Available online at www.sciencedirect.com
ScienceDirect
Chinese Chemical Letters 21 (2010) 846–849
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

# Two new 5α-adynerin-type compounds from *Parepigynum funingense*

Yan Hua [a,*], Hai Yang Liu [b], Wei Ni [b], Chang Xiang Chen [b]

[a] *Key Laboratory for Forest Resources Conservation and Use in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming 650224, China*
[b] *State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Academia Sinica, Kunming 650204, China*

Received 1 December 2009

**Abstract**

A new 5α-adynerin-type cardenolide, named funingenoside U (**1**), together with its aglycone, (17R)-3β-hydroxy-4β-acetoxy-8, 14β-epoxy-5α-card-20(22)-enolide, was isolated from the roots of *Parepigynum funingense*. Their structures were established on the basis of spectral (MS, 1D and 2D NMR) measurements and chemical evidences.


*Keywords:* *Parepigynum funingense*; Chemical constituents; 5α-Adynerin-type cardenolide

*Parepigynum funingense* Tsiang et P. T. Li (Apocynaceae), a member of a monotypic genus, is distributed widely in Yunnan Province, People's Republic of China [1]. Due to the absence of any preview chemical studies on this species, we examined an extract of the roots from *P. funingense*. In the previous paper, we reported structural elucidation of sixteen steroidal glycosides [2–4]. Our continuing phytochemical investigation into the constituent of this plant has resulted in the isolation of one new 5α-adynerin-type cardenolide, funingenosides U (**1**) and its aglycone (**2**).

## 1. Experimental

The dried roots (15 kg) of *P. funingense* were extracted with 75% EtOH three times under reflux. After removal of the solvent *in vacuo*, the aqueous solution was passed through a HPD-100 column and the absorbed materials were eluted with 65% aqueous methanol and methanol, successively. The 65% methanol eluate was concentrated *in vacuo* to give a residue (138 g), which was chromatographed on a silica gel (200–300 mesh) column and eluted with gradient mixtures of chloroform/methanol from 9:1 (v/v) to 2:1 (v/v) to afford eight fractions. Fraction 4 was subjected to CC gradient elution with ethyl acetate/methanol/water (8:1:0.1 ∼ 6:1:0.1, v/v/v), passaged over RP-18 eluted with methanol/water (6:4, v/v), and further purified by semi-pre HPLC (eluted by acetonitrile/water,

* Corresponding author.
*E-mail address:* huayan@mail.kib.ac.cn (Y. Hua).

1001-8417/$ – see front matter 
doi:10.1016/j.cclet.2010.03.034

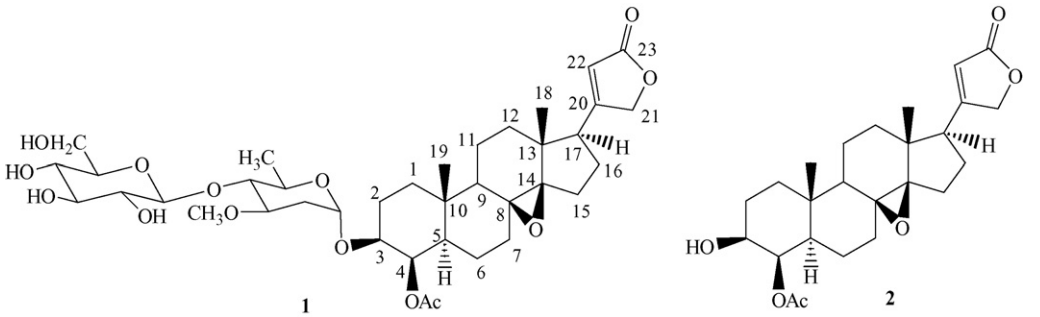

Fig. 1. Structures of compounds **1** and **2**.

33:67, v/v) to afford compound **1** (15 mg). Fraction 1 was further subjected to silica gel (200–300 mesh) column chromatography using mixtures of chloroform/methanol (20:1, v/v) and petroleum ether/acetone (3:1, v/v) as eluents to yield pure **2** (11 mg) (Fig. 1).

## 2. Result and discussion

Compound **1**, $[\alpha]_D^{26}$ − 60.3 (*c* 0.25, MeOH), was obtained as white powder, and analyzed for $C_{38}H_{56}O_{14}$ by negative-ion HRFABMS (*m/z* 735.4263 $[M - H]^-$, calcd. for $C_{38}H_{55}O_{14}$, 735.4251). Its IR spectrum exhibited absorption bands for hydroxyl (3438 $cm^{-1}$), carbonyl (1742 and 1737 $cm^{-1}$), and olefinic groups (1630 $cm^{-1}$). The $^1H$ and $^{13}C$ NMR spectra showed signals due to one acetyl group [$\delta_C$ 21.0 (q), 170.9 (s)], one carbonyl group [$\delta_C$ 171.2 (s)], two olefinic carbons [$\delta_C$ 173.9 (s), 116.9 (d)], two angular methyl groups [$\delta_C$ 16.3 (q), 15.1 (q)], and two anomeric carbons and their corresponding anomeric protons [$\delta_C$ 94.9 (d), 105.2 (d); $\delta_H$ 5.32 (br d, 1H, $J$ = 3 Hz), 5.27 (d, 1H, $J$ = 7.5 Hz)]. In the negative FABMS, significant peaks occurred at *m/z* 573 $[M - H - 162]^-$, 429 $[M - H - 162 - 144]^-$, and indicated the elimination of two hexosyl moieties. Comparison of the $^1H$- and $^{13}C$-NMR data of the aglycone with those of 5α-adynerin, 3β-β-D-diginopyranosyloxy-8,14β-epoxy-5α-card-20 (22)-enolide showed that the structures of the two aglycones were very similar except that **1** had one additional acetoxyl group [5]. The downfield resonance of H − 4 [$\delta_H$ 5.46 (br s, 1H)] and long-range correlations between the deshielded H-4 and the acetyl carbonyl carbon [$\delta_C$ 170.9 (s)], suggested that the acetoxyl group was attached at C-4. 5α-Structure was suggested by Me-19 of **1** which was shifted upfield to $\delta_C$ 15.1 [6]. The stereochemistry of H − 3 was determined to be α-oriented by the ROESY correlation between $H_\alpha$ − 5 and H − 3 which indicated their *cis* relationship. The configuration of AcO − 4 could be determined by the signal of H − 4. For a chair-like conformation of the A-ring, when the substituent at C-4 is β-oriented, $H_\alpha$ − 4 appears a broad singlet. So the signal of H − 4 (br s, 1H) confirmed the β-orientation of AcO − 4. This was further supported by the evidence that there was no correlation between H − 4 and Me-19 in the ROESY spectrum. The stereochemistry of H − C(17) was determined to be α-oriented by the ROESY correlation between $H_\alpha$ − 12 ($\delta_H$ 0.90) and H − C(17) ($\delta_H$ 2.35) which indicated their *cis* relationship (Table 1).

Acid hydrolysis of **1** with 1 mol/L HCl furnished two monosaccharides, which were determined to be D-glucose and D-oleandrose by TLC comparison with authentic samples. The $^{13}C$ NMR spectral data of D-oleandrose were consistent with those reported [4,7]. Sugar proton and carbon signals in the NMR spectra of compound **1** were assigned by $^1H$-$^1H$ COSY, HMQC, and HMQC-TOCSY spectra. In the HMBC spectrum, long-range couplings were observed for H − 1′ of the oleandrosyl unit to C-3 of the aglycone, H − 1″ of the glucosyl unit to C-4′ of the oleandrosyl unit. The anomeric configurations of D-glucosyl and D-oleandrosyl were determined to be β and α, respectively, from the coupling constants of the anomeric proton signals. On the basis of the above evidence, the structure of **1** was elucidated as (17*R*)-4β-acetoxy-3β-[(*O*-β-D-glucopyranosyl-(1 → 4)-α-D-oleandropyranosyl)-oxy]-8, 14β-epoxy-5α-card-20(22)-enolide, and was named funingenoside U.

Compound **2**, $[\alpha]_D^{26}$ − 31.2 (*c* 0.36, MeOH), was obtained as colorless needles (MeOH), mp 220–225 °C, and analyzed for $C_{25}H_{34}O_6$ by negative-ion HRFABMS (*m/z* 429.3155 $[M - H]^-$, calcd. for $C_{25}H_{33}O_6$, 429.3139). Comparison of the $^1H$ and $^{13}C$ NMR spectral data of **2** with those of **1** showed that the two structures were very similar except that there were no sugar moieties in compound **2**. The stereochemistry at the chiral centers in **2** were identical to

Table 1
$^{1}H$ and $^{13}C$ NMR spectral data of **1** and **2** (400 MHz, $C_5D_5N$, $\delta$).

| Position | **1** $\delta_C$ | **1** $\delta_H$ | **2** $\delta_C$ | **2** $\delta_H$ |
|---|---|---|---|---|
| 1 | 36.9 t | | 37.2 t | |
| 2 | 24.8 t | | 27.0 t | |
| 3 | 73.8 d | 3.79 (m, 1H) | 70.7 d | 3.88 (m, 1H) |
| 4 | 72.0 d | 5.46 ((br s, 1H) | 76.3 d | 5.61 ((br s, 1H) |
| 5 | 46.9 d | 1.28* | 47.8 d | 1.46* |
| 6 | 23.9 t | | 24.0 t | |
| 7 | 32.2 t | | 32.2 t | |
| 8 | 64.4 s | | 64.4 s | |
| 9 | 50.9 d | | 51.0 d | |
| 10 | 37.5 s | | 37.5 s | |
| 11 | 16.2 t | | 16.2 t | |
| 12 | 36.6 t | 0.9*, 1.35* | 36.7 t | 0.92*, 1.41* |
| 13 | 41.8 s | | 41.7 s | |
| 14 | 70.7 s | | 70.6 s | |
| 15 | 27.4 t | | 27.3 t | |
| 16 | 25.8 t | | 25.8 t | |
| 17 | 51.3 d | 2.35 (m, 1H) | 51.4 d | 2.36 (m, 1H) |
| 18 | 16.3 q | 0.84 (s, 3H) | 16.3 q | 0.80 (s, 3H) |
| 19 | 15.1 q | 1.24 (s, 1H) | 15.2 q | 1.24 (s, 1H) |
| 20 | 173.9 s | | 173.8 s | |
| 21 | 73.7 t | 3.94 (d, 1H, $J$ = 11.5 Hz) | 73.6 t | 3.94 (d, 1H, $J$ = 11.2 Hz) |
| | | 4.80 (d, 1H, $J$ = 11.5 Hz) | | 4.81 (d, 1H, $J$ = 11.2 Hz) |
| 22 | 116.9 d | 6.08 (s, 1H) | 116.9 d | 6.06 (s, 1H) |
| 23 | 171.2 s | | 171.0 s | |
| Ac | 170.9 s | | 170.6 s | |
| Ac | 21.0 q | 2.05 (s, 3H) | 21.2 q | 2.03 (s, 3H) |
| Ole-1′ | 94.9 d | 5.32 (br d, 1H, $J$ = 3 Hz) | | |
| 2′ | 35.2 t | | | |
| 3′ | 79.1 d | | | |
| 4′ | 82.3 d | | | |
| 5′ | 68.0 d | | | |
| 6′ | 18.9 q | 1.70 (d, 3H, $J$ = 6.5 Hz) | | |
| OMe-3′ | 56.9 q | 3.4 ((s 3H) | | |
| Glc-1″ | 105.2 d | 5.27 (d, 1H, $J$ = 7.5 Hz) | | |
| 2″ | 76.0 d | | | |
| 3″ | 78.5 d | | | |
| 4″ | 71.7 d | | | |
| 5″ | 78.5 d | | | |
| 6″ | 63.1 t | 4.32 (dd, 1H, $J$ = 11.8, 5.0 Hz) | | |
| | | 4.50 (dd, 1H, $J$ = 11.8, 2.0 Hz) | | |

* Overlapping with other signals.

that of **1**. Thus, compound **2** was concluded to be the aglycone of **1**, and its structure was determined to be (17*R*)-3β-hydroxy-4β-acetoxy-8, 14β-epoxy-5α-card-20(22)-enolide.

## Acknowledgment

The authors are grateful to the Analytical Group of the Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, for the spectral measurements.

## References

[1] Yunnan Institute of Botany, Chinese Academy of Sciences, Flora Yunnanica, Science Press, Beijing, 1983, p. 562.
[2] Y. Hua, H.Y. Liu, W. Ni, J. Nat. Prod. 66 (2003) 898.

[3] Y. Hua, H.M. Zhong, C.X. Chen, Heterocycles 60 (2003) 2133.
[4] Y. Hua, L.D. Han, C.X. Chen, Helv. Chim. Acta 87 (2004) 516.
[5] F. Abe, T. Yamauchi, Chem. Pharm. Bull. 26 (1978) 3023.
[6] T. Yamauchi, F. Abe, M. Nishi, Chem. Pharm. Bull. 26 (1978) 2894.
[7] Z.X. Zhang, J. Zhou, K. Hayashi, Chem. Pharm. Bull. 33 (1985) 1507.