Molecules **2012**, *17*, 3324-3332; doi:10.3390/molecules17033324

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

Phytoecdysteroids from the Roots of *Achyranthes bidentata* Blume

Mei Zhang [1,2], Zhong-Yu Zhou [1], Jing Wang [1,2], Yong Cao [3], Xue-Xiang Chen [3], Wei-Min Zhang [4], Li-Dong Lin [1] and Jian-Wen Tan [1,*]

[1] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

[2] Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

[3] College of Food Science, South China Agricultural University, Guangzhou 510642, China

[4] Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China

* Author to whom correspondence should be addressed; E-Mail: jwtan@scbg.ac.cn; Tel.: +86-20-3708-2054; Fax: +86-20-3708-2054.

Received: 23 February 2012; in revised form: 8 March 2012 / Accepted: 12 March 2012 / Published: 14 March 2012

Abstract: Two new phytoecdysteroids, (25*S*)-20,22-*O*-(*R*-ethylidene)inokosterone (**1**) and 20,22-*O*-(*R*-3-methoxycarbonyl)propylidene-20-hydroxyecdysone (**2**), together with six known phytoecdysteroids **3**–**8** were isolated from the roots of *Achyranthes bidentata* Blume. The new structures were established on the basis of spectroscopic studies and chemical evidences. The absolute configuration at C-25 in the structure of known compound **3** was determined by chemical and spectroscopic means.

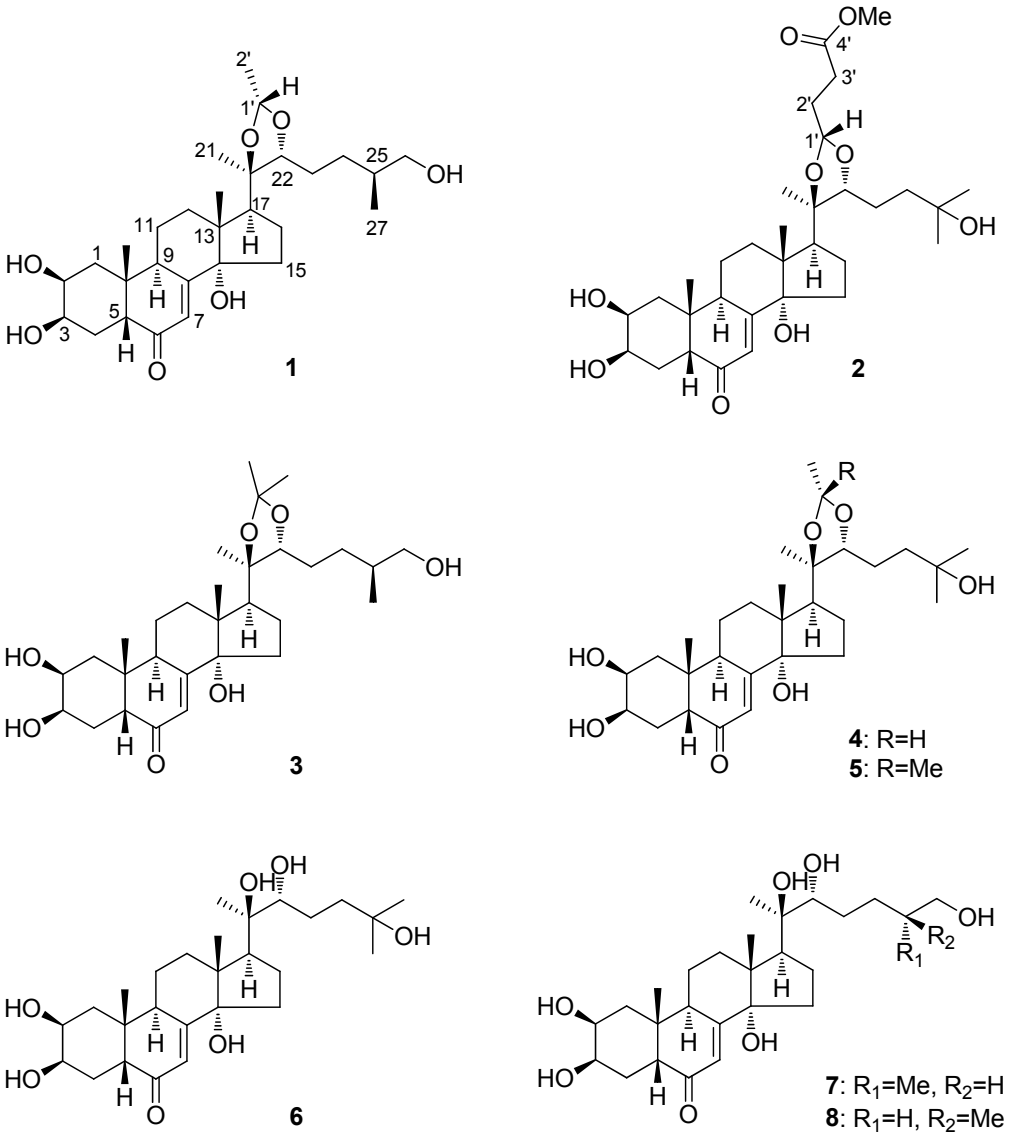
Keywords: *Achyranthes bidentata*; Amaranthaceae; phytoecdysteroids; ecdysteroids

1. Introduction

Achyranthes bidentata Blume (Amaranthaceae) is widely distributed in Asian countries like India, Korea, Japan, and China. The root of *A. bidentata* has been prescribed in the Chinese Pharmacopeia as an important herbal medicine and its multiple pharmacological effects, such as anti-osteoporosis [1,2], antitumor [3–5], anti-senility [3,6–8], anti-inflammatory [9], immunomodulatory [3,10–13] activities are well documented. Previous phytochemical investigations of *A. bidentata* have reported the isolation

of phytoecdysteroids [14–16], saccharides [17,18] and saponins [19,20], and some of them displayed diverse bioactivities. In the course of our search for potentially new and bioactive compounds from medicinal plants in China, we investigated the roots of *A. bidentata* and isolated eight phytoecdysteroids, including two new ones, (25*S*)-20,22-*O*-(*R*-ethylidene)inokosterone (**1**) and 20,22-*O*-(*R*-3-methoxy carbonyl)propylidene-20-hydroxyecdysone (**2**), and six known phytoecdysteroids **3**–**8** (Figure 1). In this paper, we report the isolation and structural elucidation of these phytoecdysteroids.

Figure 1. Chemical structures of compounds **1**–**8**.



2. Results and Discussion

Compound **1** was obtained as a white amorphous powder with a molecular formula of $C_{29}H_{46}O_7$ as established on the basis of a combined analysis of its HRESIMS and NMR spectroscopic data. The spectroscopic data (IR, UV, and NMR: see Section 3.3 and Tables 1 and 2) supported the fact that **1** was an ecdysteroid closely related to inokosterone-20,22-acetonide, a reported [21,22] ecdysteroid compound which was also obtained as compound **3** in the present study. Careful analysis of the ^{1}H- and ^{13}C-NMR spectra pointed out as major differences that the resonances for the acetone ketal

group in **3** were replaced by the signals for a C_2 acetal group [δ_H 5.05 (1H, q, H-1'), 1.29 (3H, d, H_3-2'); δ_C 102.3 (C-1'), 22.0 (C-2')]. These data led us to preliminarily establish the whole structure of **1** as 20,22-ethylideneinokosterone, of which the stereochemistry at C-25 and C-1' still needed to be determined. In the ^{1}H-^{1}H COSY spectrum, signals correlated to the connections of C-22 to C-27 were exhibited. In the NOESY spectrum, a significant correlation signal of H-1' (δ_H 5.05) with H-22 (δ_H 3.65) was observed, which indicated that the stereochemistry at C-1' in **1** should be *R* configuration (Figure 2). In order to clarify the absolute configuration at C-25 in the molecule, an acid hydrolysis of **1** was carried out, and the centre ecdysteroid unit that released from **1** was confirmed to be the same as the known compound (25*S*)-inokosterone (**8**), as evidenced by a co-TLC elution test and comparison of the NMR spectroscopic data with literature precedents [23]. This result indicated that the absolute configuration at C-25 of **1** should be *S*. Thus, the complete structure of **1** was elucidated as (25*S*)-20,22-*O*-(*R*-ethylidene)inokosterone.

Table 1. The ^{1}H-NMR (400 MHz) spectral data [δ (ppm), *J* in Hz] of **1**–**4** and **8**.

No.	δ_H (1) [a]	δ_H (2) [a]	δ_H (3) [a]	δ_H (4) [a]	δ_H (8) [b]
1	1.77 (m), 1.41(m)	1.78 (m)	1.78 (m)	1.79(m)	2.15 (m), 1.93 (m)
2	3.82 (m)	3.82 (m)	3.82 (m)	3.82 (m)	4.19 (m)
3	3.94 (m)	3.94 (m)	3.94 (m)	3.94 (m)	4.23 (m)
4	1.73 (m), 1.69 (m)	1.71 (m)	1.73 (m), 1.69 (m)	1.72 (m)	2.04 (m), 1.82 (m)
5	2.38 (m)	2.36 (m)	2.37 (m)	2.35 (m)	3.01 (dd, 13.2, 3.2)
7	5.80 (d, 2.0)	5.81 (s)	5.80 brs	5.80 (d, 2.0)	6.26 (d, 1.6)
9	3.13 (m)	3.13 (m)	3.13 (m)	3.13 (m)	3.60 (m)
11	1.79 (m), 1.67 (m)	1.79 (m), 1.68 (m)	1.79 (m), 1.67 (m)	1.78 (m), 1.68 (m)	1.88 (m), 1.73(m)
12	2.09 (m), 1.82 (m)	2.09 (m), 1.83 (m)	2.09 (m), 1.82 (m)	2.09 (m), 1.83 (m)	2.17 (m), 1.92 (m)
15	1.94 (m), 1.60 (m)	1.91 (m), 1.61 (m)	1.94 (m), 1.60 (m)	1.91 (m), 1.61 (m)	2.60 (m), 2.04 (m)
16	1.92 (m), 1.87 (m)	1.92 (m)	1.92 (m), 1.87 (m)	1.93 (m)	2.46 (m), 2.08 (m)
17	2.32 (m)	2.34 (m)	2.28 (m)	2.32 (m)	2.95 (t, 9.2)
18	0.85 (s)	0.84 (s)	0.81 (s)	0.85 (s)	1.22 (s)
19	0.95 (s)	0.95 (s)	0.95 (s)	0.96 (s)	1.07 (s)
21	1.13 (s)	1.15 (s)	1.15 (s)	1.15 (s)	1.59 (s)
22	3.65 (dd, 8.8, 3.6)	3.64 (m)	3.68 (dd, 9.6, 2.8)	3.63 (m)	3.86 (d, 10.4)
23	1.49 (m)	1.54 (m)	1.47 (m)	1.52 (m)	1.94 (m), 1.63 (m)
24	1.50 (m), 1.15 (m)	1.72 (m), 1.45 (m)	1.68 (m), 1.15 (m)	1.71 (m)	2.17 (m), 1.41 (m)
25	1.61 (m)		1.63 (m)		1.81 (m)
26	3.34 (dd, 10.4, 6.4) 3.42 (dd, 10.4, 5.6)	1.19 (s)	3.34 (dd, 10.4, 6.4) 3.42 (dd, 10.4, 5.6)	1.19 (s)	3.64 (dd, 10.0, 6.4) 3.76 (dd, 10.0, 5.2)
27	0.93 (d, 6.8)	1.20 (s)	0.93 (d, 6.4)	1.20 (s)	1.03 (d, 6.4)
1'	5.05 (q, 4.8)	4.97 (t, 4.0)		5.05 (q, 4.8)	
2'	1.29 (d, 4.8)	1.90 (m)	1.30 (s)	1.29 (d, 4.8)	
3'		2.41 (t, 7.2)	1.37 (s)		
4'-OC<u>H</u>$_3$		3.65 (s)			

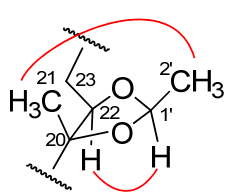
[a] Data were measured in CD_3OD; [b] Data were measured in C_5D_5N.

Table 2. The ^{13}C-NMR (100 MHz) spectral data [δ (ppm)] of compounds **1**–**4** and **8**.

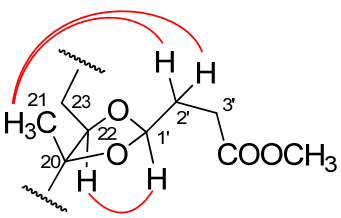
Position	δ_C (1) [a]	δ_C (2) [a]	δ_C (3) [a]	δ_C (4) [a]	δ_C (8) [b]
1	37.3 (t)	37.3 (t)	37.3 (t)	37.3 (t)	38.0 (t)
2	68.7 (d)	68.7 (d)	68.7 (d)	68.7 (d)	68.1
3	68.5 (d)	68.5 (d)	68.5 (d)	68.5 (d)	68.1
4	32.9 (t)	32.8 (t)	32.8 (t)	32.9 (t)	32.5 (t)
5	51.8 (d)	51.8 (d)	51.8 (d)	51.8 (d)	51.4 (d)
6	206.4 (s)	206.5 (s)	206.4 (s)	206.4 (s)	203.5 (s)
7	122.2 (d)	122.2 (d)	122.2 (d)	122.1 (d)	121.7 (d)
8	167.5 (s)	167.6 (s)	167.6 (s)	167.6 (s)	166.1 (s)
9	35.1 (d)	35.1 (d)	35.1 (d)	35.1 (d)	34.5 (d)
10	39.2 (s)	39.2 (s)	39.2 (s)	39.2 (s)	38.7 (s)
11	21.5 (t)	21.5 (t)	21.5 (t)	21.5 (t)	21.1 (t)
12	32.2 (t)	32.2 (t)	32.3 (t)	32.1 (t)	31.8 (t)
13	49.0 (s) *	49.0 (s) *	49.0 (s) *	49.0 (s) *	48.1 (s)
14	85.2 (s)	85.2 (s)	85.3 (s)	85.2 (s)	84.2 (s)
15	31.7 (t)	31.7 (t)	31.7 (t)	31.7 (t)	32.1 (t)
16	22.6 (t)	22.6 (t)	22.4 (t)	22.6 (t)	21.7 (t)
17	51.3 (d)	51.4 (d)	50.5 (d)	51.3 (d)	50.1 (d)
18	17.6 (q)	17.6 (q)	17.6 (q)	17.6 (q)	17.9 (q)
19	24.4 (q)	24.4 (q)	24.4 (q)	24.4 (q)	24.5 (q)
20	85.3 (s)	85.3 (s)	85.7 (s)	85.3 (s)	77.3 (s)
21	23.6 (q)	23.4 (q)	22.5 (q)	23.7 (q)	21.5 (q)
22	85.5 (d)	85.6 (d)	83.1 (d)	85.6 (d)	76.8 (d)
23	27.3 (t)	24.6 (t)	27.4 (t)	24.6 (t)	30.3 (t)
24	32.0 (t)	42.2 (t)	32.0 (t)	42.2 (t)	32.0 (t)
25	37.0 (d)	71.1 (s)	37.0 (d)	71.1 (s)	36.8 (d)
26	68.2 (t)	29.5 (q)	68.2 (t)	29.5 (q)	67.4 (t)
27	17.0 (q)	28.9 (q)	17.0 (q)	28.9 (q)	17.8 (q)
1'	102.3 (d)	103.9 (d)	108.0 (s)	102.3 (d)	
2'	22.0 (q)	31.0 (t)	29.3 (q)	22.0 (q)	
3'		29.2 (t)	27.2 (q)		
4'		175.6 (s)			
4'-O$\underline{C}$H$_3$		52.1 (q)			

[a] Data recorded in CD_3OD; [b] Data recorded in C_5D_5N; * The signal is overlapped by solvent.

Figure 2. Key NOE (—) correlations of **1** and **2**.



1



2

Compound **2** was also obtained as a white amorphous powder. HR-EIMS showed a $[M]^+$ ion at *m/z* 578.3450, corresponding to the molecular formula $C_{32}H_{50}O_9$ (calcd for $C_{32}H_{50}O_9$, 578.3449). The spectroscopic data (IR, UV, and NMR: See Section 3.3 and Tables 1 and 2) suggested **2** was an ecdysteroid closely related to 20,22-*O*-(*R*-ethylidene)-20-hydroxyecdysone, a reported [24,25] ecdysteroid which was also obtained in the present study (compound **4**). Further comparison of the ^{1}H- and ^{13}C-NMR spectra showed that the signals for the C_2 acetal group in **4** were absent in **2**. Instead, additional signals for one acetalated 3-methoxycarbonyl-propylal group [δ_H 4.97 (1H, t, H-1'), 3.65 (3H, s, 4'-OC<u>H</u>$_3$), 2.41 (3H, H$_3$-3'), 1.90 (3H, H$_3$-2'); δ_C 175.6 (C-4'), 103.9 (C-1'), 52.1 (4'-O<u>C</u>H$_3$), 31.0 (C-3'), 29.2 (C-2')] were exhibited in the spectra. ^{1}H-^{1}H COSY and HSQC spectra permitted establishment of the spin system from C-1' through C-3'. The HMBC correlations from 4'-OC<u>H</u>$_3$ (δ_H 3.65), H$_2$-3' (δ_H 2.41) and H$_2$-2' (δ_H 1.91) to C-4' (δ_C 175.6), and from H$_2$-2', H$_2$-3' to C-1' (δ_C 103.9) indicated the linkage from C-1' to 4'-O<u>C</u>H$_3$. The *R* configuration at C-1' was determined by the important NOE correlation between H-1' and H-22 (Figure 2). These findings led to the establishment of the whole structure of **2** as shown in Figure 1, and this structure was further well supported by other important ^{1}H-^{1}H COSY, HMBC and NOESY correlations. Therefore, **2** was determined as 20,22-*O*-(*R*-3-methoxycarbonyl)propylidene-20-hydroxyecdysone.

Compound **3**, showing the molecular formula $C_{28}H_{44}O_7$, was deduced to be the same ecdysteroid compound inokosterone-20,22-acetonide from the roots of *Leuzea carthamoides* recently reported in the literature [22], by comparison of its spectroscopic data (Tables 1 and 2) with reported values. However, at that time the authors had yet not clarified the absolute configuration at C-25 in the structure. In order to determine the stereochemistry at C-25 in the structure of **3**, a similar acid hydrolysis like that performed for **1** was conducted, and the free ecdysteroid that was released from **3** was confirmed to be (25*S*)-inokosterone (**8**) on the basis of a co-TLC elution test and NMR analyses, suggesting that the absolute configuration at C-25 in **3** should also be *S* configuration. Therefore, the complete structure of **3** was determined as (25*S*)-inokosterone-20,22-acetonide.

The other five known compounds were identified as 20,22-*O*-(*R*-ethylidene)-20-hydroxyecdysone (**4**) [24,25], 20-hydroxyecdysone-20,22-monoacetonide (**5**) [25,26], 20-hydroxyecdysone (**6**) [27], (25*R*)-inokosterone (**7**) [23,28] and (25*S*)-inokosterone (**8**) [23,28], by interpretation of their spectroscopic data, as well as by comparison with literature values.

Among the eight isolated compounds, **1** and **2** are two new phytoecdysteroids, each characterized by having an acetal group in the molecule. In particular, **2** is so far the first example of phytoecdysteroid acetalated at the side chain with a 4-oxobutanoic acid unit. Compounds **3**–**5** were found in *Achyranthes bidentata* roots for the first time.

3. Experimental

3.1. General

Optical rotations were measured on a Perkin-Elmer 341 polarimeter with MeOH as solvent. UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer. IR spectra (KBr) were taken on a Bruker Tensor 27 spectrophotometer in cm^{-1}. NMR spectra were recorded in C_5D_5N and CD_3OD on a Bruker DRX-400 instrument using the residual solvent peak as reference.

ESIMS were collected on an MDS SCIEX API 2000 LC/MS/MS instrument. HRESIMS data were obtained on a Water Q-TOF Premier mass spectrometer and HREIMS data were obtained on a Finigan MAT 95XP mass spectrometer. Preparative HPLC was conducted using a CXTH P3000 HPLC pump and a UV3000 UV-Vis Detector with a Fuji-C18 column (10 μm–100 A). For column chromatography (CC), silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), YMC ODS-A (50 μm, YMC Co. Ltd., Kyoto, Japan) and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden) were used. Fractions were monitored by TLC, and spots were visualized by heating the silica gel plates sprayed with 10% H_2SO_4 in ethanol.

3.2. Plant Materials

Roots of *A. bidentata* Blume were purchased from Anguo Professional Market for Chinese Materia Medica, in April 2010, and were collected in Anguo County, Hebei Province, China. Plants were authenticated by Fu-Wu Xing (South China Botanical Garden, Chinese Academy of Sciences), and a voucher specimen (No. 20100408A) was deposited in the Laboratory of Phytochemistry of South China Botanical Garden, Chinese Academy of Sciences.

3.3. Extraction and Isolation

Powder of the dry roots of *A. bidentata* (5.10 kg) was extracted with EtOH-H_2O (95:5, 10 L × 3) at room temperature three times (24 h each). The EtOH extracts were combined and concentrated *in vacuo*. Then, the resulting residue was suspended in H_2O (1.5 L) and sequentially extracted with petroleum ether (5 L × 3), EtOAc (5 L × 3) and *n*-BuOH (5 L × 3). The EtOAc layer was evaporated *in vacuo* to yield EtOAc-soluble fraction (17.5 g).

The EtOAc-soluble fraction was subjected to silica gel CC using a gradient of $CHCl_3$-MeOH (95:5–60:40, v/v) to give ten fractions (E_1–E_{10}). Fraction E_8 (3.90 g), obtained by elution with $CHCl_3$-MeOH (85:15, v/v), was further subjected to silica gel CC and successively eluted with $CHCl_3$-MeOH (20:1–10:1, v/v) to yield six sub-fractions (E_{6-1}–E_{6-6}). Sub-fraction E_{6-4} (0.370 g) was separated by an ODS column using MeOH-H_2O (60:40–100:0, v/v), followed by HPLC preparation with MeOH-H_2O (70:30, v/v) at a flow rate of 10 mL/min to afford compounds **2** (5.9 mg, t_R = 41 min), **4** (6.8 mg, t_R = 49 min), **3** (6.0 mg, t_R = 61 min), **1** (5.0 mg, t_R = 65 min), and **5** (9.3 mg, t_R = 67 min). Sub-fraction E_{6-5} (0.200 g) was purified by Sephadex LH-20 CC using MeOH as eluent, followed by preparative HPLC using MeOH-H_2O (40:60, v/v) at a flow rate of 8 mL/min to afford compounds **6** (t_R = 96 min, 20.0 mg), **7** (t_R = 114 min, 29.0 mg) and **8** (t_R = 127 min, 38.0 mg).

(25S)-20,22-O-(R-Ethylidene)inokosterone (**1**). White amorphous powder; $[\alpha]_{20}^{D}$ + 26.8 (c = 0.35, MeOH); IR (KBr) ν_{max} 3419, 2934, 1654 ,1450, 1139, 1156 cm^{-1}; UV (MeOH) λ_{max} (log ε) nm: 242 (4.14); ESIMS (+) *m/z* 529 $[M+Na]^+$, 507 $[M+H]^+$; ESIMS (−) *m/z* 505 $[M–H]^-$; HRESIMS (−) *m/z* 505.3173 $[M-H]^-$ (calcd. for $C_{29}H_{45}O_7$, 505.3160); ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz) data are shown in Tables 1 and 2.

20,22-O-(R-3-Methoxycarbonyl)propylidene-20-hydroxyecdysone (**2**). White amorphous powder; $[\alpha]_{20}^{D}$ + 34.0 (c = 0.20, MeOH); IR (KBr) ν_{max} 3423, 2964, 1737, 1654, 1382, 1139, 1058 cm^{-1}; UV

(MeOH) λ_{max} (log ε) nm: 242 (3.75); ESIMS (+) *m/z* 601 $[M+Na]^+$, 579 $[M+H]^+$; ESIMS (−) *m/z* 577 $[M-H]^-$; HREIMS *m/z* 578.3450 $[M]^+$ (calcd. for $C_{32}H_{50}O_9$, 578.3449); ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz) data are shown in Tables 1 and 2.

(25S)-Inokosterone-20,22-acetonide (**3**). White amorphous powder; ESIMS (+) *m/z* 520 $[M+Na]^+$; ESIMS (−) *m/z* 555 $[M+Cl]^-$, 519 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz) data are shown in Tables 1 and 2.

20,22-O-(R-Ethylidene)-20-hydroxyecdysone (**4**). White amorphous powder; ESIMS (+) *m/z* 545 $[M+K]^+$, 529 $[M+Na]^+$; ESIMS (−) *m/z* 541 $[M+Cl]^-$, 505 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz) data are shown in Tables 1 and 2.

(25S)-Inokosterone (**8**). White amorphous powder; ESIMS (+) *m/z* 519 $[M+K]^+$, 503 $[M+Na]^+$; ESIMS (−) *m/z* 515 $[M+Cl]^-$; ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz) data are shown in Tables 1 and 2.

*3.4. Acidic Hydrolysis of Compounds **1** and **3***

Individual solutions of **1** (2.5 mg) and **3** (3.0 mg) in 1 M H_2SO_4 (0.5 mL) were allowed to stand at room temperature for 12 h with continuous oscillation. Each reaction mixture was subsequently extracted with $CHCl_3$ (1 mL) and EtOAc (1 mL × 3), and the EtOAc-soluble fraction was concentrated to dryness to give a free ecdysteroid (1.5 mg from **1** and 1.8 mg from **3**). Both hydrolysates were identified as the known compound (25S)-inokosterone (**8**).

4. Conclusions

Two new phytoecdysteroids, (25*S*)-20,22-*O*-(*R*-ethylidene)inokosterone (**1**) and 20,22-*O*-(*R*-3-methoxycarbonyl)propylidene-20-hydroxyecdysone (**2**), were isolated from the roots of *Achyranthes bidentata* Blume, along with six known ones. Three out of the six known compounds **3**–**5** were found in this plant species for the first time. Compound **2** is so far the first example of phytoecdysteroid acetalated at the side chain with a 4-oxobutanoic acid unit. The absolute configuration at C-25 in the structure of known compound **3** was further established in this study.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/17/3/3324/s1.

Acknowledgments

We wish to acknowledge the National Natural Science Foundation of China (No.30870248) and the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-N-0804).

References and Notes

1. He, C.C.; Hui, R.R.; Tezuka, Y.; Kadota, S.; Li, J.X. Osteoprotective effect of extract from *Achyranthes bidentata* in ovariectomized rats. *J. Ethnopharmacol.* **2010**, *127*, 229–234.
2. Ren, X.C.; Xu, X.X.; Xu, D.J.; Gao, J. Effects of *Achyranthes bidentata* saponins on bone metabolism of osteoporotis rats induced by retioic acid. *Chin. J. Exp. Trad. Med. Form.* **2011**, *17*, 128–130.
3. Xiang, D.B.; Li, X.Y. Antitumor activity and immuno-potentiating actions of *Achyranthes bidentata* polysaccharides. *Acta Pharmacol. Sin.* **1993**, *14*, 556–561.
4. Hu, J.; Qi, Y.X.; Li, Q.X.; Shan, B.E. The research of extract of *Achyranthes bidentata* Blume anti-tumor activity. *Chin. J. Microbiol. Immunol.* **2005**, *25*, 415–418.
5. Wang, Y.F.; Wang, Q.D.; Liu, C.J.; Jiang, J.H.; Sun, W.X.; Xia, W.; Wu, Y. Antitumor activity of the crude saponins from *Achyranthes bidentata*. *J. Henan Med. Univ.* **1997**, *32*, 4–6.
6. Tan, F.; Deng, J. Analysis of the constitutes and antisenile function of *Achyranthes bidentata* polysaccharides. *Acta Bot. Sin.* **2002**, *44*, 795–798.
7. Deng, H.B.; Cui, D.P.; Jiang, J.M.; Feng, Y.C.; Cai, N.S.; Li, D.D. Inhibiting effects of *Achyranthes bidentata* polysaccharide and *Lycium barbarum* polysaccharide on nonenzyme glycation in D-galactose induced mouse aging model. *Biomed. Environ. Sci.* **2003**, *16*, 267–275.
8. Zhang, Z.Y.; Wang, S.K.; Guo, L.K. Aging changes of neurotensin-like and dynorphin-like neurons in the central nucleus of amygdala of the rat anti-aging effect of radix *Achyranthis bidentatae*: Immunohistochemical and image quantitative analytical study. *Chin. J. Anat.* **1996**, *19*, 123–127.
9. Yi, S.; Ken, Y. New anti-inflammatory ergostane-type ecdysteroids from the sclerothium of *Polyporus umbellatus*. *Biol. Med. Chem. Lett.* **2008**, *18*, 3417–3420.
10. Chen, Q.H.; Liu, Z.Y.; He, J.H. *Achyranthes bidentata* polysaccharide enhances immune response in weaned piglets. *Immunopharmacol. Immunotoxicol.* **2009**, *31*, 253–260.
11. Xiang, D.B.; Li, X.Y. Effects of *Achyranthes bidentata* polysaccharides in interleukin-1 and tumor necrosis factor-alpha production from mouse peritoneal macrophages. *Acta Pharmacol. Sin.* **1993**, *14*, 332–336.
12. Li, Z.K.; Li, D.D. The immunodulatory effect of *Achyranthes bidentata* polysaccharides. *Yao Xue Xue Bao* **1997**, *32*, 881–887.
13. Li, C.C.; Hu, X.G.; Zhang, W.X.; Xie, L.W.; Zhang, H.Y.; Dong, L. Eosinophils apoptosis, fas mRNA and bcl-2 mRNA expressions in asthma model of young rat and effects of *Achyranthes bidentata* polysaccharides. *Chin. J. Pediatr.* **2003**, *41*, 657–660.
14. Wang, Q.; Yang, L.; Jiang, H.; Wang, Z.B.; Yang, B.Y.; Kuang, H.X. Three new phytoecdysteroids containing a furan ring from the roots of *Achyranthes bidentata* Bl. *Molecules* **2011**, *16*, 5989–5997.
15. Li, X.; Zhao, W.T.; Meng, D.L.; Qiao, A.M. A new phytosterone from the roots of *Achyranthes bidentata*. *Fitoterapia* **2007**, *78*, 607–608.
16. Meng, D.L.; Li, X.; Wang, J.H.; Li, W. A new phytosterone from *Achyranthes bidentata* Bl. *J. Asian Nat. Prod. Res.* **2005**, *7*, 181–184.

17. Yu, B.; Tian, Z.Y.; Hui, Y.Z. Structural study on a bioactive fructan from the root of *Achytanthes bidentata* Bl. *Chin. J. Chem.* **1995**, *13*, 539–544.
18. Chen, X.M.; Xu, Y.J.; Tian, G.Y. Physical-chemical properties and structure elucidation of abPS isolated from the root of *Achyranthes bidentata*. *Yao Xue Xue Bao* **2005**, *40*, 32–35.
19. Li, J.X.; Hareyama, T.; Tezuka, Y.; Zhang, Y.; Miyahara, T.; Kadota, S. Five new oleanolic acid glycosides from *Achyranthes bidentata* with inhibitory activity on osteoclast formation. *Planta Med.* **2005**, *71*, 673–679.
20. Mitaine-Offer, A.C.; Marouf, A.; Hanquet, B.; Birlirakis, N.; Lacaille-Dubois, M.A. Two triterpene saponins from *Achyranthes bidentata*. *Chem. Pharm. Bull.* **2001**, *49*, 1492–1494.
21. Dinan, L. Phytoecdysteroids: Biological aspects. *Phytochemistry* **2001**, *57*, 3346–3351.
22. Buděšínský, M.; Vokáč, K.; Harmatha, J.; Cvăcka, J. Additional minor ecdysteroid components of *Leuzea carthamoides*. *Steroids* **2008**, *73*, 502–514.
23. Zhu, T.T.; Liang, H.; Zhao, Y.Y.; Wang, B. Isolation and structure identification of C25 epimers of inokosterone from *Achyranthes bidentata* Blume. *Yao Xue Xue Bao* **2004**, *39*, 913–916.
24. Odinokov, V.N.; Kumpun, S.; Galyautdinov, I.V.; Evrard-todeschi, N.; Veskina, N.A.; Khalilov, L.M.; Girault, J.P.; Dinan, L.; Maria, A.; Lafont, R. Low-polarity phytoecdysteroids from the juice of *Serratula coronata* L. *Collect. Czech. Chem. Commun*. **2005**, *70*, 2038–2052.
25. The Ecdysone Handbook, 3rd ed. Available online: http://www.ecdybase.org/ (accessed on 8 March 2012).
26. Píš, J.; Buděšínský, M.; Vokáč, K.; Laudová, V.; Hatmatha, J. Ecdysteroids from the roots of *Leuzea carthamoides*. *Phytochemistry* **1994**, *37*, 707–711.
27. Vokáč, K.; Buděšínský, M.; Harmatha, J.; Kohoutová, J. Ecdysteroid constituents of the mushroom *Tapinella panuoides*. *Phytochemistry* **1998**, *49*, 2109–2114.
28. Hikino, H.; Mohri, K.; Hikino, Y.; Arihara, S.; Takemoto, T. Inokosterone, an insect metamorphosing substance from *Achyranthes fauriei*: Absolute configuration and synthesis. *Tetrahedron* **1976**, *32*, 3015–3021.

Sample Availability: Samples of the compounds are available from the authors.