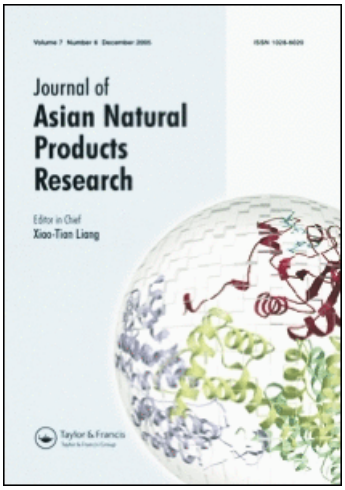

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A NEW PHYTOECDYSONE FROM THE ROOTS OF *RHAPONTICUM UNIFLORUM*

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A new ecdysteroid named rhapontisterone R_1 (**1**) together with two known phytoecdysones, rhapontisterone (**2**) and ecdysterone (**3**) were isolated from the roots of *Rhaponticum uniflorum* (L.) DC. The new compound was shown to be 2β,3β,11α,14α,20ξ,22ξ-hexahydroxy-stigma-7,24(28)-dien-6-oxo-28,25-carbolactone. The structure has been determined primarily on the basis of physico-chemical properties and spectral analysis.

Keywords: *Rhaponticum uniflorum*; Phytoecdysone; Rhapontisterone R_1 (**1**); Rhapontisterone (**2**); Ecdysterone (**3**)

INTRODUCTION

The roots of *Rhaponticum uniflorum* is a traditional Chinese medicine for "clearing heat and toxic materials" [1]. It was reported that eight phytoecdysones have been isolated from this plant [2,3]. In this paper we wish to report the isolation and structural determination of a new phytoecdysone rhapontisterone R_1 (**1**).

RESULTS AND DISCUSSION

Dried and minced roots of *R. uniflorum* were extracted with ethanol. The extract was separated on silica gel column followed by reversed-phase

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HPLC (Rp-18) yielding the new rhapontisterone R_1 (**1**). **2** and **3** were identified as rhapontisterone and ecdysterone by comparison of their ^1H and ^{13}C NMR data with those reported in the literature.

Rhapontisterone R_1 (**1**), white powder, was obtained in 0.00015% yield. **1** gave positive response to Liebermann–Burchard reaction. A molecular ion at m/z 534 $[M]^+$ in the ESI-MS spectrum together with the ^1H and ^{13}C NMR data allowed us to propose the molecular formula $C_{29}H_{42}O_9$. The UV spectrum [λ_{max}(MeOH): 244 nm] of **1** exhibited typical absorption of α,β-unsaturated carbonyl moiety. The ^1H NMR and ^{13}C NMR spectra of **1** (δ 6.32 br.s, δ 203.9, 164.0, 122.4) showed signals characteristic of an ecdysteroid-type skeleton [2]. The ^1H NMR spectrum of **1** showed the presence of five tertiary methyl groups (δ 1.27, 1.32, 1.63, 1.28, 1.36). The ^1H NMR and ^{13}C NMR spectra indicated that an α,β-unsaturated γ-lactone (δ 6.27, δ 176.9, 172.4, 115.4, 87.54) existed in the structure. Comparison with the NMR spectrum data of ajugasterone C [4], showed that the chemical shifts of the ^1H and ^{13}C NMR signals from C-1 to C-23 in **1** agreed with those of ajugasterone C, but the carbon signals at δ 37.1 and 28.1 (C-24 and C-25) in the spectrum of ajugasterone C were replaced by δ 176.9 and δ 87.5 in **1**. Thus the γ-lactone was linked on the side chain.

In the HMQC spectrum, the carbon signal of C-28 (δ 115.4) correlated with the proton signal of H-28 (δ 6.27). In the ^1H-^1H COSY spectrum, the proton signal of H-28 showed long-range correlation with the proton signals of H-23 (δ 2.71, 2.52), besides, a correlation between H-22 (δ 4.17) and H-23 was also observed. In the HMBC spectrum (see Fig. 1), the proton signals of H-26 and H-27 correlated with the carbon signals of C-25 (δ 87.54) and C-24 (δ 176.9); the proton signal of H-28 (δ 6.27) was in correlation with the carbon signals of C-24 (δ 176.9), C-23 (δ 30.5), C-25 (δ 87.54), C-29 (172.4),

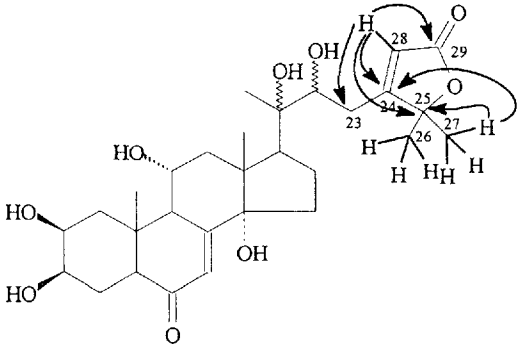

FIGURE 1 HMBC correlation of compound **1**.

but the carbon signal of C-29 (δ 172.4) only correlated with H-28, confirming the site of cyclization of the γ-lactone was at C-25, thus the structure was identified as in Fig. 1. According to the literature [2], the chemical shifts and splitting patterns of the proton signals and the chemical shifts of the carbon signals of C-2, C-3, C-11, C-14 are consistent with those of rhapontisterone. Then the formulation of **1** was established as 2β,3β,11α,14α,20ξ,22ξ-hexahydroxy-stigma-7,24(28)-dien-6-oxo-28,25-carbolactone.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were measured on a Yamaco micro-hot-stage and are uncorrected. UV spectra were taken in MeOH on Shimadzu UV-260 spectrophotometers. ESI-MS was obtained with VG-70SE mass spectrometer. All NMR spectra were recorded on Bruker-ARX-300 spectrometer, using TMS as an internal standard. For HPLC, a Shimadzu CTO-6A apparatus with ODS-5 (20 mm i.d., ×25 cm) column and UV-detector was used. Silica gel for chromatography was produced by Qingdao Ocean Chemical Factory.

Plant Material

The roots of *R. uniflorum* were bought in Shenyang. The plant material was identified by Prof. Xu Chunquan (Shenyang Pharmaceutical University).

Extraction and Isolation

Dried and minced roots of *R. uniflorum* (3 kg) were extracted with hot 70% EtOH, the EtOH extract was mixed with H_2O and extracted successively with petrolum ether, $CHCl_3$, EtOAc and n-BuOH. A part of the n-BuOH extract was chromatographed on a silica gel column eluted with $CHCl_3$ and MeOH gradient (100 : 0.5–100 : 10). Fractions 544–550 crystallized in $CHCl_3$–MeOH to yield ecdysterone (**3**, 150 mg). Fractions 563–579 and 580–598 eluted with $CHCl_3$–MeOH (100 : 4) were separated by preparative HPLC on an ODS-5 column (20 mm i.d., ×25 cm, flow rate: 2 ml/min, detect wave: 242 nm) with MeOH–H_2O (64% and 53%, respectively) as eluent to yield compound **2** and compound **1**.

Rhapontisterone R_1 (**1**) White powder, UV λ_{max}(MeOH): 244 nm. ESI-MS: 534 $[M]^+$, ^{1}H NMR (300 MHz, in C_5D_5N): δ 4.56 (1H, m, H-2),

4.22 (1H, m, H-3), 3.04 (1H, m, H-5), 6.32 (1H, br.s, H-7), 3.90 (1H, m, H-9), 4.59 (1H, m, H-11), 3.04 (1H, m, H-17), 1.27 (3H, s, H-18), 1.32 (3H, s, H-19), 1.63 (3H, s, H-21), 4.17 (1H, m, H-22), 1.28 (3H, s, H-26), 1.36 (3H, s, H-27), 6.27 (1H, br.s, H-28). ^{13}C NMR (75.4 MHz, in C_5D_5N): see Table I.

Rhapontisterone (**2**) White powder, m.p. 234–236°C, λ_{max}(MeOH): 242 nm. ^{1}H NMR (300 MHz, in C_5D_5N): δ 4.56 (1H, m, H-2), 4.25 (1H, m, H-3), 3.05 (1H, m, H-5), 6.27 (1H, br.s, H-7), 4.59 (1H, m, H-11), 3.01 (1H, m, H-17), 1.25 (3H, s, H-18), 1.30 (3H, s, H-19), 1.54 (3H, s, H-21), 4.14 (1H, m, H-22), 3.90 (1H, m, H-24), 1.73 (1H, m, H-25), 1.02 (6H, s, H-26,27). ^{13}C NMR (75.4 MHz, in C_5D_5N): see Table I.

Ecdysterone (**3**) White needle, m.p. 240–243°C, λ_{max}(MeOH): 242 nm. ^{1}H NMR (300 MHz, in C_5D_5N): δ 6.23 (1H,br.s,H-7), 1.20 (3H,s,H-18), 1.07 (3H,s,H-19), 1.57 (3H,s,H-21), 1.37 (6H,s,H-26,27) ^{13}C NMR (75.4 MHz, in C_5D_5N): see Table I.

TABLE I ^{13}C NMR spectral data for compounds **1**, **2**, **3** and ajugasterone C (in C_5D_5N)

No.	*Rhapontisterone* R_1	*Ecdysterone*	*Rhapontisterone*	*Ajugasterone C*
1	39.6	38.0	39.5	39.6
2	68.8	68.2	68.4	68.4
3	68.2	68.2	68.1	68.2
4	32.9	32.5	32.9	32.9
5	52.5	51.5	52.5	52.1
6	203.9	203.7	203.9	203.9
7	122.45	121.8	122.3	122.3
8	164.0	166.3	164.2	164.2
9	42.8	34.6	42.8	42.8
10	39.9	38.8	39.5	39.9
11	68.4	21.3	68.8	68.9
12	44.1	31.9	44.2	44.2
13	48.3	48.2	48.1	48.2
14	84.2	84.4	84.2	84.3
15	32.0	32.1	31.9	32.0
16	21.6	21.6	21.6	21.5
17	50.0	50.2	49.9	50.0
18	18.9	18.0	18.9	18.9
19	24.7	24.6	24.9	24.8
20	75.3	77.0	76.6	76.8
21	21.7	21.8	21.6	21.6
22	76.5	77.7	77.6	76.8
23	30.5	27.6	35.8	30.2
24	176.9	42.7	76.8	37.0
25	87.5	69.8	34.0	28.2
26	24.9	30.1	17.0	23.3
27	24.9	30.1	18.9	22.4
28	115.4			
29	172.4			

Acknowledgements

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