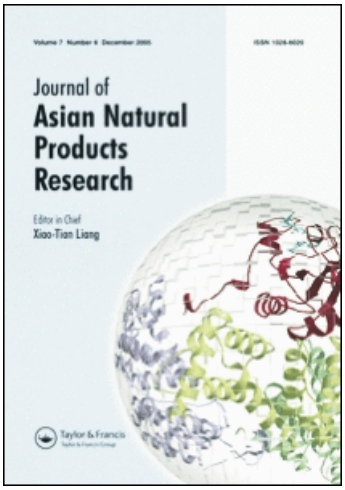

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Cheng-Yu Tan[a]; Jin-Hui Wang[b]; Xian Li[b]
[a] The Natural Products and Glycoconjugate Research Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China [b] Research Department of Natural Products, Shenyang Pharmaceutical University, Shenyang, China

Online publication date: 12 May 2010

To cite this Article Tan, Cheng-Yu , Wang, Jin-Hui and Li, Xian(2003) 'Phytoecdysteroid constituents from *Cyanotis arachnoidea*', Journal of Asian Natural Products Research, 5: 4, 237 — 240
To link to this Article: DOI: 10.1080/1028602031000105830
URL: http://dx.doi.org/10.1080/1028602031000105830

Journal of Asian Natural Products Research, 2003 VOL. 5 (4), pp. 237–240

PHYTOECDYSTEROID CONSTITUENTS FROM *CYANOTIS ARACHNOIDEA*

CHENG-YU TAN[a,*], JIN-HUI WANG[b] and XIAN LI[b]

[a]*The Natural Products and Glycoconjugate Research Group, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China;* [b]*Research Department of Natural Products, Shenyang Pharmaceutical University, Shenyang, China*

(Received 15 November 2002; Revised 22 January 2003; In final form 14 February 2003)

Four phytoecdysteroids that have only 19 or 21 carbons, named 11α-hydroxyrubrosterone (**1**), dihydroxyrubrosterone (**2**), rubrosterone (**3**) and poststerone (**4**), were isolated from the whole plant of *Cyanotis arachnoidea* C.B. Clarke. Among them, **1** was a new compound. Their structures were elucidated by spectroscopic methods.

Keywords: *Cyanotis arachnoidea*; Commelinaceae; Phytoecdysteroids; 11α-Hydroxyrubrosterone

INTRODUCTION

Cyanotis arachnoidea C.B. Clarke, which belongs to the Commelinaceae family, is a Chinese herbal medicine used for recovering from weakness, reducing sweat, stimulating the blood circulation and relaxing the muscles and joints [1]. A few reports have appeared on this plant [2–5]. This paper describes the isolation and structural elucidation of four phytoecdysteroids, which have 19 or 21 carbons.

RESULTS AND DISCUSSION

Compound **1** was isolated as white crystals from MeOH and gave a positive response to the Liebermann–Burchard reaction. Its molecular formula of $C_{19}H_{26}O_6$ was determined by HREI-MS, *m*/*z* 350.1718 $[M]^+$ (calcd. 350.1729), this being consistent with the ^1H and ^{13}C NMR spectral analysis. The ^1H and ^{13}C NMR signals of **1** were assigned by means of a 2D NMR experiment, including HMBC and HMQC analysis. The ^{13}C NMR spectrum showed 19 carbon signals, of which an α,β-unsaturated carbonyl at δ 203.7, 161.3 and 122.6 indicated the presence of an ecdysteroid-type skeleton [6]. Conversely, the ^1H NMR spectrum had a resonance characteristic of an olefinic proton (H-7) at δ 6.32 as a doublet. In the HMBC spectrum (Fig. 1), the signal of H-7 at δ 6.32 showed correlations with resonances at δ 52.7

*Corresponding author. Tel.: +86-411-4379061. E-mail: tanchyu@sina.com

ISSN 1028-6020 print/ISSN 1477-2213 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1028602031000105830

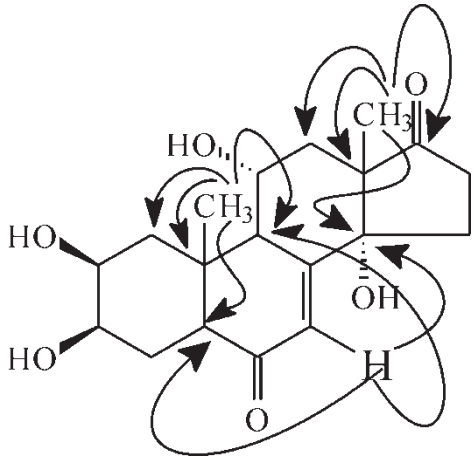

FIGURE 1 HMBC correlations of **1**.

(C-5), 43.5 (C-9) and 79.5 (C-14); the signal of H-18 at δ 0.90 showed correlations with the resonances at δ 36.2 (C-12), 53.3 (C-13), 79.5 (C-14) and 216.5 (C-17); and the signal of H-19 at δ 1.29 showed correlations with the resonances at δ 39.7 (C-1), 52.7 (C-5), 43.5 (C-9) and 39.8 (C-10). In the HMQC spectrum, the signal of C-9 at δ 43.5 had a correlation with the resonance of H-9 at δ 3.82 (1H, dd, $J = 2.2$, 8.6 Hz), which in turn displayed long-range correlations with the resonances at δ 39.8 (C-10), 52.7 (C-5) and 68.3 (C-11). The stereochemistry of **1** was defined by comparison of the ^{13}C NMR spectral data with those of the known compounds ajugasterone C and rubrosterone [7–9], and in the NOESY spectrometry, the signal of H-11 at δ 4.21 had a strong NOE enhancement with the signal of H-18 at δ 0.90 and H-19 at δ 1.29, which suggested that 11-OH was α-oriented. So the structure of compound **1** was identified as 2β,3β,11α,14α-tetrahydroxyandrost-7-en-6,17-dione (11α-hydroxy-rubrosterone).

Compound **2** was isolated as white needles from MeOH. It gave a positive response to the Liebermann-Burchard reaction. The ^{1}H NMR spectrum showed a resonance characteristic of an olefinic proton (H-7) at δ 6.23 as a broad singlet, and two methyl signals at δ 1.01, 1.08. The ^{13}C NMR spectrum showed 19 carbon signals, of which an α,β-unsaturated carbonyl at δ 203.6, 165.3 and 121.2 indicated the presence of an ecdysteroid-type skeleton [6]. In addition, the ^{13}C NMR spectrum also gave four signals, at δ 68.1, 68.1, 78.3, 82.4, that indicated the presence of four oxygenated carbons. Compared with the ^{13}C NMR data of compound **3** [8], which had only 19 carbons (Table I), the chemical shifts of C_1–C_{11} were similar to the corresponding data of **3**, while the chemical shifts of C_{12}–C_{18} were found to be quite different, especially, the signal at δ 217.2 was replaced by the signal of a hydroxyl group at δ 78.3. Therefore, we presumed that **2** contained C_{17}—OH. Comparing **2** with the known compound dihydrorubrosterone [10], the NMR data of two compounds were in accord. So **2** was identified as dihydrorubrosterone (2β,3β,14α,17-tetrahydroxyandrost-7-en-6-one).

EXPERIMENTAL

General Experimental Procedures

Melting points were measured on a Yanaco micro-hot-stage and are uncorrected. HREI-MS were performed on a Micromass Autospec-Ultima NT (Micromass UK), EI-MS spectra were

performed on a VG-5050E mass spectrometer. The optical rotation was measured on a Perkin-Elmer 241 polarimeter. ^{1}H and ^{13}C spectra (in Py-d_5 at room temperature) were obtained with a Bruker-ARX-300 instrument using TMS as internal standard and operating at 300 MHz for ^{1}H, and 75 MHz for ^{13}C. Column chromatographic separations were performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd., China), and Al_2O_3 (Shanghai Xincheng Fine Chemical Co. Ltd., China). Preparative TLC was detected by UV illumination, and TLC for identification was sprayed with 10% H_2SO_4 EtOH reagent.

Plant Material

Dried plant of *C. arachnoidea* C.B. Clarke (2.5 kg) was collected in Kunming City, Yunnan Province, and was identified by Professor Chun-Quan Xu (Shenyang Pharmaceutical University). A voucher specimen (No. 81) was deposited in the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and Isolation

Dried whole plant of *C. arachnoidea* (2.5 kg) was extracted with 70% EtOH. The EtOH extract was then concentrated, and extracted successively with light petroleum, EtOAc and n-BuOH. The EtOAc extract was subjected to CC on Al_2O_3 and eluted with $CHCl_3$–CH_3OH (100:15 → 100:25) to provide fractions 1, 2. Fraction 1 was followed by silica-gel column chromatography with $CHCl_3$–CH_3OH (100:3) and ODS open column with CH_3OH–H_2O (1:9) to yield compounds **4** (4 mg) and **3** (30 mg); Fraction 2 was subjected to silica-gel column chromatography eluted with $CHCl_3$–CH_3OH (100:5), followed by preparative HPLC [CH_3CN:H_2O (20:80)] to give compounds **1** (3 mg) and **2** (2.8 mg).

11α-Hydroxyrubrosterone (**1**): white needles (MeOH), mp 251–253°C, $[\alpha]_D = +1.07$ (MeOH). HREI-MS *m/z* 350.1718 $[M]^+$ (calcd. 350.1729), EI-MS *m/z* 350 $[M]^+$ (18), 332 $[M - H_2O]^+$ (77), 314 $[M - 2H_2O]^+$ (25), 304 $[M - H_2O - CO]^+$ (23), 286 $[M - 2H_2O - CO]^+$ (21), 271 $[M - 2H_2O - CO - CH_3]^+$ (22), 249 (35), 227 (64), 185

TABLE I ^{13}C NMR data of **1**–**4**

Carbon no.	**1**	**2**	**3**	**4**
1	39.7	38.0	37.8	38.0
2	68.1	68.1	68.0	68.1
3	68.0	68.1	68.0	68.0
4	32.9	32.5	32.5	32.5
5	52.7	51.5	51.6	51.4
6	203.7	203.6	203.3	203.3
7	122.6	121.2	122.0	122.1
8	161.3	165.3	163.0	164.4
9	43.5	34.9	35.1	34.4
10	39.8	38.8	38.8	38.7
11	68.3	21.0	20.1	21.1
12	36.2	31.6	29.0	31.9
13	53.3	48.0	53.3	48.1
14	79.5	82.4	79.5	83.9
15	33.7	30.4	33.6	31.3
16	29.1	29.0	24.5	21.9
17	216.5	78.3	217.2	59.5
18	17.9	16.0	17.2	17.1
19	24.9	24.5	24.7	24.4
20				209.2
21				30.5

(93), 161 (48), 91(97), 77 (63), 69 (41), 55 (100), 43 (90). ^{1}H NMR δ (ppm): 3.41 (1H, dd, $J = 4$, 12.6 Hz, Hα-1), 2.02 (1H, m, He-1), 4.54 (2H, m, H-2, 3), 3.08 (1H, m, H-5), 6.32 (1H, d, $J = 2.2$ Hz, H-7), 3.82 (1H, dd, $J = 2.2$, 8.6 Hz, H-9), 4.21 (1H, m, H-11), 3.05, 2.46 (each 1H, m, H-12), 2.62 (2H, m, H-15), 2.24, 2.31 (2H, m, H-16), 0.90 (3H, s, H-18), 1.29 (3H, s, H-19); ^{13}C NMR see Table I.

Dihydrorubrosterone (**2**): white crystals (MeOH), mp > 300°C, ^{1}H NMR δ (ppm): 6.23 (1H, br. s, H-7), 5.01 (1H, m, H-17), 1.01 (3H, s, H-18), 1.08 (3H, s, H-19); ^{13}C NMR see Table I.

Rubrosterone (**3**): white crystals (MeOH–H_2O), mp 245–247°C, ^{1}H NMR δ (ppm): 6.24 (1H, br. s, H-7), 0.81 (3H, s, H-18), 1.00 (3H, s, H-19); ^{13}C NMR see Table I.

Poststerone (**4**): white needles (MeOH), mp 172–173°C, ^{1}H NMR δ (ppm): 4.14 (1H, m, H-2), 4.24 (1H, br. s, H-3), 6.18 (1H, d, $J = 2.2$ Hz, H-7), 0.70 (3H, s, H-18), 1.02 (3H, s, H-19), 2.14 (3H, s, H-21); ^{13}C NMR see Table I.

Acknowledgements

Special thanks are due to the Analytical Detection Center, Shenyang Pharmaceutical University, for recording the optical rotation, EI-MS and NMR spectra. We also thank Professor Chun-quan Xu for plant identification.

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