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华麻花头根中的蜕皮甾酮类成分

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摘要：从华麻花头（*Serratula chinensis* S. Moore）根中分得 7 种蜕皮甾酮类化合物，经光谱分析和化学方法，分别鉴定为：20- 羟基蜕皮松 (1)，podecdysone C (2)，3- 氧 - 乙酰基 -20- 羟基蜕皮松 (3)，20- 羟基蜕皮松 -20,22 - 缩丁醛 (4)，shidasterone (5)，atrotosterone C (6) 和 carthamosterone (7)，其中 20- 羟基蜕皮松 -20,22 - 缩丁醛为一新的化合物。

关键词：麻花头属；华麻花头；蜕皮甾酮类化合物；20- 羟基蜕皮松 -20,22 - 缩丁醛
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Ecdysteroids from the Roots of *Serratula chinensis*

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Abstract: Seven ecdysteroids, 20-hydroxyecdysone (**1**), podecdysone C (**2**), 3-O-acetyl-20-hydroxyecdysone (**3**), 20-hydroxyecdysone-20, 22-butylidene acetal (**4**), shidasterone (**5**), atrotosterone C (**6**) and carthamosterone (**7**), were isolated from the roots of *Serratula chinensis* S. Moore. All compounds except compound **1** were isolated from this plant for the first time, and compound **4** was found to be a new ecdysteroid.

Key words: *Serratula*; *Serratula chinensis*; Ecdysteroid; 20-hydroxyecdysone-20,22-butylidene acetal

Serratula chinensis S. Moore (Compositae) is a perennial herbaceous plant growing mainly in South China[1], where its roots are used as one of the substitutes of the traditional Chinese medicine, "Sheng-Ma" (*Cimicifuga foetida* L.)[2]. The occurrence of 20-hydroxyecdysone from the plant was previously reported [3], but its further chemical composition was unknown. In order to discover new bioactive compounds, we investigated its chemical constituents. From ethanol extract of the roots, seven ecdysteroids have been isolated. The structures of the compounds (Fig.1) were elucidated on the basis of spectroscopic analysis and chemical correlation.

1 Results and discussion

By combined analysis of UV, ^{1}H and ^{13}C NMR spectral data, compounds **1**–**7** were determined to be ecdysteroids with same structural nuclei and different side chains. Six known ecdysteroids were identified to be 20-hydroxyecdysone (**1**), podecdysone C (**2**), 3-O-acetyl-20-hydroxyecdysone (**3**), shidasterone (**5**), atrotosterone C (**6**) and carthamosterone (**7**), respectively, as physical and spectral data were in good agreement with those already reported in the literatures[4-8].

Compound **4** was obtained as colorless amorphous solid. The ESI-MS gave a base ion peak at m/z 557 $[M+Na]^+$, indicating the molecular weight of 534. By combined analysis of ESI-MS, ^{13}C NMR and the DEPT data, its molecular formula was suggested as $C_{31}H_{50}O_7$. Its ^{1}H and ^{13}C NMR spectra were similar to those of 20-hydroxyecdysone (**1**), except the presence of four additional carbon signals at δ 104.4 (d), 38.1 (t), 17.9 (t) and 14.4 (q) in the ^{13}C NMR spectrum and four additional proton signals at δ 5.09 (1H, t, J = 4.8 Hz), 1.73 (2H, m), 1.52 (2H, m) and 0.92 (3H, t, J = 7.6 Hz) in the ^{1}H NMR spectrum. The

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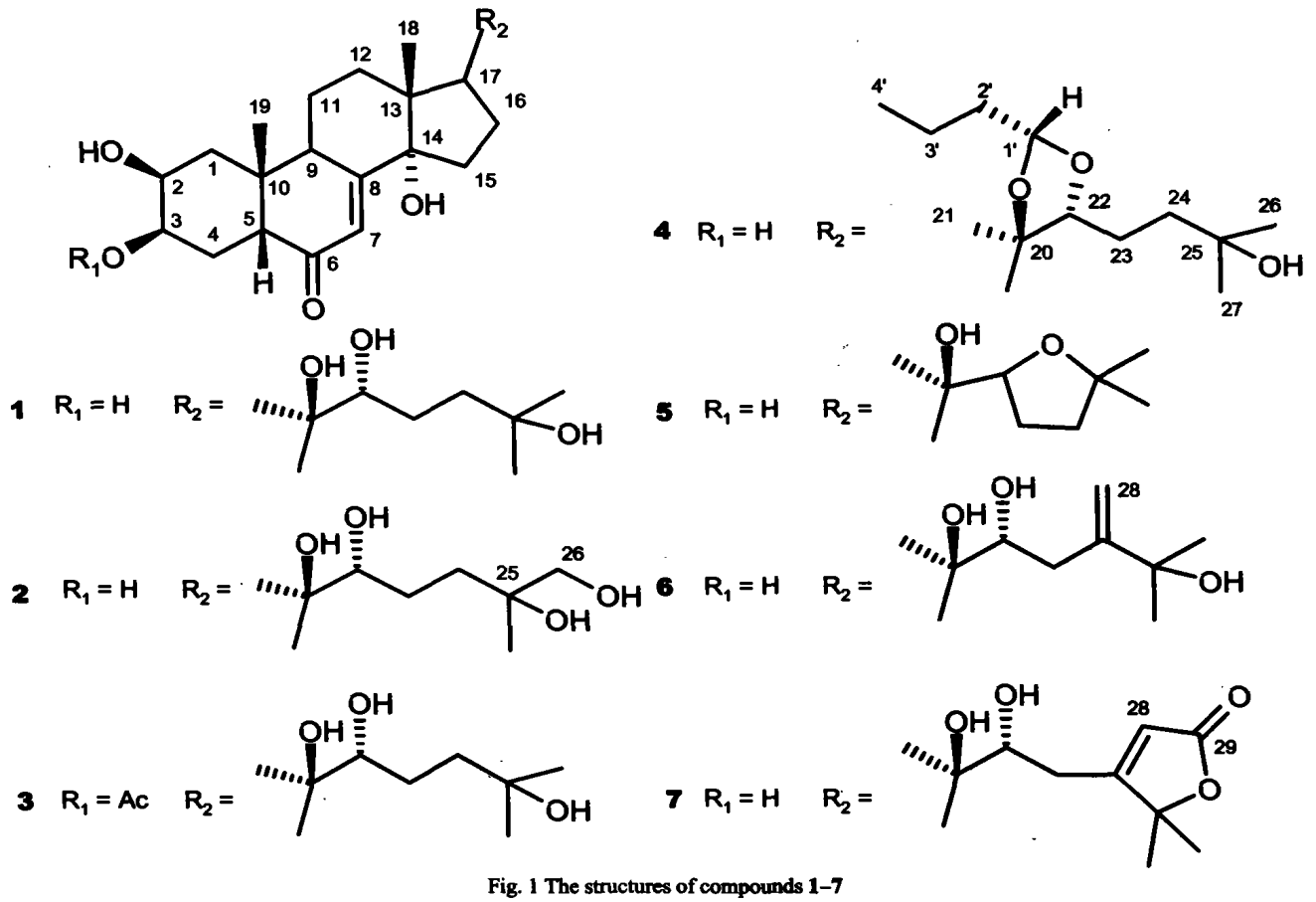

Fig. 1 The structures of compounds 1–7

carbon signal at δ 104.4 and the proton signal at δ 5.09 suggested the presence of an acetalic methine. By careful analysis of the ^{1}H-^{1}H and ^{13}C-^{1}H COSY data, a group of *n*-butylidene acetal was deduced. The downfield shifts of ^{13}C NMR signals for C-20 and C-22 to δ 84.2 and 84.7, respectively, suggested that the *n*-butylidene acetal group was connected to C-20 and C-22. This was supported by the acid hydrolysis of compound **4** to afford 20-hydroxyecdysone (**1**). Compound **4** was thus determined to be 20-hydroxyecdysone-20, 22-butylidene acetal.

To determine the stereochemistry of the acetalic carbon in compound **4**, NOESY measurement was carried out. In the NOESY spectrum of compound **4**, the presence of a strong cross peak between H-22 (δ 3.87) and H-1' (δ 5.09), and the absence of a cross peak between H-21 (δ 1.32) and H-1', indicated the H-1' was at β orientation (Fig. 2). As the *R* and *R* configuration has been assigned for C-20 and C-22 [9], respectively, the absolute configuration of C-1' in compound **4** could be determined as *R*.

Though compound **4** has not been reported before, it is supposed to be an artificial product from condensation of 20-hydroxyecdysone and butanal in *n*-BuOH, as the compound could not be detected in EtOH extract.

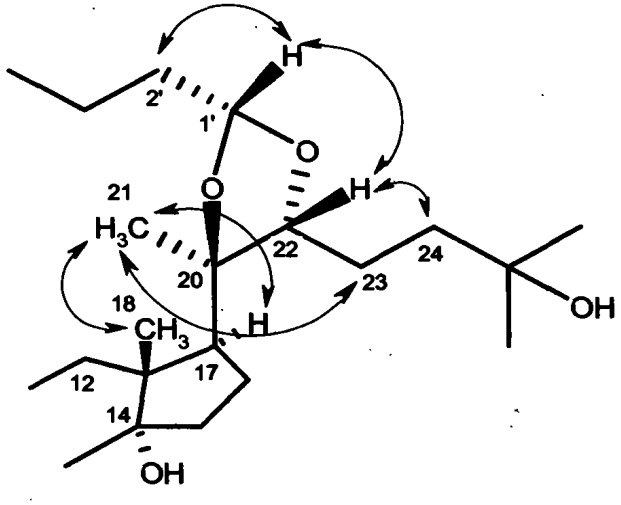

Fig. 2 Significant correlations for the side chain of compound **4** in the NOESY spectrum

Table 1 ^{13}C NMR spectral data of compounds 1–7

C	1	2	3	4	5	6	7
1	37.3 t	37.3 t	38.4 t	38.1 t	37.4 t	38.0 t	38.0 t
2	68.5 d	68.5 d	67.1 d	68.1 d	68.5 d	68.2 d	68.2 d
3	68.7 d	68.7 d	71.8 d	68.1 d	68.7 d	68.2 d	68.2 d
4	32.8 t	32.8 t	32.5 t	32.5 t	32.9 t	32.5 t	32.6 t
5	51.8 d	51.7 d	52.5 d	51.4 d	51.8 d	51.5 d	51.5 d
6	206.5 s	206.5 s	205.4 s	203.7 s	206.5 s	203.6 s	203.8 s
7	122.1 d	122.1 d	122.0 d	121.9 d	122.1d	121.8 d	121.9 d
8	168.0 s	168.0 s	168.3 s	165.7 s	168.0 s	166.2 s	166.1 s
9	35.1 d	35.1 d	35.2 d	34.5 d	35.1 d	34.8 d	34.5 d
10	39.2 s	39.3 s	39.2 s	38.7 s	39.2 s	38.8 s	38.8 s
11	21.5 t	21.5 t	21.5 t	21.1 t	21.5 t	21.5 t	21.7 t
12	32.5 t	32.5 t	30.3 t	31.7 t	32.3 t	32.1 t	32.1 t
13	48.6 s	48.6 s	48.6 s	47.8 s	48.6 s	48.2 s	48.3 s
14	85.2 s	85.2 s	85.2 s	84.2 s	85.3 s	84.3 s	84.3 s
15	31.8 t	31.8 t	31.8 t	31.6 t	31.7 t	31.8 t	31.9 t
16	21.5 t	21.5 t	21.5 t	22.4 t	21.8 t	21.5 t	21.7 t
17	50.5 d	50.5 d	50.5 d	50.9 d	51.8 d	50.1 d	50.1 d
18	18.0 q	18.1 q	18.0 q	17.3 q	18.1 q	18.0 q	18.0 q
19	24.4 q	24.4 q	24.4 q	24.5 q	24.4 q	24.5 q	24.6 q
20	77.9 s	77.9 s	77.9 s	84.2 s	77.0 s	76.9 s	76.7 s
21	21.1 q	21.1 q	21.1 q	23.4 q	20.7 q	21.2 q	21.2 q
22	78.4 d	78.4 d	78.4 d	84.7 d	85.5 d	78.0 d	75.4 d
23	27.3 t	26.5 t	27.4 t	24.4 t	28.4 t	34.5 t	30.6 t
24	42.4 t	37.1 t	42.4 t	42.2 t	39.6 t	156.2 s	172.7 s
25	71.3 s	73.6 s	71.3 s	69.4 s	81.8 s	72.3 s	87.8 s
26	29.7 q	70.7 t	29.7 q	30.2 q	29.0 q	30.8 q	24.9 q
27	29.0 q	23.6 q	29.0 q	29.9 q	28.4 q	30.2 q	24.9 q
28	—	—	—	—	—	109.6 t	115.5 d
29	—	—	—	—	—	—	177.2 s
1'	—	—	—	104.4 d	—	—	—
2'	—	—	—	38.1 t	—	—	—
3'	—	—	—	17.9 t	—	—	—
4'	—	—	—	14.4 q	—	—	—
$COCH_3$	—	—	172.6 s	—	—	—	—
$COCH_3$	—	—	21.1 q	—	—	—	—

2 Experimental

General Melting points were uncorrected. UV spectra were obtained using a Perkin Elmer lambda 25 UV/VIS spectrophotometer. IR spectra were recorded on a WQF-410 FT-IR spectrophotometer. Optical rotations were measured with a Perkin Elmer 343 polarimeter. NMR spectra were measured on a Bruker DRX-400 instrument (^1H at 400 MHz; ^{13}C at 100 MHz) using TMS as an internal standard. ESI-MS spectra were obtained with a MDS SCIEX API 2000 LC/MS/MS spectrometer by direct inlet using MeOH as solvent. Silica gel for CC and silica gel 60 GF_{254} for preparative TLC were from Qingdao Marine Chemical Ltd., China. MeOH was used as the eluant in Sephadex LH-20 CC. HPTLC was performed on precoated silica gel plates (Merck, Kieselgel 60 F_{254}) using EtOAc-MeOH-$NH_3 \cdot H_2O$ (concentrated) (10:0.5:0.2) as the developing solvent and spotted with 10% H_2SO_4 in EtOH followed by heating.

Plant material The roots of *S. chinensis* were collected in Lechang county, Guangdong province, China, in Autumn 2001, and identified by Prof. Ze-xian Li at South China Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation The ground dry roots (4.8 kg) of *S. chinensis* were extracted by percolation with 95% EtOH three times at room temperature. The EtOH solution was concentrated to syrup (247 g) *in vacuo*. This syrup was suspended in H_2O and the aqueous suspension was extracted successively with petroleum (500 ml ×4), $CHCl_3$ (500 ml ×5) and *n*-BuOH (500 ml ×5).

The *n*-BuOH extract, upon concentration under

reduced pressure, afforded 78 g of light brown syrup. This syrup was further fractionated by a Diaion HP-20 CC eluted sequentially with 20% EtOH, 50% EtOH and 97% EtOH, yielding three fractions (D–F). Fraction D was subjected to a silica gel CC using $CHCl_3$-MeOH (6:1) as the eluant followed by recrystallization, yielding compound **1** (4 258 mg) and compound **2** (184 mg). Fraction F was subjected to a silica gel CC eluted with $CHCl_3$-MeOH (20:1) and repeated Sephadex LH-20 CC to yield compound **3** (480 mg) and compound **4** (378 mg).

The $CHCl_3$ extract, on concentration, gave a brown syrup (15 g). This syrup was subjected to a silica gel (100–120 mesh) CC, eluted with $CHCl_3$-MeOH mixture of increasing polarity [(98:2) to (9:1)], yielding three fractions (A–C). Fraction B, after chromatography over a silica gel (200–300 mesh) column using $CHCl_3$-MeOH (92:8) as the eluant, followed by a Sephadex LH-20 CC, afforded compound **5** (22 mg). Fraction C was sequentially chromatographed on a silica gel column eluted with $CHCl_3$-MeOH (9:1) mixture, a Sephadex LH-20 column, and a preparative thin layer using EtOAc-MeOH-NH_3•H_2O (concentrated) (10:0.5:0.2) as the developing solvent, yielding compound **6** (15 mg) and compound **7** (50 mg).

20-hydroxyecdysone (1) $C_{27}H_{44}O_7$, colorless needles, mp 243–245℃ (MeOH), $[\alpha]_D^{28}$ + 59.3° (c 0.3, MeOH); ESI-MS m/z (%): 503 $[M+Na]^+$ (100); IR ν_{max}^{KBr} cm^{-1}: 3423, 2966, 1655, 1382, 1057; UV λ_{max}^{MeOH} nm (log ε): 242.3 (4.06); ^1H NMR (CD_3OD) δ :0.88, 0.96, 1.18, 1.189, 1.194 (each 3H, s, Me ×5), 3.13 (1H, m, H-9), 3.83 (1H, br d, J =11.2 Hz, H-2α), 3.94 (1H, br s, H-3α), 5.80 (1H, d, J =2.0 Hz, H-7); ^{13}C NMR (CD_3OD): see Table 1.

Podecdysone C (2) $C_{27}H_{44}O_8$, colorless needles, mp 160–165℃ (MeOH), $[\alpha]_D^{28}$ + 53.0° (c 0.37, MeOH); ESI-MS m/z (%): 519 $[M+Na]^+$ (100); UV λ_{max}^{MeOH} nm(log ε):241.8 (4.13); ^1H NMR (CD_3OD) δ : 0.88, 0.96, 1.13, 1.19 (each 3H, s, Me × 4), 3.13 (1H, m, H-9), 3.36 (2H, s, H-26), 3.83 (1H, br dd, J =3.6, 11.6 Hz, H-2α), 3.94 (1H, br s, H-3α), 5.80 (1H, d, J = 2.0 Hz, H-7); ^{13}C NMR (CD_3OD): see Table 1.

3-O-Acetyl-20-hydroxyecdysone (3) $C_{29}H_{46}O_8$, amorphous solid, $[\alpha]_D^{28}$ + 51.8° (c 0.114, MeOH); ESI-MS m/z (%):545 $[M+Na]^+$ (100); UV λ_{max}^{MeOH} (log ε): 242.4 (4.22); ^1H NMR (CD_3OD) δ : 0.89, 0.99, 1.18, 1.191, 1.196 (each 3H, s, Me × 5), 2.11(3H, s, Ac), 3.16 (1H, m, H-9), 3.97 (1H, br dd, J =3.6, 11.6 Hz, H-2α), 5.14 (1H, br s, H-3α), 5.82 (1H, d, J = 2.0 Hz, H-7); ^{13}C NMR (CD_3OD): see Table 1.

20-hydroxyecdysone-20,22-butylidene-acetal (4) $C_{31}H_{50}O_7$, colorless amorphous solid, $[\alpha]_D^{28}$ +55.0° (c 0.1, MeOH); ESI-MS m/z (%): 573 $[M+K]^+$ (21), 557 $[M+Na]^+$ (100), 535 $[M+H]^+$ (22); IR ν_{max}^{KBr} cm^{-1}: 3425, 2964, 1653, 1380, 1057; UV λ_{max}^{MeOH} nm (log ε): 241.8 (4.00); ^1H NMR (C_5D_5N) δ :0.92 (3H, t, J =7.6 Hz, H-4'), 1.04, 1.05, 1.32, 1.37, 1.38 (each 3H, s, H-18, H-19, H-21, H-27, H-26), 1.52 (2H, m, H-3'), 1.73 (2H, m, H-2'), 2.79 (1H, m, H-17), 3.00 (1H, dd, J = 3.2, 12.8 Hz, H-5), 3.55 (1H, br t, J =8.0 Hz, H-9), 3.87 (1H, br d, J =9.6 Hz, H-22), 4.17 (1H, br dd, J = 3.6, 11.6 Hz, H-2α), 4.23 (1H, br s, H-3α), 5.09 (1H, t, J = 4.8 Hz, H-1'), 6.26 (1H, d, J = 2.0 Hz, H-7); ^{13}C NMR (C_5D_5N): see Table 1.

Acid hydrolysis of compound 4 The solution of compound **4** in 2 ml of methanolic HCl (6%) was refluxed for 1 hour. The reaction mixture was diluted with 3 ml of water and evaporated to remove methanol. The aqueous solution was extracted with *n*-BuOH. The *n*-BuOH layer, after evaporation, afforded 20-hydroxyecdysone, which was identified by the direct comparison on TLC with compound **1**.

Shidasterone (5) $C_{27}H_{42}O_6$, amorphous solid, $[\alpha]_D^{28}$ + 31.4°(c 0.086, MeOH); ESI-MS m/z (%): 485 $[M+Na]^+$ (100); UV λ_{max}^{MeOH} nm (log ε): 240.8 (4.15); ^1H NMR (CD_3OD) δ : 0.84, 0.95, 1.21, 1.23, 1.24 (each 3H, s, Me×5), 3.13 (1H, m, H-9), 3.83 (1H, br dd, J =3.2, 11.4 Hz, H-2α), 3.92 (1H, br d, J=10.0 Hz, H-22), 3.94 (1H, br s, H-3α), 5.80 (1H, d, J=2.0 Hz, H-7); ^{13}C NMR (CD_3OD): see Table 1.

Atrotosterone C (6) $C_{28}H_{44}O_7$, colorless needles, mp 219–225℃ (MeOH), $[\alpha]_D^{28}$ + 44.0° (c 0.116, MeOH); ESI-MS m/z (%): 515 $[M+Na]^+$ (100); UV λ_{max}^{MeOH} nm (log ε): 242.3 (4.08); ^1H NMR (C_5D_5N) δ : 1.07, 1.21, 1.52, 1.59, 1.59 (each 3H, s, Me×5), 3.60 (1H, br t, J =8.8 Hz, H-9), 4.09 (1H, br d, J =8.8 Hz, H-22), 4.18 (1H, br d, J =11.6 Hz, H-2α), 4.23 (1H, br s, H-3α), 5.12 (1H, br s, H-28a), 5.29 (1H, br s,

H-28b), 6.26 (1H, d, J=2.0 Hz, H-7); ^{13}C NMR (C_5D_5N): see Table 1.

Carthamosterone (7) $C_{29}H_{42}O_8$, amorphous solid, $[\alpha]_D^{28}$ +46.1° (c 0.436, MeOH); ESI-MS m/z (%): 541 $[M+Na]^+$ (100); UV λ_{max}^{MeOH} nm (log ε): 240.8 (4.14); ^{1}H NMR (C_5D_5N) δ: 1.07, 1.22, 1.34, 1.41, 1.65 (each 3H, s, Me×5), 3.56 (1H, br t, J = 8.4 Hz, H-9), 4.16 (1H, br d, J=10.0 Hz, H-2α), 4.16 (1H, br d, J=10.0 Hz, H-22), 4.24 (1H, br s, H-3α), 6.26 (1H, d, J =2.0 Hz, H-7), 6.30 (1H, s, H-28); ^{13}C NMR (C_5D_5N): see Table 1.

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