Z = 4$; and $\rho_{calc} = 1.294$ Mg m^{-3}. A total of 6183 reflections were collected to a maximum 2θ value of 53.98° by using the Φ/ω 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^{-2} to 10^2 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^{-1}).

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^{-1}.

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[2]) 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.

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