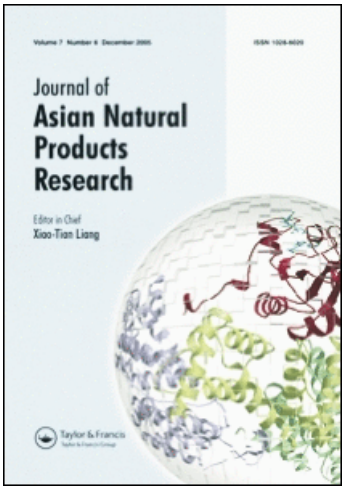

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Note

A new phytosterone from *Achyranthes bidentata* Bl.

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A new phytosterone named achyranthesterone A (**1**), along with the three known compounds stachysterone D (**2**), β-ecdysone (**3**) and polypodine B (**4**) have been isolated from the roots of *Achyranthes bidentata* Bl. The structure of the new compound was determined as 2β,3β,14α,20*S*,21,22*R*,25-heptahydroxycholest-7-en-6-one on the basis of physico-chemical properties and spectral methods.

Keywords: *Achyranthes bidentata* Bl; Phytosterone; Achyranthesterone A; HMBC

1. Introduction

Achyranthes bidentata Bl. is a traditional Chinese medicine that has the effect of immunomodulation [1], antitumor [2] and antisenility activities [3]. This paper describes the isolation and structural elucidation of a new phytosterone, achyranthesterone A (**1**).

2. Results and discussion

Achyranthesterone A (**1**) was obtained as white needles from EtOAc–MeOH and gave a positive response to the Liebermann–Burchard reaction. The molecular formula was suggested to be $C_{27}H_{44}O_8$ (*m/z* 497.1, $[M + H]^+$) by ESI-MS along with ^1H and ^{13}C NMR data. The UV spectrum shows a typical absorption of an α,β-unsaturated carbonyl moiety at 240.8 nm. An olefinic proton signal at δ 6.25 as a doublet ($J = 3$ Hz) in the ^1H NMR spectrum together with carbon signals at δ 203.5, 165.9 and 121.8 in the ^{13}C NMR spectrum indicate an ecdysteroid-type skeleton. The ^1H NMR spectrum has four methyl signals at δ 1.35, 1.35, 1.20 and 1.02. The ^{13}C NMR spectrum has signals of 27 carbon atoms, among which seven are oxygenated (δ 84.3, 78.8, 78.6, 69.6, 68.2, 68.1 and 66.8), indicating the presence of seven hydroxyls.

In the ^1H–^1H COSY spectrum, the signal at δ 6.25 (H-7) shows a long-range correlation with the signal at δ 3.60 (1H, m), thus δ 3.60 was assigned to H-9. In the HSQC spectrum,

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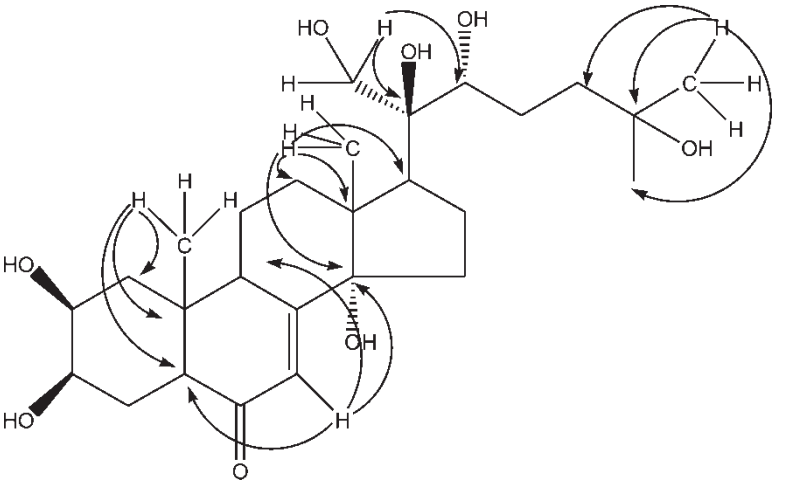

Figure 1. HMBC correlations of compound **1**.

the signal at δ 3.60 is correlated with δ34.5 (C-9). In the HMBC spectrum (figure 1), the signal of H-7 at δ 6.25 shows long-range correlations with δ 34.5 (C-9), 51.4 (C-5) and 84.3 (C-14). The ^{1}H–^{1}H COSY spectrum showed correlations between H-5 (δ 3.00, 1H, dd, $J_1 = 3.0$, $J_2 = 12$ Hz) and H-4 (δ1.98, 1H, m); H-4 and H-3 (δ 4.22, 1H, br.s); H-3 and H-2 (δ 4.18, 1H, m); H-2 and H-1 (δ 2.10, 1.90, 1H, m). There are also correlations between H-9 (δ 3.60, 1H, m) and H-11 (δ 1. 80, 1H, m, 1.68, 1H, m); H-11 and H-12 (δ 2.16, 1H, m, 1.96, 1H, m). In the HMBC spectrum, the signal of H-19 at δ 1.02 shows long-range correlations with three carbon signals at δ 51.4 (C-5), 38.7 (C-10) and 38.0 (C-1); the signal of H-18 at δ 1.20 shows long-range correlations with the four carbon signals at δ 84.3 (C-14), 47.9 (C-17), 47.8 (C-13) and 31.6 (C-12). In the HSQC spectrum, the signal of C-17 at δ 47.9 showed a correlation with δ 3.11 (H-17, 1H, m). Correlations between H-17 and H-16 (2.15, 1H, m); H-16 and H-15 (1.96, 1H, m) were also be observed in the ^{1}H–^{1}H COSY spectrum.

Comparison of the ^{13}C NMR data of compound **1** with those of β-ecdysone [4] reveal that the chemical shifts of carbon signals of C-1–C-17 have no obvious differences, indicating that rings A–D of compound **1** are similar to those of β-ecdysone. In the side-chain, the chemical shifts of C-23–C-27 also accord with the data of β-ecdysone. The only difference is that C-22, C-20 are downfield shifted (+1.2, +1.7, respectively). The signal of δ 21.7 (C-21) in β-ecdysone disappeared, while another carbon signal of δ 66.8 is observed. In the ^{1}H NMR, the signals at δ 1.57 (H-21) of β-ecdysone also disappeared, instead of which there are two signals at δ 4.42 (1H, d, $J = 19.2$) and 4.39 (1H, d, $J = 19.2$). Both signals at δ 4.42 and 4.39 correlate with the carbon signal at δ 66.8 in the HSQC spectrum and the HMBC spectrum, δ 4.42 and 4.39 show long-range correlations with δ 78.8 (C-22) and 78.6 (C-20). Thus the signals at δ 4.42 and 4.39 are for the two protons of H-21 and the signal at δ 66.8 is the C-21. Thus, the C-21 methyl was replaced by a hydroxymethyl group in compound **1**. At the same time, there are long-range correlations between H-27, 26 (δ 1.35, 6H, s) and 69.6 (C-25), 42.8 (C-24), 30.1 (C-27) and 30.0 (C-26) in the HMBC spectrum (figure 1). In the HSQC spectrum, the signal at δ 78.8 (C-22) correlates with δ 4.08 (H-22). The ^{1}H–^{1}H COSY spectrum shows correlations between H-22 and H-23 (δ 2.18, 1H, m); H-23 and H-24 (δ 1.81, 1H, m). From the view of biosynthesis, the configurations at C-20, C-22 of all phytosterones from nature are *R*,*R* respectively. Thus, it could be deduced that the configuration of compound **1** at C-20 should be *S* because of the hydroxymethyl group at C-21

Table 1. ^{13}C NMR data of compounds **1**–**4** (in C_5D_5N).

No	*1*	*2*	*3*	*4*
1	38.0	36.6	38.0	34.9
2	68.2	66.8	68.2	68.0
3	68.1	66.6	68.1	69.9
4	32.4	30.7	32.5	36.0
5	51.4	50.1	51.4	79.9
6	203.5	202.7	203.4	200.9
7	121.8	120.5	121.7	119.9
8	165.9	165.2	166.1	166.9
9	34.5	33.2	34.5	38.3
10	38.7	37.7	38.7	44.7
11	21.1	20.5	21.1	21.1
12	31.6	30.3	31.8	32.1
13	47.8	46.6	48.1	48.1
14	84.3	83.0	84.2	84.0
15	31.4	30.3	32.0	31.7
16	21.6	20.5	21.5	21.4
17	47.9	50.1	50.1	50.0
18	17.9	17.2	17.9	17.9
19	24.5	23.9	24.5	17.2
20	78.6	74.7	76.9	76.8
21	66.8	20.5	21.7	21.7
22	78.8	84.1	77.6	77.6
23	27.9	27.1	27.5	27.5
24	42.8	38.3	42.7	42.7
25	69.6	79.8	69.6	69.5
26	30.0	28.6	30.0	30.0
27	30.1	28.0	30.1	30.1

and the configuration at C-22 should be *R*. In any event, a further study on the absolute configuration at C-20 and C-22 is needed. Therefore, the structure of compound **1** is 2β,3β,14α,20*S*,21,22*R*,25-heptahydroxycholest-7-en-6-one, which was named achyranthesterone A. ^1H and ^{13}C signal assignments of **1** were made by examination of the HSQC and ^1H–^1H COSY spectra. The results were confirmed by the data of HMBC spectrum and are summarized in table 1.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yanaco micro-hot-stage and are uncorrected. ESI-MS was performed on a LCQ spectrometer (Finnigan Co. Ltd., U.S.A.). All NMR spectra (in C_5D_5N at room temperature) were recorded on a Bruker-ARX-300 spectrometer using TMS as internal standard. The optical rotation was measured on a Perkin-Elmer 241 polarimeter. The UV spectrum was recorded on a Shimadzu UV-260 UV-Vis spectrometer. PHPLC was performed on a Shimadzu CTO-6A, using Shimadzu Shim-pack PREP-ODS (i.d. 25 mm × 21.6 cm). Silica gel and Al_2O_3 for chromatography were produced by Qingdao Haiyang Chemical Group Co. Ltd., China and Shanghai Xincheng Fine Chemical Co. Ltd., China, respectively. The light petroleum used had the boiling point range 60–90°C.

3.2 Plant material

The plant material was purchased in Shenyang TCM Corporation (Shenyang), and was identified by Professor Chunquan Xu (Shenyang Pharmaceutical University). A voucher

specimen (No. 2000, 1013) has been deposited in the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

Dried powder of the roots of *Achyranthes bidentata* Bl. (2.5 kg) was extracted with 75% EtOH. The extracts were then concentrated *in vacuo*, and extracted successively with EtOAc and n-BuOH. The EtOAc extract was subjected to column chromatography on Al_2O_3 and gradiently eluted with $CHCl_3$–CH_3OH to provide 8 fractions. Fractions 1–3 ($CHCl_3$–CH_3OH 100:0–100:10) were collected and chromatographed on a silica-gel column eluted with light petroleum–EtOAc (100:20) to yield **2** (8 mg); Fractions 4–6 ($CHCl_3$–CH_3OH 100:15-100:30) were separated by column chromatography on silica gel using $CHCl_3$–MeOH. The eluate of 100:4 yielded **3** (20 mg) and the eluate of 100:5 was further separated by HPLC [CH_3CN–H_2O (16:100)] to yield **1** (3 mg) and **4** (8 mg).

Achyranthesterone A (**1**): white needles (EtOAc–MeOH). mp $>$ 300°C. UV λ_{max} (MeOH): 240.8 nm, ESI-MS: *m/z* 497.1 $[M + H]^+$, 479.0 $[M + H - H_2O]^+$, 461.0 $[M + H - 2H_2O]^+$, 443.1 $[M + H - 3H_2O]^+$, 425.1 $[M + H - 4H_2O]^+$, $[\alpha]_D^{30} = +13.0$ (*c* 0.001, MeOH). ^{1}H NMR (in C_5D_5N, 300 MHz) δ (ppm): 1.90 (1H, m, H-1), 2.10 (1H, m, H-1), 4.18 (1H, m, H-2), 4.22 (1H, m, H-3), 1.98 (1H, m, H-4), 1.88 (1H, m, H-4), 3.00 (1H, dd, $J_1 = 3.0$, $J_2 = 12$ Hz, H-5), 6.25 (1H,d, $J = 3$ Hz, H-7), 3.60 (1H, m, H-9), 1.68 (1H, m, H-11), 1.80 (1H, m, H-11), 2.16 (1H, m, H-12), 1.96 (1H, m, H-12), 2.56 (1H, m, H-15), 1.96 (1H, m, H-15), 2.51 (1H, m, H-16), 2.08 (1H, m, H-16), 3.11 (1H, m, H-17), 1.20 (3H, s, H-18), 1.02 (3H, s, H-19), 4.42 (1H, d, $J = 19.2$ Hz, H-21), 4.39 (1H, d, $J = 19.2$ Hz, H-21), 4.08 (1H, m, H-22), 2.34 (1H, m, H-23), 2.18 (1H, m, H-23), 2.32 (1H, m, H-24), 1.81 (1H, m, H-24), 1.35 (6H, s, H-26, 27); ^{13}C NMR (in C_5D_5N, 75 MHz) (table 1).

Stachysteron D (**2**): white needles (MeOH), mp. 246–248°C. ^{1}H NMR (in C_5D_5N, 300 MHz) δ (ppm): 5.63 (1H, br.s, H-7), 0.71 (3H, s, H-18), 0.83 (3H, s, H-19), 1.07 (3H, s, H-21), 1.17 (6H, s, H-26, 27); ^{13}C NMR (in C_5D_5N, 75 MHz) (table 1).

β-Ecdysone (**3**): white needles (EtOAc-MeOH, mp. 240–242°C, ^{1}H NMR (in C_5D_5N, 300 MHz) δ (ppm): 6.27 (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 (in C_5D_5N, 75 MHz) (table 1).

Polypodine B (**4**): white needles (EtOAc-MeOH), mp. 253–256°C. ^{1}H NMR (in C_5D_5N, 300 MHz) δ (ppm): 6.28 (1H, br. s, H-7), 1.16 (3H, s, H-19), 1.21(3H, s, H-18), 1.59 (3H, s, H-21), 1.36 (6H, s, H-26, 27); ^{13}C NMR (in C_5D_5N, 75 MHz) (table 1).

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