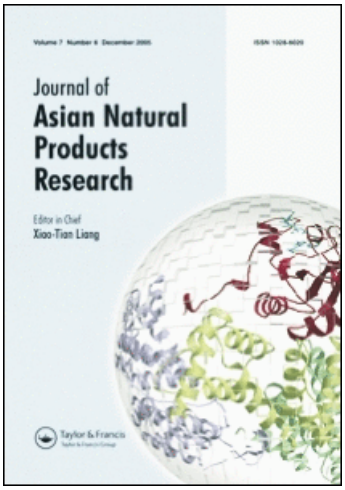

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Two new cycloartane-type triterpenoids 25-hydroxyl-9,19-cycloart-22-ene-3-one (**1**) and (23*Z*)-9,19-cycloart-23-ene-3α,25-diol (**2**) along with 9,19-cycloart-25-ene-3β, 24ξ-diol (**3**) and cycloeucalenol (**4**) have been isolated from the leaves and stems of *Fritillaria hupehensis* Hsiao et K.C. Hsia. Their structures were elucidated on the basis of spectroscopic analysis.

Keywords: *Fritillaria hupehensis*; triterpenoid; 25-hydroxyl-9,19-cycloart-22-ene-3-one; (23*Z*)-9,19-cycloart-23-ene-3α,25-diol

1. Introduction

Fritillaria hupehensis Hsiao et K.C. Hsia is a liliaceous plant growing in the southwest district of Hubei Province, China. Its bulbs have been recorded in Pharmacopoeia of the People's Republic of China as a principal traditional Chinese medicine named 'Hubeibeimu', and *ent*-kaurane diterpenoids and *C-nor-D-homo* steroidal alkaloids were isolated from the bulbs [1]. In our continuing studies on the chemical constituents of the leaves and stems of the plant, four cycloartane-type triterpenoids were isolated. This paper describes the isolation and structural elucidation of two new cycloartane-type triterpenoids 25-hydroxyl-9,19-cycloart-22-ene-3-one (**1**) and (23*Z*)-9,19-cycloart-23-ene-3α,25-diol (**2**) (Figure 1).

2. Results and discussion

Compound **1** was obtained as a white powder (petroleum–EtOAc), mp 161–163°C. The Liebermann–Burchard reaction was positive. Compound **1** showed a molecular ion at *m/z* 440.3638 in its HR-EI-MS, corresponding to the molecular formula $C_{30}H_{48}O_2$. Compound **1** gave strong IR absorption bands at 1708 cm^{-1}, suggesting the presence of ketone group(s). The ^1H NMR spectrum of **1** revealed a pair of doublets at δ 0.51 and 0.78 (d, $J = 4.2$ Hz), respectively, typical of a C-9, C-10 cyclopropyl methylene group of a 3-oxocycloartane [2]. The ^1H NMR spectrum also showed signals due to six tertiary (δ 0.92, 0.97, 1.09, 1.10, 1.29, and 1.30, all singlets) methyl groups and a secondary (δ 0.85, d, $J = 6.4$ Hz) methyl group. The methyl proton signals at δ 1.29

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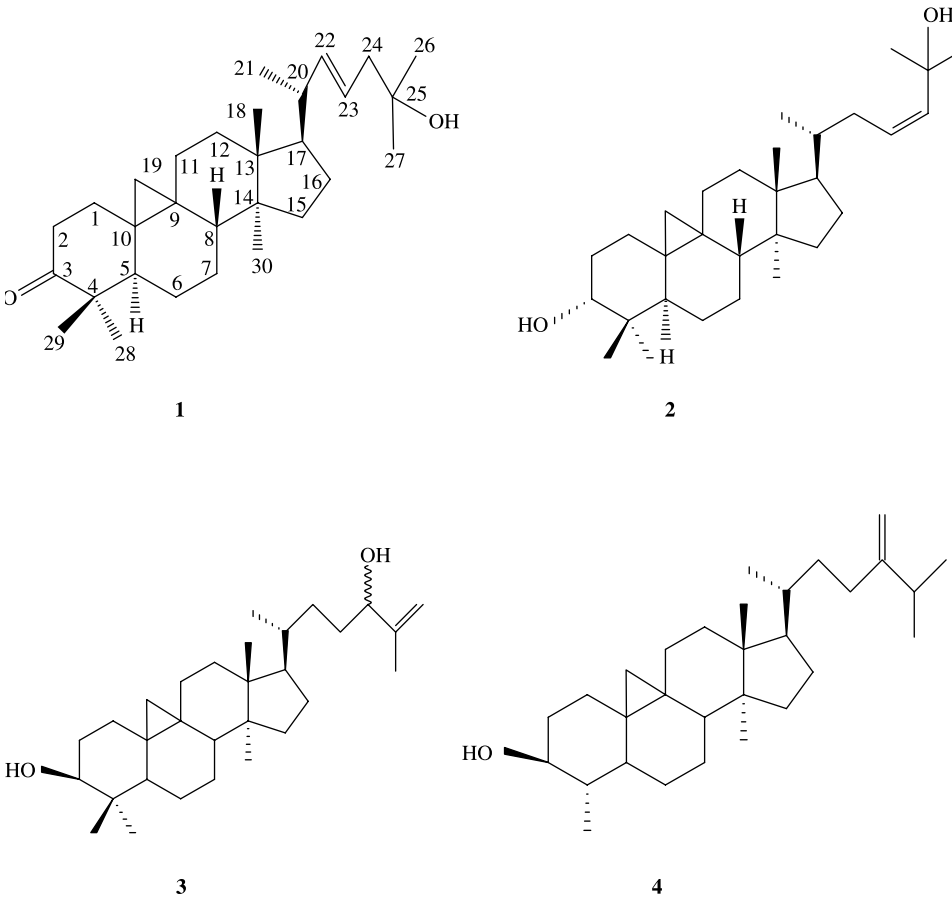

Figure 1. The structures of compounds **1**–**4**.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (100 MHz, $CDCl_3$).

No.	**1**	**2**	**5**	No.	**1**	**2**	**5**
1	33.7	32.4	26.4	16	28.8	26.6	31.9
2	37.1	31.3	28.0	17	52.9	52.2	52.0
3	216.2	77.9	76.8	18	18.4	18.4	18.0
4	49.6	41.1	40.5	19	30.3	29.9	29.8
5	48.3	47.4	47.1	20	37.3	36.8	37.4
6	21.9	21.4	21.1	21	18.7	18.5	18.2
7	26.8	28.3	27.4	22	125.9	39.5	125.6
8	47.4	48.1	47.9	23	140.2	141.6	139.4
9	20.9	19.9	20.0	24	39.8	124.5	39.0
10	27.0	26.3	26.1	25	70.8	69.7	70.7
11	27.3	26.5	26.0	26	29.9	30.8	29.8
12	32.8	35.8	35.6	27	30.0	30.8	29.9
13	46.2	45.5	45.3	28	19.7	19.5	19.3
14	48.8	49.0	48.8	29	14.5	14.8	19.0
15	36.4	33.0	32.8	30	25.9	26.2	25.4

and 1.30 were indicative of the presence of an α-hydroxy isopropyl moiety, so the hydroxyl should be located at C-25 [3,4]. The multiplet at δ 5.82 for two olefinic protons can only be accommodated for a C-22 double bond [3,4]. The ^{13}C NMR spectrum of **1** showed 30 carbon signals (Table 1). The multiplicity assignments were made by 2D NMR and DEPT experiments, which revealed the presence of 7 methyl, 10 methylene, 4 methine, 2 olefinic carbons, and a carbonyl carbon. Analyses of ^{1}H and ^{13}C NMR spectral data of **1** and comparison with those of 9,19-cycloart-22-ene-3α,25-diol (**5**) clearly indicated that the difference from the two compounds should be confined to a functional group at C-3, namely the carbonyl in **1** instead of the hydroxyl group in 9,19-cycloart-22-ene-3α,25-diol [3], which was further confirmed by the HMBC correlations between the carbon signal at δ_C 216.2 and the proton signals at δ_H 1.52 (H-1β), 1.70 (H-5), 0.89 (H-28), and 1.11 (H-29). On the basis of the above evidence, the structure of **1** was concluded to be 25-hydroxyl-9,19-cycloart-22-ene-3-one.

Compound **2** was obtained as colorless needles (petroleum–EtOAc), mp 210–213°C. The Liebermann–Burchard reaction was positive. Compound **2** showed a molecular ion at *m/z* 442.3807 in the HR-EI-MS, indicating a molecular formula of $C_{30}H_{50}O_2$. The IR spectrum showed the presence of the hydroxyl groups (3315 cm^{-1}). The ^{1}H NMR spectrum of **2** displayed two upfield-shifted doublets at δ 0.55 and 0.31 (d, $J = 4.0$ Hz) assignable to a cyclopropyl methylene group (H_2-19), characteristic of cycloartane-type triterpenoids [4]. The ^{1}H NMR spectrum also showed signals due to six methyl signals at δ 0.89 (s, 3H, H-28), 1.00 (s, 3H, H-18), 1.11 (s, 3H, H-29), 1.23 (s, 3H, H-30), and 1.32 (s, 6H, H-26, 27) and one secondary methyl signal at δ 0.96 (d, 3H, $J = 6.3$ Hz, H-21), a signal due to the proton attached to the oxygen-bearing carbon at δ 3.47 (br s), an olefinic signal at δ 5.96 (m, 2H, $W_{1/2} = 8.0$ Hz). The configuration of the double bond was assigned as *Z*, since the olefinic proton signals appeared as narrow multiplets with half bandwidth of 8.0 Hz [5]. The signal at δ 1.32 indicated the presence of an α-hydroxy isopropyl moiety, so the hydroxyl was located at C-25 [6–8]. The broad singlet at δ 3.47 is characteristic of the proton geminal to a 3α-axial hydroxyl in cycloartanes [3]. Analyses of ^{1}H and ^{13}C NMR spectral data of **2** and comparison with those of **5** clearly indicated that the difference from the two compounds should be confined to the location and configuration of the double bond of the side chain. The HMBC spectrum of **2** showed correlations between the proton signals at δ_H 2.29 (H-22) and 1.32 (H-26, 27) with the carbon signals at δ_C 124.5 (C-23) and 141.6 (C-24), which suggested that the double bond located at C-23. This was also confirmed by the ^{1}H–^{1}HCOSY spectrum, which showed the correlations of H-23, H-24 (δ_H 5.96) to H-22 (δ_H 1.89, 2.29). On the basis of the above evidence, the structure of **2** was determined to be (23*Z*)-9,19-cycloart-23-ene-3α,25-diol.

In addition, the known compounds 9,19-cycloart-25-ene-3β,24ξ-diol (**3**) and cycloeucalenol (**4**) were also isolated and identified by the spectral analysis [6–10].

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-4 micromelting point apparatus and are uncorrected. IR spectra were measured on a Shimadzu IR-460 spectrophotometer. Mass spectra were determined on a VG Auto spec-300 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400D NMR spectrometer, with TMS as the internal standard. Silica gel H (160–200 mesh; Qingdao Marine Chemical Group Co., Qingdao, China) was used for column chromatography and SiO_2

GF_{254} (Qingdao Marine Chemical Group Co.) for TLC.

3.2 Plant material

The leaves and stems of *F. hupehensis* were collected from Enshi County, Hubei Province, China, and identified by Prof. Jizhou Wu (Faculty of Pharmaceutical Sciences, Tongji Medical College). Voucher specimens (No. 20050504) have been deposited in the Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.

3.3 Extraction and isolation

The freshly collected plant material (3 kg) was extracted four times with EtOH, and the ethanol extract (224.3 g) was dissolved in 2% HCl. Then, the residue was further subjected to partitioning between H_2O and EtOAc, the EtOAc-soluble fraction was evaporated to dryness *in vacuo* and subjected to column chromatography over silica gel eluted with petroleum ether–EtOAc (10:1–1:10) to obtain 10 fractions. Fraction 8 (2.5 g) was separated by silica gel column eluted with petroleum ether–EtOAc (1:1–1:10). The eluate (0.8 g) obtained in petroleum ether–EtOAc (1:9) yielded compounds **1**–**4**.

*3.3.1 Compound **1***

A white powder, mp 161–163°C. IR (KBr) ν_{max}: 1708 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.51 (1H, d, $J = 4.2$ Hz, 19-H), 0.78 (1H, d, $J = 4.2$ Hz, 19-H), 0.85 (3H, d, $J = 6.4$ Hz, 21-CH_3), 0.92 (3H, s, 18-CH_3), 0.97 (3H, s, 30-CH_3), 1.09 (3H, s, 28-CH_3), 1.10 (3H, s, 29-CH_3), 1.29 (3H, s, 26-CH_3), 1.30 (3H, s, 27-CH_3), 5.82 (2H, m, 22, 23-H). ^{13}C NMR (100 MHz, $CDCl_3$) spectral data: see Table 1. HR-EI-MS *m/z*: 440.3638 $[M]^+$ (calcd for $C_{30}H_{48}O_2$, 440.3654).

*3.3.2 Compound **2***

Colorless needles, mp 210–213°C. IR (KBr) ν_{max}: 3315 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.31 (1H, d, $J = 4.2$ Hz, 19-H), 0.55 (1H, d, $J = 4.2$ Hz, 19-H), 0.89 (3H, s, 28-CH_3), 1.00 (3H, s, 18-CH_3), 1.11 (3H, s, 29-CH_3), 1.23 (3H, s, 30-CH_3), 1.32 (6H, s, 26, 27-CH_3), 0.96 (3H, d, $J = 6.3$ Hz, 21-CH_3), 5.96 (2H, $W_{1/2} = 8.0$ Hz, 23, 24-H), 3.47 (1H, br s, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$) spectral data: see Table 1. HR-EI-MS *m/z*: 442.3807 $[M]^+$ (calcd for $C_{30}H_{50}O_2$, 440.3811).

References

[1] The State Pharmacopoeia commission of the P.R. China, *Chinese Pharmacopeia [S]* (Chemical Industry Press, Beijing, 2005), Vol. 1, p. 286.
[2] A. Inada, H. Murayta, Y. Inatomi, and T. Nakaniski, *J. Nat. Prod.* **58**, 1143 (1995).
[3] N. Rasool, A. Qasim Khan, V. Uddin Ahmad, and A. Malik, *J. Nat. Prod.* **54**, 889 (1991).
[4] J.H. Ju, D. Liu, and J.S. Yang, *Chin. J. Magn. Reson.* **18**, 79 (2001).
[5] J. Kitajima, K. Kimizuka, and Y. Tanaka, *Chem. Pharm. Bull.* **46**, 1408 (1998).
[6] H.M. Hua, B.L. Hou, W. Li, X. Li, and Y. Zhang, *Chin. Tradit. Herb. Drugs* **31**, 409 (2000).
[7] G.Y. Li, J.H. Wang, N. Li, and X. Li, *J. Shengyang Pharm. Univ.* **20**, 252 (2003).
[8] Y.L. Zhong, J.Y. Su, and L.M. Zeng, *Chem. J. Chin. Univ.* **14**, 214 (1993).
[9] M. Della, A. Fiorentino, P. Monaco, and L. Previtera, *Phytochemistry* **35**, 1017 (1994).
[10] Y. Yu and J.K. Liu, *Acta Bat. Yunnan*. **21**, 53 (1994).