Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, P.R. China

Ecdysteroids from *Rhaponticum uniflorum*

YONG-HONG ZHANG and HAN-QING WANG

Five phytoecdysteroids were isolated from the roots of *Rhaponticum uniflorum*. They were identified as ecdysterone, ajugasterone C, ajugasterone C-20,22-monoacetonide, ajugasterone C-2,3,20,22-diacetonide and 5-deoxykaladasterone-20,22-monoacetonide by means of spectroscopic data. This is the first report of ajugasterone C-2,3,20,22-diacetonide and 5-deoxykaladasterone-20,22-monoacetonide isolated from a natural source.

1. Introduction

In China, there are two species of *Rhaponticum* (Compositae). *Rhaponticum uniflorum* (L.) DC. is distributed in northern China. Its roots have been used as a Chinese traditional medicine for the treatment of fever and intoxications [1]. Since the 1980s many researchers have studied *Rhaponticum uniflorum* [2–3]. Phytoecdysteroids and other compounds were isolated from this plant [4–6]. We report here the isolation and structural elucidation of two new ecdysteroids, as well as the isolation of 3 known ecdysteroids, from the roots of *R. uniflorum.*

2. Investigations, results and discussion

The dried powdered roots of *R. uniflorum* were extracted with methanol at room temperature. The extract was fractionated successively by petrol, ethyl acetate and n-butanol. The ethyl acetate extract was further separated by silica gel chromatography to give two new ecdysteroids, ajugasterone C-2,3,20,22-diacetonide (**4**) and 5-deoxykaladasterone-20,22-monoacetonide (**5**), and one known ecdysteroid, ajugasterone C-20,22-monoacetonide (**3**) [7]. The n-butanol extract was also purified by silica gel chromatography to give two known ecdysteroids, ecdysterone (**1**) [5] and ajugasterone C (**2**) [5].

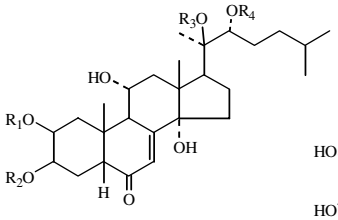
Compound **4**, $C_{33}H_{52}O_7$. The IR spectrum of compound **4** contained absorption bands of hydroxy groups (3431 cm^{-1}) and of a keto group conjugated with a double bond (1662 cm^{-1}). Its UV spectrum (λ_{max}^{MeOH} 243 nm) also confirmed the presence of a 7-en-6-keto group in the steroid nucleus. In the EI MS the peaks at *m/z* 379, 343, 325, 317 and 299 confirmed the existence of an ajugasterone C ring skeleton [8]. A strong peak at *m/z* 185 could be the result of the side chain ion derived from cleavage between C-17 and C-20. The ^{13}C NMR spectrum (Table) showed that the compound is quite similar to ajugasterone C except that six more peaks corresponding to the ketal groups were observed (δ 108.09, 106.81, 26.76, 26.83, 29.04 and 30.87), and the C-2, C-3, C-20, C-22 signals had shifted to a lower field. The MS of compound **4** showed the peaks of the molecular ion with *m/z* 560. This is higher by 40 amu than the molecular weight of compound **3**. Accordingly, the structure of compound **4** is ajugasterone C-2,3,20,22-diacetonide.

Ajugasterone C-2,3,20,22-diacetonide (**4**) is a native compound [9]. This follows from the fact that no acetone was used in the extraction and subsequent treatment of the extractive substances.

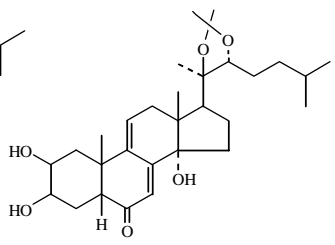
Compound **5** was identified as 25-deoxy-9(11)-dehydro-20-hydroxyecdysone-20,22-mono acetonide based on the following evidence. Its UV spectrum in MeOH showed a strong maximum at λ_{max}287 nm; such a maximum value is characteristic of a conjugated dienone and was previously found for kaladasterone [10, 11] and 5-deoxykaladasterone [12]. The mass spectrum gave a $[M]^+$ at *m/z* 502. The

Table: ^{13}C NMR spectral data of compounds 4–5 (100 MHz, 4 in $CDCl_3$, 5 in CD_3OD)

C	4	5
1	39.98	43.19
2	72.64	68.05
3	71.59	67.81
4	36.35	37.22
5	52.09	51.07
6	203.29	203.18
7	122.13	119.24
8	161.14	154.24
9	41.88	141.14
10	38.58	37.13
11	68.09	132.01
12	42.39	38.31
13	47.14	47.36
14	84.42	84.93
15	31.65	31.22
16	21.16	22.35
17	48.79	50.01
18	17.70	17.74
19	23.59	27.08
20	83.90	83.16
21	21.84	22.11
22	81.57	82.23
23	27.12	27.48
24	36.38	37.62
25	28.22	28.70
26	22.45	22.76
27	22.58	22.88
Ketal group		
−O−C−O−	108.09	107.22
	106.81	
CH_3	26.76	29.39
CH_3	26.83	29.68
CH_3	29.04	
CH_3	30.87	



2 R_1=R_2=R_3=R_4=H
3 R_1=R_2=H , R_3R_4=CMe_2
4 R_1R_2=CMe_2 , R_3R_4=CMe_2



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presence of fragments at *m/z* 361, 343 (361–18) and 325 (343–18) is characteristic of an ecdysteroid bearing a 20,22 diol and no 25-OH [10]. ^{1}H NMR spectroscopy (1D and 2D-COSY experiments) allowed us to assign the 25-deoxy structure based on the chemical shifts and the doublet pattern of 26-Me and 27-Me. The $\Delta^{9(11)}$ structure was established from the disappearance of the 9-H signal and the appearance of a new olefinic proton signal at δ 6.22 coupled with three signals 2.73 (J = 2 Hz), 2.43 (J = 6.5 Hz) and 7-H signal by a small long-range coupling (observed with a 2D-COSY experiment). The observed downfield shifts of the 19-Me, 1-H and the small upfield shifts of 2-H, 3-H and 7-H are in accordance with a $\Delta^{9(11)}$ structure. The ^{13}C NMR spectrum (Table) showed that the compound is quite similar to 5-deoxykaladasterone [11] except that three more peaks corresponding to the ketal group were observed (δ 107.22, 29.39, 29.68), and the C-20, C-22 signals had shifted to a lower field. Thus, the structure of compound **5** was determined as 5-deoxykaladasterone-20,22-monoacetonide.

3. Experimental

3.1. Equipment

M.p.s.: Kofler-microscope (Reichert) uncorr. Optical rotation: Polarimeter 241 (Perkin Elmer) solvent $CHCl_3$ or MeOH. IR-spectra were recorded on a Nicolet-5DX IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AM-400, solvent $CDCl_3$, CD_3OD, C_5D_5N, using TMS as internal standard. EIMS and FABMS were determined on a ZAB-HS mass spectrometer.

3.2. Plant material

The roots of *R. uniflorum* were purchased from the company of Chinese medicinal materials in province Gansu, China and were identified by Prof.Yin-Shou Zhou, Lanzhou Medical College. A voucher specimen No. 98001 was deposited in the Herbarium of the Department of Pharmacy, Lanzhou Medical College, Lanzhou, P.R. China.

3.3. Extraction and isolation

The air-dried roots of *R. uniflorum* (3 kg) were exhaustively extracted with MeOH at room temperature, the extract was concentrated under reduced pressure. The resulting residue was suspended in H_2O, extracted with petrol, EtOAc and n-BuOH, respectively. The EtOAc extract (50 g) was subjected to CC on a silica gel column (1000 g, 200–300 mesh) using petrol/EtOAc gradient as eluent to give fractions I–V according to TLC analysis. Compound **4** (48 mg) was obtained from fraction II (8 : 1), compound **5** (26 mg) from fraction IV (4 : 1) and compound **3** (57 mg) from fraction V (1 : 1), and purified by rechromatography on silica gel followed by recrystallization. The n-BuOH extract (40 g) was also subjected to CC on a silica gel column using $CHCl_3$/MeOH gradient as eluent to give fractions I–IV. Compound **2** (31 mg) was obtained from fraction I (10 : 1), compound **1** (62 mg) from fraction II (8 : 1), and purified by rechromatography on silica gel followed by recrystallization.

3.4.1. Ecdysterone (**1**)

White needles, m.p. 236–238 °C (EtOAc/MeOH); $[\alpha]_D^{20}$ +62.0 (c 0.9, MeOH); UV λ_{max}^{MeOH} nm: 242; IR υ_{max}^{KBr} cm^{-1}: 3479, 3289, 3226, 2959, 2875, 1658, 1640, 1468, 1447, 1384, 1229, 1145, 1053, 920, 878, 688; FABMS: 503 $[M + Na]^+$, 487 $[M + Li]^+$; EIMS *m/z* (%): 462 $[M-H_2O]^+$ (2), 444 $[M-2H_2O]^+$ (5), 426 $[M-3\,H_2O]^+$ (16), 408 $[M-4\,H_2O]^+$ (3), 363 $[M-C_6H_{13}O_2]^+$ (58), 345 $[M-C_6H_{13}O_2-H_2O]^+$ (100), 327 $[M-C_6H_{13}O_2-2\,H_2O]^+$ (35), 161 $[C_8H_{17}O_3]^+$ (7), 143 $[C_8H_{17}O_3-H_2O]^+$ (15), 125 $[C_8H_{17}O_3-2\,H_2O]^+$ (17), 107 $[C_8H_{17}O_3-3\,H_2O]^+$, 117 $[C_6H_{13}O_2]^+$ (4), 99 $[C_6H_{13}O_2-H_2O]^+$ (42), 80 $[C_6H_{13}O_2-2\,H_2O]^+$ (32); ^{1}H NMR (400 MHz, C_5D_5N) δ: 1.01 (3 H, s, 19-H), 1.21 (3 H, s, 18-H), 1.36 (6 H, s, 26, 27-H), 1.58 (3 H, s, 21-H), 3.01 (2 H, m, 5,17-H), 3.02 (1 H, m, 5-H), 3.58 (1 H, m, 9-H), 3.77 (1 H, dd, 22-H), 3.90 (2 H, m, 2,3-H), 6.26 (1 H, d, J = 1.8 Hz, 7-H).

3.4.2. Ajugasterone C (**2**)

White powder, m.p. 196–198 °C (EtOAc/MeOH). UV λ_{max}^{MeOH} nm: 240. IR υ_{max}^{KBr} cm^{-1}: 3430, 1658, 1641, 1440, 1384, 1059, 976. FABMS *m/z*: 503 $[M + Na]^+$, 487 $[M + Li]^+$. EIMS *m/z*: 462 $[M-H_2O]^+$, 444 $[M-2\,H_2O]^+$, 426 $[M-3H_2O]^+$, 379 $[M-C_6H_{13}O]^+$, 361 $[M-C_6H_{13}O-H_2O]^+$, 343 $[M-C_6H_{13}O-2H_2O]^+$, 145 $[C_8H_{17}O_2]^+$, 127 $[C_8H_{17}O_2-H_2O]^+$, 109 $[C_8H_{17}O_2-2\,H_2O]^+$, 101 $[C_6H_{13}O]^+$, 83 $[C_6H_{13}O-H_2O]^+$; ^{1}H NMR (CD_3OD) δ: 5.80 (1 H, brs, 7-H), 4.05 (2 H, m, 2,3-H), 3.58 (2 H, m, 9, 22-H), 3.24 (1 H, m, 11-H), 2.57 (1 H, dd, 5-H), 1.24 (3 H, s, 21-H), 1.05 (3 H, s, 18-H), 0.93 (6 H, d, J = 7 Hz, 26, 27-H), 0.85 (3 H, s, 19-H).

3.4.3. Ajugasterone C-20,22-monoacetonide (**3**)

White needles, m.p. 248–250 °C (EtOAc/MeOH); $[\alpha]_D^{20}$ +60.8 (c 1.3, MeOH); UV λ_{max}^{MeOH} nm: 237. IR υ_{max}^{KBr} cm^{-1}: 3400, 1658, 1468, 1377, 1039. FABMS *m/z*: 543 $[M + Na]^+$, 527 $[M + Li]^+$; EIMS *m/z*: 520 $[M]^+$, 505 $[M-CH_3]^+$, 502 $[M-H_2O]^+$, 487 $[M-CH_3-H_2O]^+$, 469 $[M-CH_3-2H_2O]^+$, 462, 444, 427, 379, 361, 344, 325, 317, 299, 266, 185, 127, 109, 59. ^{1}H NMR (CD_3OD) δ: 5.79 (1 H, d, J = 1.8 Hz, H-7), 3.99 (1 H, m, H-2), 3.94 (1 H, m, H-3), 4.08 (1 H, m, H-11), 3.65 (1 H, m, H-22), 3.13 (1 H, m, H-9), 0.91 (6 H, d, J = 3.6 Hz, H-26, 27), 0.79, 1.04, 1.17, (each 3 H, s, H-18, 19, 21), 1.30, 1.38 (each 3 H, s, ketal group Me × 2).

3.4.4. Ajugasterone C-2,3,20,22-diacetonide (**4**)

White needles, m.p. 134–136 °C (Petrol/EtOAc); $[\alpha]_D^{20}$ +58.2 (c 1.0, MeOH); UV λ_{max}^{MeOH} nm: 243. IR υ_{max}^{KBr} cm^{-1}: 3431, 1662, 1459, 1056. EIMS *m/z*: 560 $[M]^+$, 545 $[M-CH_3]^+$, 542 $[M-H_2O]^+$, 502, 484, 460, 419, 379, 361, 357, 325, 317, 306, 299, 185, 142, 127. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.84 (1 H, d, J = 2.4 Hz, H-7), 4.52 (1 H, m, H-2), 4.29 (1 H, m, H-3), 4.12 (1 H, m, H-11), 3.60 (1 H, m, H-22), 2.83 (1 H, m, H-9), 0.90 (6 H, d, J = 6.5 Hz, H-26, 27), 0.78, 1.04, 1.16 (each 3 H, s, H-18, 19, 21), 1.32, 1.33, 1.41, 1.49 (each 3 H, s, ketal group Me × 4). ^{13}C NMR: see Table.

3.4.5. 5-Deoxykaladasterone-20,22-monoacetonide (**5**)

White powder, m.p. 235–237 °C (EtOAc/MeOH); $[\alpha]_D^{20}$ +54.8 (c 0.8, MeOH); UV λ_{max}^{MeOH} nm: 287. IR υ_{max}^{KBr} cm^{-1}: 3400, 1665. FABMS *m/z*: 525 $[M + Na]^+$, 509 $[M + Li]^+$, EIMS *m/z*: 487 $[M-15]^+$, 469 $[M-CH_3-H_2O]^+$, 444, 426, 402, 379, 361, 343, 325, 326, 317, 301, 299, 211, 185. ^{1}H NMR (400 MHz, CD_3OD) δ: 3.69 (1 H, m, H-2), 3.76 (1 H, m, H-3), 2.40 (1 H, m, H-5), 5.66 (1 H, brs, H-7), 6.22 (1 H, dd, J = 6.4, 2 Hz, H-11), 2.73 (1 H, m, J = 2 Hz, H-12), 2.43 (1 H, m, J = 6.5 Hz, H-12), 2.45 (1 H, m, H-17), 0.81, 1.06, 1.16 (each 3 H, s, H-18, 19, 21), 0.88, 0.89 (each 3 H, d, J = 6.7 Hz, H-26, 27), 1.28, 1.35 (each 3 H, s, ketal group Me × 2); ^{13}C NMR: see Table.

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Prof. Han-Qing Wang
Lanzhou Institute of Chemical Physics
Chinese Academy of Sciences
Lanzhou, 730000
PR of China