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## 弯蕊开口箭中一个新的甾体皂甙元\*

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**摘要** 从云南民间抗炎药弯蕊开口箭(*Tupistra wattii* Hook.f.)的新鲜根状茎中分离到1个新的甾体皂甙元,命名为弯蕊开口箭甙元A(wattigenin A),其结构通过光谱分析鉴定为螺甾-25(27)-烯-1 $\beta$ ,2 $\beta$ ,3 $\beta$ ,4 $\beta$ ,5 $\beta$ ,6 $\beta$ -六醇;同时还分离到1个已知的甾体皂甙元,兰茂甙元D(ranmogenin D)。

**关键词** 弯蕊开口箭, 百合科, 甾体皂甙元, 弯蕊开口箭甙元A

## A STEROIDAL SAPOGENIN FROM TUPISTRA WATTII

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**Abstract** A new steroidal sapogenin named wattigenin A was isolated from the fresh rhizoms of *Tupistra wattii* Hook.f.(Liliaceae) together with a known one, ranmognin D. On the basis of spectral evidence, the structure of wattigenin A was elucidated as spirost-25(27)-ene-1 $\beta$ ,2 $\beta$ ,3 $\beta$ ,4 $\beta$ ,5 $\beta$ ,6 $\beta$ -hexol.

**Key words** *Tupistra wattii*, Liliaceae, Steroidal sapogenin, Wattigenin A

Steroidal sapogenins and saponins as a kind of chemical marker are widely distributed in many Liliaceous plants. *Tupistra wattii* Hook.f. is mainly distributed in the southeast of Yunnan, Sichuan, Guizhou, Guangdong and Guangxi of China as well as Bhutan and India. It is used as a folk medicine in Yunnan for the treatment of several inflammatory diseases such as pharyngitis, tonsillitis, bronchitis and cystitis as well as wound bleeding, injuries from falls, fractures and strains<sup>[1]</sup>. As a part of our continued chemical studies on steroidal compounds existing in Liliaflorae plants<sup>[2]</sup>. We examined the methanol extracts of this plant.

The chloroform soluble portion of the methanol extracts was repeatedly chromatographed on silica gel and preparative HPLC to give compounds 1 and 2.

Based on the analyses and comparison of IR, EI-MS and negative FAB-MS, <sup>1</sup>H and <sup>13</sup>C NMR spectra with reported data, compound 2 was proved to be ranmogenin D which was previously isolated

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Table 1  $^{13}\text{C}$  and  $^1\text{H}$  NMR data of  
1 (in pyridine- $d_5$ , ppm)

C	$\delta_{\text{C}}$	$\delta_{\text{H}}$ *
1	79.5	4.31 br s
2	67.2	4.23 br s
3	75.5	4.79 br s
4	69.6	4.31 br s
5	78.7	
6	69.6	4.90 br s
7	35.5	1.49, 2.10
8	30.1	2.39 br d(9.1)
9	45.4	1.32
10	45.5	
11	21.6	1.46(2H) **
12	40.0	1.03, 1.62 br d(12.2)
13	40.7	
14	56.2	1.10
15	32.3	1.45, 2.07
16	81.4	4.57 q-like (7.2)
17	63.2	1.83
18	16.5	0.85
19	16.1	1.93
20	42.0	1.85
21	14.9	1.06 d (6.9)
22	109.5	
23	33.3	1.71 (2H) **
24	29.0	2.23 br d(11.2), 2.67
25	144.5	
26	65.1	4.01 d(12.0), 4.44d (12.0)
27	108.6	4.76 br s, 4.79 br s

\* Coupling patterns well resolved are expressed with coupling constants in Hz in parentheses.

\*\* The two geminal protons resonate in the same position.

from *Tupistra aurantiaca* <sup>[3]</sup>.

**Compound 1**, mp 267—270°C (dec.) showed a quasimolecular ion peak at  $m/z$  493 $[\text{M}(\text{C}_{27}\text{H}_{42}\text{O}_8)-\text{H}]$  in the negative FAB-MS. In the EI-MS spectrum, it showed a molecular ion at  $m/z$  494 $[\text{M}]^+$  and fragment ions at  $m/z$  458 $[\text{M}-2\text{H}_2\text{O}]^+$ , 403 $[\text{M}-5\text{H}_2\text{O}-\text{H}]^+$ , 385 $[\text{M}-6\text{H}_2\text{O}-\text{H}]^+$ , suggesting the presence of polyhydroxyl groups. The IR spectrum displayed absorption bands for hydroxyl groups (3400  $\text{cm}^{-1}$ , br) and a spirostanol skeleton (980, 925, 880, 856  $\text{cm}^{-1}$ ). Comparison of the  $^{13}\text{C}$  spectrum of 1 with those of reported steroidal sapogenins <sup>[4,5]</sup> revealed that it was a polyhydroxyl steroidal sapogenin with a double bond between C-25 and C-27 [ $\delta$  144.5 (s, C) and 108.6 (t,  $\text{CH}_2$ )]. In addition, the  $^{13}\text{C}$  NMR signals of 1 are almost superimposable on those of 25(R)-5 $\beta$ -spirostane-1 $\beta$ , 2 $\beta$ , 3 $\beta$ , 4 $\beta$ , 5 $\beta$ , 6 $\beta$ -hexol except the signals of F ring <sup>[5]</sup>. The double bond at C-25 and C-27 was also supported by the  $^1\text{H}$  NMR spectrum in which the normal H-27 methyl signal was absent instead of the presence of two broad siglets at  $\delta$  4.76 and 4.79. Furthermore, 2D NMR experiments were used to confirm the proposed structure. A comprehensive analysis of  $^{13}\text{C}$ - $^1\text{H}$  COSY,  $^1\text{H}$ - $^1\text{H}$  COSY and COLOC spectra allowed the complete assignments of the  $^{13}\text{C}$  and  $^1\text{H}$  signals as shown in Table 1. Therefore, compound 1 was formulated as spirost 25(27)-ene-1 $\beta$ , 2 $\beta$ , 3 $\beta$ , 4 $\beta$ , 5 $\beta$ , 6 $\beta$ -hexol, and was named wattigenin A.

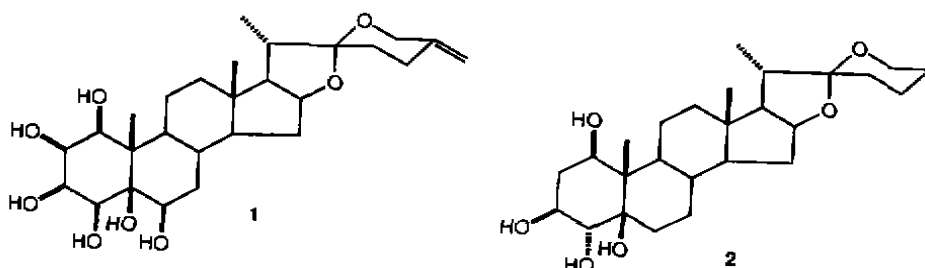
## EXPERIMENT

**Mps: uncorr.** Optical rotation were recorded on a J-20C polarimeter. IR spectra were measured on IR-450 spectrophotometer in KBr pellets.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were measured on a Bruker spectrosin AM-400 spectrometer using TMS as an internal standard. FAB-MS and EI-MS spectra were measured on an Autospec mass spectrometer. Column chromatography was carried out with silica gel (200—300 mesh) and ODS (HPLC). TLC was conducted on precoated Kieselgel 60 F<sub>254</sub>HPTLC plates (0.2 mm, Merck) and detected by spraying 10%  $\text{H}_2\text{SO}_4$  followed by heating.

**Plant material** The fresh rhizoms of *Tupistra wattii* Hook.f. were collected in Wenshan, Yunnan and identified by Prof. Li H. A voucher specimen is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and Isolation** The fresh rhizoms (244 g) were extracted with MeOH under reflux. After removal of the solvent by evapn, the combined extracts (56 g) were suspended in  $\text{H}_2\text{O}$  and successively extracted with  $\text{CHCl}_3$  and 1-BuOH. The  $\text{CHCl}_3$  layer was evapd *in vacuo* to give a residue (9.2 g), which

was subjected to silica gel column, eluting with  $\text{CHCl}_3$ -MeOH (20 : 1 to 5 : 1) to give fractions I—IV. Fr. I was chromatographed on a silica gel column using  $\text{CHCl}_3$ -MeOH (15 : 1 to 10 : 1) and crystallized with  $\text{CHCl}_3$ -MeOH to give compound 2 (25 mg). Fr. II was repeatedly chromatographed on a silica gel column with  $\text{CHCl}_3$ -MeOH (10 : 1 to 6 : 1), a reversed silica column of RP-8 with 70% MeOH and finally purified with preparative HPLC (Beckman gold system, YMC-Pack 312 ODS column 250 × 16 mm), using 75% MeOH at 5—8 mL/min as mobile phase to afford compound 1 (21 mg).



**Wattigenin A (1)** White amorphous powder, mp 267—270°C (dec.);  $[\alpha]_D^{25} -41.5$  ( $c = 0.49$ ,  $\text{C}_3\text{H}_5\text{N}$ ).  $\text{IR}_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$ : 3400 (br), 980, 925, 880, 856 (intensity 925 > 880). EI-MS  $m/z$  (70 eV): 494 $[\text{M}(\text{C}_{27}\text{H}_{42}\text{O}_8)]^+$ , 458 $[\text{M}-2\text{H}_2\text{O}]^+$ , 403 $[\text{M}-5\text{H}_2\text{O}-\text{H}]^+$ , 385 $[\text{M}-6\text{H}_2\text{O}-\text{H}]^+$ , 357 $[\text{M}-6\text{H}_2\text{O}-2(\text{CH}_3)+\text{H}]^+$ , 137 $[\text{base}]^+$ ; FAB-MS(neg.)  $m/z$ : 493 $[\text{M}-\text{H}]^-$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data see Table 1.

**Ranmogenin D (2)** Colorless needles from  $\text{CHCl}_3$ -MeOH, mp 303—305°C (dec.).  $\text{IR}_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$ : 3300 (br), 970, 940, 905, 885 (intensity 905 > 885). EI-MS  $m/z$  (70 eV): 464 $[\text{M}(\text{C}_{27}\text{H}_{44}\text{O}_6)]^+$ , 446 $[\text{M}-\text{H}_2\text{O}]^+$ , 428 $[\text{M}-2\text{H}_2\text{O}]^+$ , 414 $[\text{M}-2\text{H}_2\text{O}-\text{CH}_3+\text{H}]^+$ , 139 $[\text{base}]^+$ . FAB-MS(neg.)  $m/z$ : 463 $[\text{M}-\text{H}]^-$ .  $^1\text{H}$  NMR (pyridine- $d_5$ ):  $\delta$ 0.86(3H, s, H-18), 1.08(3H, d,  $J = 7\text{Hz}$ , Me-27), 1.16(3H, d,  $J = 6\text{Hz}$ , H-21), 1.59(3H, s, H-19), 3.38(1H, br d,  $J = 11\text{Hz}$ , H-26), 4.05(1H, br d,  $J = 11\text{Hz}$ , H-26'), 4.27(1H, br s, H-16).  $^{13}\text{C}$  NMR (pyridine- $d_5$ ):  $\delta$ 73.9(C-1), 33.5(C-2), 71.3(C-3), 68.2(C-4), 78.5(C-5), 30.5(C-6), 28.6(C-7), 35.2(C-8), 45.5(C-9), 45.9(C-10), 21.6(C-11), 40.2(C-12), 40.8(C-13), 56.4(C-14), 32.3(C-15), 81.5(C-16), 63.2(C-17), 16.7(C-18), 13.9(C-19), 42.1(C-20), 15.0(C-21), 109.9(C-22), 31.2(C-23), 29.0(C-24), 30.0(C-25), 67.0(C-26) and 17.4(C-27).

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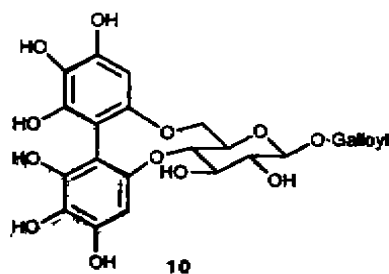
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### 作者更正

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