

## 架棚中酚类化合物的研究\*

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**摘要** 从架棚(*Ceratostigma minus* Stapf ex Prain)的乙酸乙酯部分分离得到14个酚类化合物。经波谱技术,特别是2D-NMR技术鉴定,其中化合物 plumbocatechins A (1)和 B(2)为新化合物。其他12个化合物被分别鉴定为 plumbolactone A (3)、plumbagic acid (4)、isoshinanolone (5)、epiisoshinanolone (6)、N-trans-caffeoyltyramine、N-trans-feruloyltyramine、apocynin、vanillic acid、syringic acid、gallo catechin、(+)-catechin 和 1,2,6-tri-O-galloylglucose。

**关键词** 架棚, 蓝雪科, 酚类化合物, Plumbocatechins A 和 B

## PHENOLICS FROM *CERATOSTIGMA MINUS* \*

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**Abstract** Fourteen phenolics were isolated from the whole plant of *Ceratostigma minus* Stapf ex Prain. Two of them were elucidated as new compounds, plumbocatechins A (1) and B (2). The others were identified as known compounds, plumbolactone A (3), plumbagic acid (4), isoshinanolone (5), epiisoshinanolone (6), N-trans-caffeoyl-tyramine, N-trans-feruloyltyramine, apocynin, vanillic acid, syringic acid, gallo catechin, (+)-catechin and 1,2,6-tri-O-galloylglucose. The structure elucidation of the two new compounds were mainly achieved by 2D-NMR techniques.  
**Key words** *Ceratostigma minus*, Plumbaginaceae, Phenolics, Plumbocatechins A and B

The isolations of several compounds, viz. plumbasides A ~ C, plumbagin, quercetin, myricetin, quercetin 3-O-glucoside, myricetin 3-O-rhamnoside and maltol-O-glucoside from *Ceratostigma minus* were previously reported<sup>[1,2]</sup>. As a continuation of our research on bioactive compounds, another fourteen compounds from the ethyl acetate soluble fraction have been isolated in the same plant. Two of them, plumbocatechins A (1) and B (2), have been characterized as new compounds, while the others were identified as the following known compounds, viz. plumbolactone A

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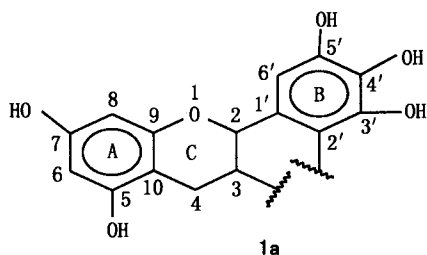
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(3), plumbagic acid (4), isoshinanolone (5), epiisoshinanolone (6), *N-trans*-caffeoyltyramine, *N-trans*-feruloyltyramine, apocynin, vanillic acid, syringic acid, galocatechin, (+)-catechin and 1,2,6-*tri-O*-galloylglucose. The structure elucidation of the two new compounds were mainly accomplished by 2D-NMR techniques.

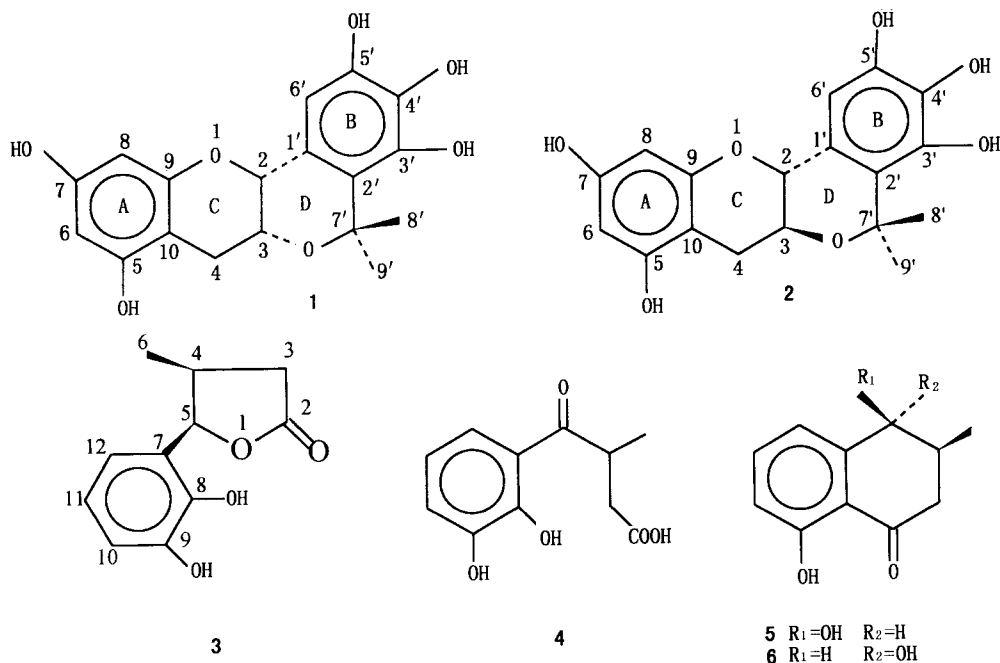
## RESULTS AND DISCUSSION

Plumbocatechin A (1) was obtained as an amorphous powder. EIMS study clearly revealed its molecular weight of 346 and HREIMS of molecular ion established its empirical formula as  $C_{18}H_{18}O_7$ . The  $^{13}C$ -NMR spectrum exhibited 18 resolved signals, comprising  $2 \times CH_3$ ,  $1 \times CH_2$ ,  $5 \times CH$  and  $10 \times$  quaternary carbons, as distinguished by DEPT spectra. Eighteen proton signals were present in the  $^1H$ -NMR spectrum and five proton signals at  $\delta$  7.5 ~ 8.5 were disappeared in the  $D_2O$  exchange experiment, indicating that compound 1 contains five exchangeable protons. This result is consistent with the fact revealed by DEPT spectrum analysis (Table 1). The IR spectrum did not show the presence of carbonyl group, suggesting that five exchangeable protons may be assigned to five hydroxyl groups. Two aromatic rings were inferred by the chemical shifts of twelve carbon signals appeared at the aromatic range in the  $^{13}C$ -NMR spectrum. The aromatic region of the  $^1H$ -NMR spectrum showed a classical pattern for a 1,2,3,5-tetrasubstituted aromatic ring [5.77 (1H, d,  $J = 2.3$  Hz), 5.97 (1H, d,  $J = 2.3$  Hz)] and a pentasubstituted aromatic ring (6.46, 1H, s). The above information suggested that the chemical formulation of compound 1 is  $C_{18}H_{13}O_2(OH)_5$ . IR absorption bands at  $3402\text{ cm}^{-1}$  for hydroxyls, and  $1629$ ,  $1608$  and  $1517\text{ cm}^{-1}$  for aromatic ring suggested that compound 1 is likely a phenolic compound. Observation of  $^{13}C$ -NMR resonance for twelve aromatics accounted for a total of 8 out of the 10 degrees of unsaturation required for this formula. The remaining degrees of unsaturation was assumed to be resulted from the presence of two additional oxygen-containing heterocycles. Interpretation of  $^1H$ ,  $^{13}C$ , DEPT, HMBC (Table 2) and  $^1H$ - $^1H$  COSY NMR spectra led to the proposal of the partial structure 1a, with only a-OC(CH<sub>3</sub>)<sub>2</sub> group left unaccounted for. The connection of two parts was made on the basis of the chemical shifts of C-3, C-2' and C-7'. The planar structure of compound 1 was thus proposed and further confirmed by H-2 $\beta$  correlating to C-9, H-3 $\beta$  correlating to C-7' and H-8' and H-9' correlating to both C-2' and C-7' in the HMBC spectrum. The C-1' was assigned to  $\delta$  125.40 and C-2' to 122.28, as H-8' and H-9' appeared to exhibit HMBC cross peaks to C-2' only, whereas H-6' and H-2 $\beta$  correlated to both. Although their chemical shifts were very close, C-7 and C-9 were respectively assigned to  $\delta$  157.23 and 157.31 based on H-2 $\beta$  HMBC cross peak correlating to C-9 only, and H-6 HMBC cross peaks correlating to the C-7 and C-5, and H-8 HMBC cross peaks correlating to the C-7 and C-9, whereas H-6 and H-8 did not correlate to C-9 and C-5, respectively.



In an attempt to determine the stereochemistry of the C-2 and C-3, we performed a NOESY experiment. The strong correlation between H-2 $\beta$  and H-3 $\beta$  indicated a *cis*-configuration. H-3 $\beta$  correlating to both H-4 $\alpha$  and H-4 $\beta$  inferred that H-3 $\beta$  took an equatorial bond as the C-ring probably took a chair-conformation. As consistent with the structure 1, a correlation was observed between H-2 $\beta$  and H-4 $\beta$  which took an axial bond, indicating that H-2 $\beta$  is also an axial configuration, and C-ring definitely took a chair-conformation. H-2 $\beta$  and H-6' were shown to be very close in space with a long ranged correlation between two protons.

Plumbocatechin B (2) was also obtained as an amorphous powder. With HREIMS of its molecular ion, the elemental formula as  $C_{18}H_{18}O_7$  was established. The  $^{13}C$ -NMR spectrum displayed 18



resolved signals, including 2 methyls, 1 methylene, 5 methines and 10 quaternary carbons. Two aromatic rings were displayed by the chemical shifts of twelve carbon signals appeared at the aromatic range in the  $^{13}\text{C}$ -NMR spectrum. The IR spectrum showed absorption bands at  $3392\text{ cm}^{-1}$  for hydroxyls, and  $1630$ ,  $1604$  and  $1516\text{ cm}^{-1}$  for aromatic rings. Eighteen proton signals were exhibited in  $^1\text{H}$ -NMR. Five of the irrational proton signals (each peak area is less than one proton) exhibiting between  $\delta 7.4 \sim 8.5$  were disappeared in the  $\text{D}_2\text{O}$  exchange experiment, suggesting that five hydroxyl groups were present as the IR spectrum did not show the presence of carbonyl group. The chemical formulation of compound **2** is thus arranged to be  $\text{C}_{18}\text{H}_{13}\text{O}_2(\text{OH})_5$ . The above mentioned spectral data suggested that the planar structure of compound **2** is identical with that of compound **1**, indicating that two compounds are stereoisomers differing at the C-2 or C-3.

The interpretation of coupling constants of H-2 (1H, d,  $J_{2\beta,3\alpha} = 9.1\text{ Hz}$ ) and H-3 $\alpha$  (1H, ddd,  $J_{3\alpha,4\beta} = 10.6$ ,  $J_{2\beta,3\alpha} = 9.1$ ,  $J_{3\alpha,4\alpha} = 5.9\text{ Hz}$ ) inferred that both H-2 and H-3 took an axial-bond, proposing that H-2 and H-3 are respectively in  $\beta$ -orientation and  $\alpha$ -orientation, and C-ring and D-ring are combined in a *trans*-configuration. This proposal was further confirmed by an excellent NOESY experiment. No correlation was observed between H-2 $\beta$  and H-3 $\alpha$ , supporting a *trans*-orientation. Correlations between H-2 $\beta$  and H-4 $\beta$ , and between H-3 $\alpha$  and H-4 $\alpha$ , are also consistent with the structure **2**.

The other twelve known compounds were distinguished as plumbolactone A (**3**)<sup>[3]</sup>, plumbic acid (**4**)<sup>[4]</sup>, isoshinanolone (**5**)<sup>[5]</sup>, epiisoshinanolone (**6**)<sup>[5]</sup>, *N-trans*-caffeoyltyramine, *N-trans*-feruloyltyramine<sup>[6]</sup>, apocynine<sup>[7]</sup>, vanillic acid<sup>[8]</sup>, syringic acid<sup>[9]</sup>, galocatechin<sup>[10]</sup>, (+)-catechin<sup>[11]</sup> and 1,2,6-*tri*-galloylglucose<sup>[12]</sup> according to the physical constants and spectral data (MS,  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR).

## EXPERIMENTAL

**General Experimental Procedures** Melting points were obtained on a Kofler apparatus and were uncorrected. IR spectral data were measured on a FT-5DX instrument with KBr disks. EIMS were obtained on an Auto-Spec mass spectrometer.  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR were recorded on Bruker AM-

400 and Bruker AMX-600 instruments with TMS as an internal standard and D<sub>6</sub>-acetone and C<sub>5</sub>D<sub>5</sub>N as solvents.

Table 1 <sup>13</sup>C-NMR data of compounds 1 and 2 (D<sub>6</sub>-acetone) \*

Carbons	Compound 1	Compound 2	Carbons	Compound 1	Compound 2
1			1'	125.40	126.18
2	71.68	74.21	2'	122.28	122.30
3	63.83	66.80	3'	143.01	142.82
4	26.30	27.92	4'	134.17	133.00
5	156.76	157.43 <sup>b</sup>	5'	144.37	144.83
6	95.69	95.90 <sup>c</sup>	6'	109.53	104.57
7	157.23	156.77 <sup>b</sup>	7'	75.60	76.10
8	95.94	96.62 <sup>c</sup>	8'	24.56	25.10
9	157.31	157.83 <sup>b</sup>	9'	28.91	29.10
10	99.47	101.04			

\* Assignments are partly based on the DEPT and 2D-NMR techniques. b, c. Assignments in the same column may be exchanged.

Table 2 Long range <sup>1</sup>H-<sup>13</sup>C correlation data from the HMBC of compound 1

<sup>1</sup> H	J <sub>2</sub>	J <sub>3</sub>	<sup>1</sup> H	J <sub>2</sub>	J <sub>3</sub>
2β	C-1'	C-9, C-3	8	C-7, C-9	C-6, C-10
3β	C-4	C-7'	6'	C-1'	C-2
4	C-10, C-3	C-5	8'	C-7'	C-2'
6	C-5, C-7	C-10, C-8	9'	C-7'	C-2'

**Plant Material** *Ceratostigma minus* Stapf ex Prain was collected at Dali county of Yunnan Province, China. It was authenticated by Prof. Zhongwen LIN at Kunming Institute of Botany, The Chinese Academy of Sciences, where a voucher specimen was deposited.

**Extraction and Partition** The air-dried powdered whole herb of *C. minus* (7 kg) was extracted three times with 95% EtOH at room temperature. The solvent was evaporated under reduced pressure to give 405 g residue. The crude extract was then dissolved in water (4 000 mL). The aqueous solution was partitioned with petroleum ether, ethyl acetate and n-butanol to afford three fractions P (80 g), E (140 g) and N (78 g), respectively. The aqueous solution was condensed under reduced pressure to obtain fraction W (102 g).

**Isolation and Purification** Fraction E (140 g) was chromatographed on a column of silica gel (1 500 g) eluting with petroleum ether-acetone (8:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2 and acetone) to give fractions 1~50. The frs. 3~8 were combined and crystallized in hexane-ether to give plumbagin (2.1 g). The frs. 10~14 were combined and chromatographed on silica gel column eluting with petroleum ether-acetone (8:1, 6:1 and 4:1) to obtain a mixture of isoshinanolone and epiisoshinanolone (5.2 g, <sup>1</sup>H-NMR spectrum indicated that the mixture was in the ratio of 1:7). It was further rechromatographed on silica gel column eluting with cyclohexane-isopropanol (20:1) to yield isoshinanolone (350 mg) and epiisoshinanolone (2.7 g). The frs. 15~19 were combined and chromatographed on silica gel column to give plumbagic acid (4.6 g). The frs. 21~24 were combined and chromatographed on MCI-gel CHP 20P eluting with methanol-water (7:3) to give apocynin (207 mg), vanillic acid (49 mg), and crude plumbolactone A which was then recrystallized in hexane-acetone to yield 1.3 g white prisms. The frs. 30~36 were combined and chromatographed on MCI-gel CHP 20P eluting with methanol-water (7:3) to give syringic acid (500 mg) and a mixture of amides which was then rechromatographed on silica gel column eluting with petroleum ether-ethyl acetate (4:1) to afford *N-trans*-caffeoyltyramine (77 mg) and *N-trans*-feruloyltyramine (13 mg). The frs. 38~46 were combined and chromatographed on MCI-gel CHP 20P eluting with

methanol-water (7:3) to give (+)-catechin (402 mg), gallocatechin and a mixture of plumbocatchins A and B. The mixture was further chromatographed on RP-18 silica gel eluting with methanol-water (6:4) to yield plumbocatchin A (240 mg) and plumbocatchin B (407 mg) with a minor impurity. Plumbocatchin B was further purified by silica gel chromatography eluting with petroleum ether-acetone (3:1) to obtain 301 mg pure compound. The frs. 48 ~ 50 were combined and chromatographed on MCI-gel CHP 20P column eluting with methanol-water (1:1) to give a major compound 1,2,6-tri-O-galloylglucose (1.7 g).

**Plumbocatchin A (1)**  $C_{18}H_{18}O_7$  (HRMS, found 346.137 1, requires 346.138 4), amorphous powder, IR (KBr)  $\nu_{max} \text{ cm}^{-1}$ : 3 402, 1 629, 1 602, 1 517, 1 645 and 1 143;  $^1\text{H-NMR}$  ( $D_6$ -Acetone): 8.45 (<1H, brs., OH), 8.15 (<1H, s, OH), 7.93 (<1H, s, OH), 7.70 (<1H, brs., OH), 7.45 (<1H, brs., OH), 4.40 (1H, s, H-2 $\beta$ ), 4.22 (1H, dd,  $J = 1.6, 5.1$  Hz, H-3 $\beta$ ), 2.85 (1H, dd,  $J = 5.1, 17.5$  Hz, H-4 $\beta$ ), 2.76 (1H, dd,  $J = 1.6, 17.5$  Hz, H-4 $\alpha$ ), 5.77 (1H, d,  $J = 2.3$  Hz, H-6), 5.97 (1H,  $J = 2.3$  Hz, H-8), 6.46 (1H, s, H-6'), 1.60 (3H, s,  $\text{CH}_3$ -7' $\beta$ ), 1.54 (3H, s,  $\text{CH}_3$ -7' $\alpha$ ). EIMS  $m/z$  (%): 346[M]<sup>+</sup>(55), 331[M-CH<sub>3</sub>]<sup>+</sup>(100). 193(95), 149(35) and 139(77).

**Plumbocatchin B(2)**  $C_{18}H_{18}O_7$  (HRMS, found 346.138 8, requires 346.138 4), amorphous powder, IR (KBr)  $\nu_{max} \text{ cm}^{-1}$ : 3 392, 1 630, 1 604, 1 516 and 1 141;  $^1\text{H-NMR}$  ( $D_6$ -Acetone): 8.37 (<1H, s, OH), 8.33 (<1H, brs., OH), 8.12 (<1H, s, OH), 7.60 (<1H, brs., OH), 7.34 (<1H, s, OH), 4.40 (1H, d,  $J = 9.1$  Hz, H-2 $\beta$ ), 3.80 (1H, m, H-3 $\alpha$ ), 2.97 (1H, dd,  $J = 5.9, 15.4$  Hz, H-4 $\alpha$ ), 2.42 (1H, dd,  $J = 10.6, 15.4$  Hz, H-4 $\beta$ ), 5.95 (1H, d,  $J = 2.0$  Hz, H-6), 6.05 (1H,  $J = 2.0$  Hz, H-8), 6.74 (1H, s, H-6'), 1.65 (3H, s,  $\text{CH}_3$ -7' $\alpha$ ), 1.63 (3H, s,  $\text{CH}_3$ -7' $\beta$ ). EIMS  $m/z$  (%): 346[M]<sup>+</sup>(55), 331[M-CH<sub>3</sub>]<sup>+</sup>(100), 193(60) and 139(50).

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