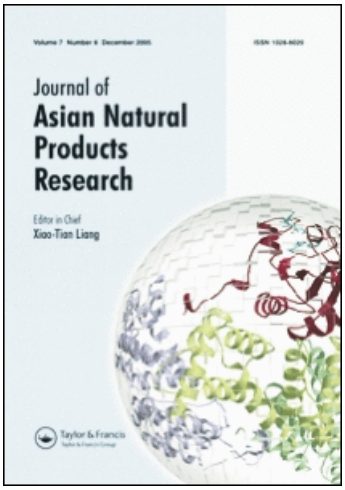

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A NEW PHYTOSTERONE FROM *CYANOTIS ARACHNOIDEA*

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From *Cyanotis arachnoidea* C.B. Clarke, a new phytosterone named cyanosterone A (**1**), along with three known compounds—β-ecdysone (**2**), ajugasterone C (**3**) and β-sitosterol (**4**) were isolated. The structure of the new compound was determined as 3β,14α,20*R*,22*R*-tetrahydroxy-5α-cholest-7-en-6-one on the basis of physico-chemical properties and spectral analysis.

Keywords: *Cyanotis arachnoidea*; Cyanosterone A; HMBC spectrum; NOE enhancement

INTRODUCTION

Cyanotis arachnoidea C.B. Clarke is a herbal medicine which has many effects such as "recovering weakness, getting rid of humidity, stimulating blood circulation and relaxing the muscles and joints" [1]. In this paper, we wish to report the isolation and structural determination of the new phytosterone, cyanosterone A (**1**).

RESULTS AND DISCUSSION

Cyanosterone A (**1**) was isolated as white needles. Its melting point is 272–274°C. **1** gave a positive response to Liebermann–Burchard reaction. The peak at *m*/*z* 449 $[M+H]^+$ in the ESI-MS spectrum, along with ^1H and ^{13}C-NMR data suggested a molecular formula $C_{27}H_{44}O_5$ for **1**. The UV spectrum showed a typical maximum absorption of α, β-unsaturated carbonyl moiety at 243 nm. The olefinic proton signal at δ 6.20 (1H, brs) in the ^1H-NMR spectrum together with the carbon signals at δ 200.3, 165.1 and 122.9 in the ^{13}C-NMR spectrum indicated the presence of an ecdysteroid-type skeleton.

The ^1H-NMR spectrum gave five methyl signals at δ 1.59, 1.23, 0.92, 0.82 and 0.82. The ^{13}C-NMR spectrum gave four signals at δ 83.9, 76.8, 76.8 70.1, which indicated the presence of four oxygenated carbons. In the $^1H-^1H$-COSY spectrum, the signal of H-7 at δ

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6.20 showed a long-range correlation with the signal at δ 3.03 (1H, m), thus δ 3.03 was the signal of H-9. In the HMQC spectrum, the signal of H-9 was correlated with the carbon signal at δ 46.6 (C-9). In the HMBC spectrum (see Fig. 1), the proton signal of H-7 at δ 6.20 showed the long-range correlations with the carbon signals at δ 46.6 (C-9), 53.9 (C-5) and 83.9 (C-14). In the HMQC spectrum, the signal of C-5 showed the correlation with the signal of H-5 at δ 2.21 (1H, m). In the ^{1}H–^{1}H-COSY spectrum, the signal of H-5 was correlated with H-4 at δ 2.63 (IH, m) and δ 1.94 (1H, m) which in turn showed the correlation with the signal of H-3 at δ 3.80. In the HMQC spectrum, the signal of H-3 correlated with the signal of C-3 at δ 70.1. In the NOESY spectrum, the signal of H-5 at δ 2.21 had a strong NOE enhancement with the signal of H-9 at δ 3.03, at the same time, the correlations between H-3 at δ 3.80 and H-5 at δ 2.21 were observed. According to the models (see Fig. 2), only model A is probable, which suggested that H-5 and H-3 were α-oriented.

In the HMBC spectrum, the signal of H-18 at δ 1.23 showed long-range correlations with four carbon signals at δ 83.9 (C-14), 47.9 (C-13), 50.1 (C-17) and 31.9 (C-12). The methyl signal of H-19 showed long-range correlations with the signals at δ 53.9 (C-5), δ 46.6 (C-9), δ 38.7 (C-10) and δ 37.2 (C-1). The methyl signal of H-21 showed long-range correlations with the signals at δ 50.1 (C-17) and 76.8 (C-20, C-22).

Compared with the ^{13}C-NMR data of β-ecdysone [2] (see Table I), the chemical shifts of C_{11}–C_{18} were in accordance with the corresponding data of β-ecdysone, which meant that C-and D-rings were similar and compared with ajugasterone C [3,4], the chemical shifts of C_{20}–C_{27} were in accord with ajugasterone C. Therefore, compound **1** had the same side-chain as that of ajugasterone C.

In addition, compared with the ^{13}C-NMR data of β-ecdysone, the chemical shifts of carbon signals at ring A and ring B of compound **1** were quite different. The signals of C-4, C-6 and C-19 were upfield shifted, respectively (− 1.0, − 3.5, − 11.5), while the signals of C-3, C-5, C-9 were downfield shifted (+1.9, +2.4, +10.0). These data along with the observations in the NOESY spectrum supported the junctions between A- and B-rings of these two compounds were different, thus in **1**, the A/B ring fusion was "*trans*-" (5α-H) (see Fig. 2). Therefore, the structure of compound **1** was 3β,14α,20*R*,22*R*-tetrahydroxy-5α-cholest-7-en-6-one, the carbon and proton signals were summarized (see Table I).

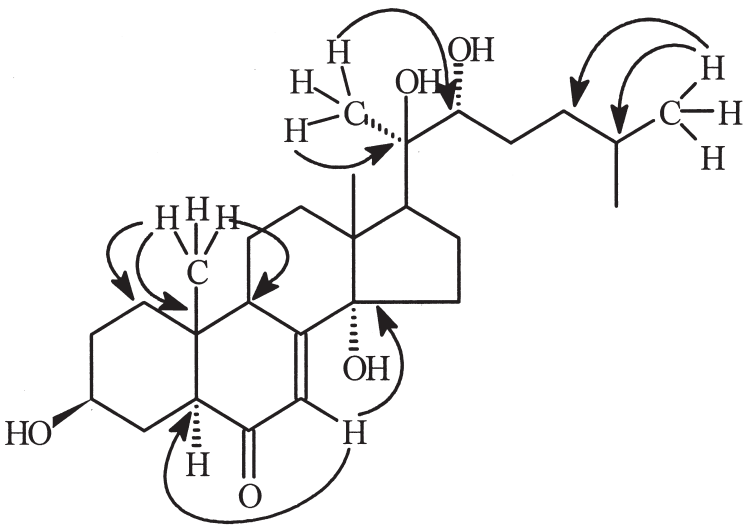

FIGURE 1 HMBC correlations of compound **1**.

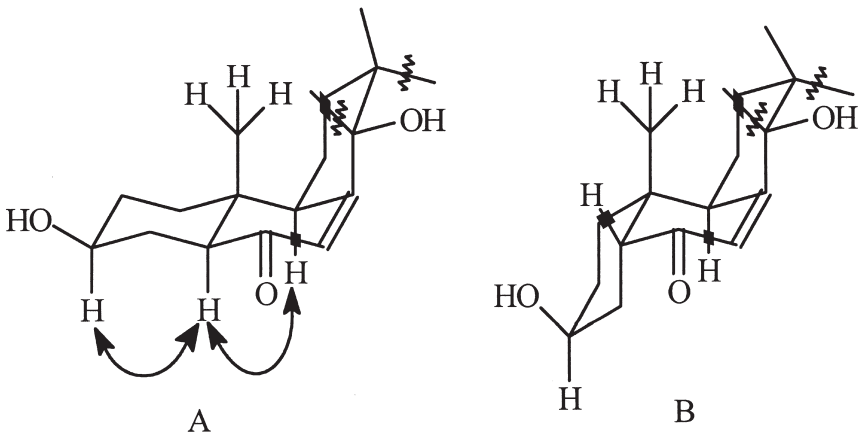

FIGURE 2 NOE enhancements of compound **1**.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were measured on a Yamaco micro-hot-stage and are uncorrected. All NMR spectra were recorded on Bruker-ARX-300 spectrometer, using TMS as internal standard. UV spectrum was recorded on Shimadzu UV-260 UV–Vis spectrometer. ESI-MS was performed on VG-70SE mass spectrometer. The optical rotation was measured on

TABLE I The ^{13}C-NMR spectral data for compounds **1**–**3** (in C_5D_5N)

No.	**1**	**2**	**3**
1	37.3	38.0	39.6
2	30.3	68.2	68.4
3	70.1	68.2	68.2
4	31.5	32.5	32.9
5	53.9	51.5	52.5
6	200.2	203.7	203.9
7	122.9	121.8	122.3
8	165.1	166.3	164.2
9	46.6	34.6	42.8
10	38.7	38.8	39.9
11	21.1	21.3	68.9
12	31.9	31.9	44.2
13	47.9	48.2	48.2
14	83.9	84.4	84.3
15	31.7	32.1	31.9
16	21.4	21.6	21.5
17	50.1	50.2	50.0
18	17.9	18.0	18.9
19	13.1	24.6	24.9
20	76.8	77.0	76.8
21	21.6	21.8	21.5
22	76.8	77.0	76.8
23	30.0	27.6	30.3
24	37.2	42.7	37.2
25	28.2	69.8	28.2
26	23.3	30.1	23.3
27	22.4	30.1	22.4

Perkin–Elmer 241 polarimeter. Silica gel and Al_2O_3 chromatography were produced by Qingdao Ocean Chemical Group Co. of China and Shanghai Xincheng fine chemical Co. Ltd, respectively.

Plant Material

The plant material was collected in Kunming city, Yunnan Province, and was identified by Prof. Xu Chunquan (Shenyang Pharmaceutical University). A voucher specimen (No.81) is deposited in the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and Isolation

Dried whole grass *C. arachnoidea* (2.5 kg) was extracted with hot 70% ethanol. The extract was concentrated, then was extracted successively with petroleum ether, EtOAc and *n*-BuOH. The part of the EtoAc extract was subjected to column chromatography on Al_2O_3 and eluted with $CHCl_3$ to yield compound **4**; eluted with $CHCl_3$–CH_3OH (20:1) to provide Fraction 1; eluted with $CHCl_3$–CH_3OH (4:1) to afford compound **2** (100 mg) and **3** (15 mg). Fraction 1 was followed by column chromatography on silica gel to yield compound **1** (5 mg) using petroleum ether–EtOAc (10:3) as eluent.

Cyanosterone A (**1**): white needles (EtOAc–MeOH), mp 272–274°C. UV λ_{max} (MeOH): 242.8 nm, ESI-MS: 449 $[M+H]^+$, $[\alpha]_D = +12.7$ (MeOH). ^{1}H-NMR (300 MHz, in C_5D_5N): δ 3.80 (1H, m, H-3), δ2.21 (1H, m, H-5), δ 6.20 (1H, brs, H-7), δ 3.03 (1H, ddd, H-9), δ 2.94 (1H, m, H-17),δ 1.23 (3H, s, H-18), δ 0.92 (3H, s, H-19), δ 1.59 (3H, s, H-21), δ3.83 (1H, m, H-22), δ 1.53 (1H, m, H-25), δ 0.82 (6H, d, $J = 3.3$ Hz, H-26, 27). ^{13}C-NMR (75 MHz, in C_5D_5N) (see Table I).

β-Ecdysone (**2**): white needles (EtOAc–MeOH), mp 240–242°C. ^{13}C-NMR (75 MHz, in C_5D_5N) (see Table I).

Ajugasterone C (**3**): white crystals. mp 196–198°C. ^{1}H-NMR (300–MHz, in C_5D_5N): δ 6.30 (1H, brs, H-7), δ 4.21 (1H, brs, H-11), δ 3.86 (1H, dd, H-9), δ 3.78 (1H, d, H-22), δ 1.57 (3H, s, H-21), δ 1.32 (3H, s, H-19), δ 1.28 (3H, s, H-18), δ 0.79 (6H, d, $J = 4.5$ Hz, H-26, 27). ^{13}C-NMR (75 MHz, in C_5D_5N) (see Table I).

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