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C_{21} steroidal glycosides with cytotoxic activity from *Cynanchum taihangense*

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ABSTRACT

Four new C_{21} steroidal glycosides, cynataihosides A (**1**), B (**2**), C (**5**) and D (**6**), together with two known compounds, cynanoside J (**3**) and glaucoside H (**4**), were isolated from the 95% ethanol extract of *Cynanchum taihangense*. The aglycone of cynataihoside A (**1**) was also a new compound, and that of cynataihoside B (**2**) was unusual. Their structures were elucidated on the basis of 1D- and 2D- NMR spectroscopic data, HR-ESI–MS analysis and qualitative chemical methods. The compounds were subjected to detect the cytotoxicity against three human tumor cell lines (HL-60, THP1 and Caco2). Compound **2** selectively showed potent cytotoxic activity on Caco2 cells with IC_{50} value of 1.23 μM, being more effective than the positive control (5-fluorouracil, IC_{50} = 6.76 μM). Compound **5** was more sensitive to THP1 cell line than HL-60 and Caco2 cell lines with IC_{50} values of 7.85 μM. Moreover, the structure-activity relationship of the C_{21} steroidal glycosides on the cell lines was analyzed.

1. Introduction

C_{21} steroidal glycosides, with the pregnane derivatives as their basic skeletons, have exhibited a broad range of biological activities, including antitumor (Peng et al., 2008), immunosuppressive (Zhang et al., 2015), anti-inflammatory (Yu and Zhao, 2016) and antiviral activities (Yan et al., 2014), etc. Their chemical structures are classified into polyhydroxypregnane-type and seco-pregnae-type glycosides (Lin et al., 1995; Warashina and Noro, 1995). Of the two types, the latter possesses higher oxidation degree and has been found in some species of *Cynanchum* in Asclepiadaceae, which contain the plants with high medicinal value and being used in traditional Chinese medicines for thousands of years, such as *C. stauntonii* ("Bai-Qian" in Chinese) (Yu and Zhao, 2016), *C. atratum* ("Bai-Wei" in Chinese) (Zhang et al., 1985a, 2015), *C. paniculatum* ("Xu-Chang-Qing" in Chinese) (Zhao et al., 2016), *C. amplexicaule* ("He-Zhang-Xiao" in Chinese) (Chen et al., 2008), and so on.

Cynanchum taihangense ("Tai-Hang-Bai-Qian" in Chinese) (Tsiang and Li, 1974), a plant belongs to *Cynanchum* in family Asclepiadaceae, has a close relation with those medical plants (Qiu et al., 1989). It is an herbaceous vine with erect lower part and fractionally twining upper part of stem, and chiefly distributed in Shanxi Province, China. In addition, it is a kind of traditional Chinese medicine, which has the effect of eliminating pathogenic heat from the blood, inducing diuresis for treating stranguria, and boil detoxification treatment (Kong et al., 2015). Up to now, no chemical structure investigation has been reported previously on this species. As our interest in the potential biologically active and structurally unique natural products, four new seco-pregnane glycosides, cynataihoside A (**1**), cynataihoside B (**2**), cynataihoside C (**5**) and cynataihoside D (**6**), together with two known compounds, cynanoside J (**3**) and glaucoside H (**4**) were obtained from the EtOH extract of *C. taihangense*. The aglycone of **1** was also a new compound, and from the recently reports, the aglycone of **2** is not common among those of C_{21} steroidal glycosides. Their structures see Fig. 1. Herein, structural elucidation of the new compounds and cytotoxicity of all compounds for human cancer cell lines (HL-60 or HL-60, THP1 and Caco2) were described.

2. Results and discussion

2.1. Structure elucidation

Cynataihoside A (**1**) was afforded as white amorphous powder, $[\alpha]_D^{20}$ −102.5 (*c* 0.2, MeOH). Its positive HR-ESI–MS showed an ion peak at *m/z* 847.4049 $[M + Na]^+$ (calcd for $C_{42}H_{64}NaO_{16}$, 847.4087), indicating a molecular formula of $C_{42}H_{64}O_{16}$. The ^1H NMR spectrum of **1**

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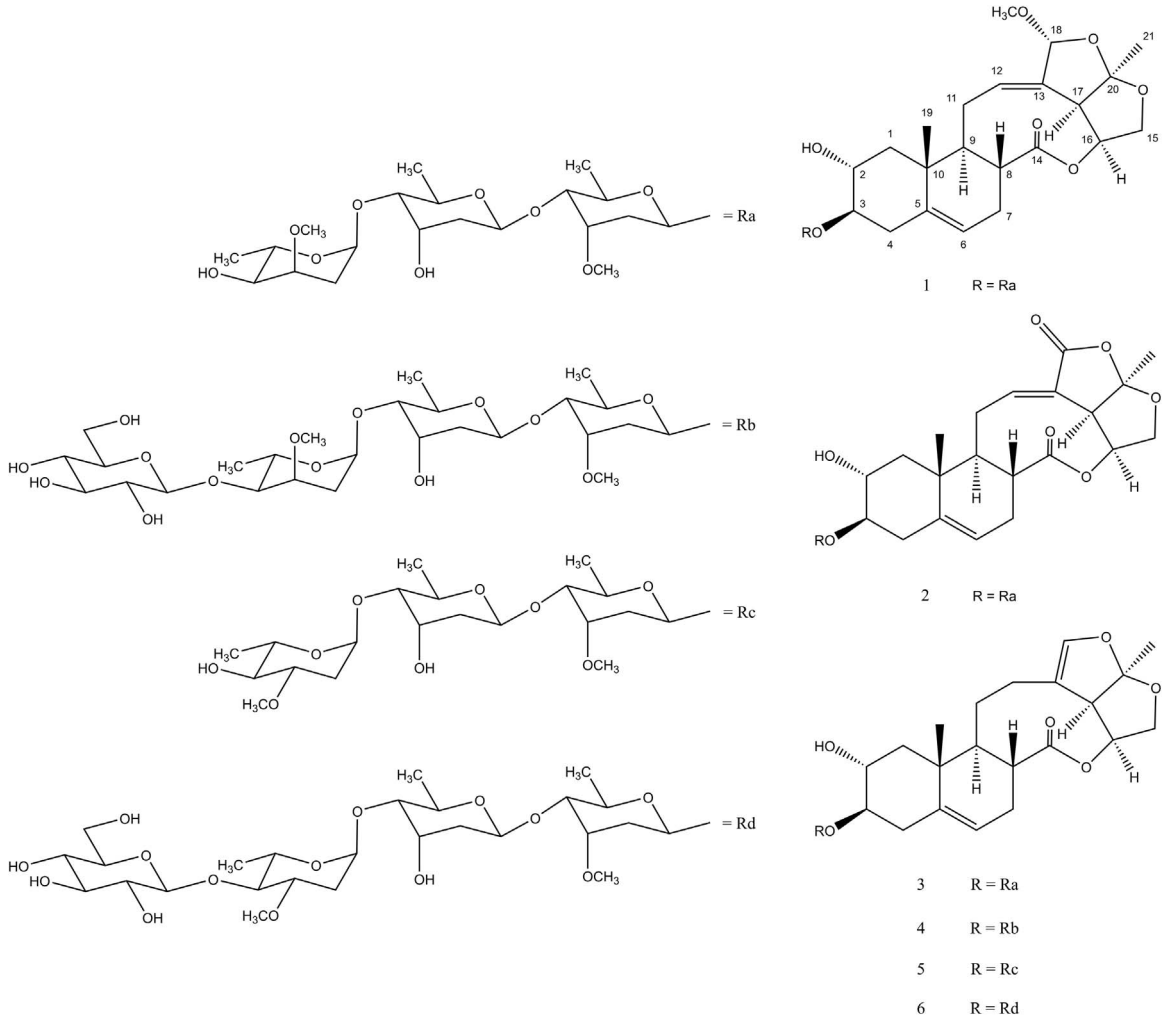

Fig. 1. Structures of compounds **1–6**.

showed the presence of tertiary methyl groups [δ_H 1.02 (3H, s, H-19) and 1.70 (3H, s, H-21)], one methoxy group [δ_H 3.47 (3H, s, 18 $-OCH_3$)], three oxygen-substituted methine protons [δ_H 3.58 (1H, m, H-3), 3.96 (1H, ddd, J = 12.0, 9.0, 4.7 Hz, H-2) and 5.70 (1H, ddd, J = 8.1, 7.7, 5.0 Hz, H-16)], two oxygen-substituted methylene protons [δ_H 4.39 (1H, dd, J = 10.0, 7.7 Hz, H_a-15) and 4.10 (1H, dd, J = 10.0, 5.0 Hz, H_b-15)], two olefinic proton [δ_H 5.36 (1H, m, H-6) and 5.45 (1H, m, H-12)] and one acetal methine proton [δ_H 5.58 (1H, s, H-18)]. The ^{1}H and ^{13}C NMR data (Table 1) of the aglycone part of **1** were similar to those of cynanside A (Kim et al., 2013), except for the replacement of the carbon signals at δ_c 45.1 (C-1), δ_c 69.7 (C-2) and δ_c 84.5 (C-3), with the peaks at δ_c 37.1 (C-1), δ_c 30.1 (C-2) and δ_c 77.0 (C-3). These implied that the carbon C-2 was hydroxylated. This conclusion was further confirmed by detailed 1D NMR and 2D NMR spectral analysis. Thus, the aglycone of **1** was also a new compound, and named as cynataihogenin A. The sugar chain was linked at its C-3 hydroxyl group. The sugar moiety was assumed to be α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-cymaropyranosyl because of the same ^{13}C NMR data as those of cynanoside J (Warashina and Noro, 2006), and was confirmed by the spectroscopic analyses and acid hydrolysis. The proton signals showed three anomeric proton signals at [δ_H 5.17 (1H, dd, J = 9.5, 1.7 Hz, H-1′), 5.22 (1H, dd, J = 9.5, 1.7 Hz, H-1″) and 5.08 (1H, dd, J = 4.0, 2.0 Hz, H-1‴)], indicating the existence of three sugars whose anomeric configurations were deduced by J values as β-, β- and α- form, respectively. The linkage of the three sugars were determined by HMBC correlations from δ_H 5.08 (H-1‴ of α-cymarose) to δ_C 80.6 (C-4″ of β-digitoxopyranose), from δ_H 5.22 (H-1″ of β-digitoxopyranose) to δ_C 82.6 (C-4′ of β-cymarose) and from δ_H 5.17 (H-1′ of β-cymarose) to δ_C 82.6 (C-3) (Fig. 2). Acid hydrolysis of **1** afforded two sugars: cymaroses and digitoxose, which were identified by comparison of their R_f values with those reported in the literatures (Chen etal., 2008). In addition, the relative stereochemistry of **1** was elucidated through the NOESY experiments: H-19/H-2, H-11b, H-18 (β-orientated); H-24/H-18-OMe (α-orientated); H-17/H-24 (α-orientated); H-1′/H-5′, H-3′-OCH_3 (α-orientated); H-1″/H-5″ (α-orientated); H-3‴/H-1‴, H-6‴-Me (α-orientated). Thus, the structure of **1** was established as 2α,3β-hydroxy-18α-methoxy-15,20α:18,20β-diepoxy-13,14:14,15-disecopregna-5,12-dien-14-oic acid 16-oxylactone 3-*O*-α-L-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-cymaropyranoside, and named as cynataihoside A.

Cynataihoside B (**2**) was isolated as white amorphous powder, with a molecular formula of $C_{41}H_{60}O_{16}$ by the $[M + Na]^+$ ion peak at m/z 831.3770 $[M + Na]^+$ (Calcd for $C_{41}H_{60}NaO_{16}$ 831.3774) in HR-ESI–MS. The ^{1}H NMR data showed the diagnostic signals of steroidal glycoside, with a 14,15-secopregnane-type skeleton aglycone typically by two tertiary methylic groups at δ_H 1.15 (3H, s, H-19) and 1.63 (3H, s, H-21), and with three deoxysugars by three anomeric proton signals at [δ_H 5.17 (1H, dd, J = 9.5, 1.7 Hz, H-1′), 5.22 (1H, dd, J = 9.5, 1.7 Hz, H-1″) and 5.08 (1H, br d, 2.4 Hz, H-1‴)]. With the corresponding ^{13}C NMR data, it was apparent that **2** possessed the same sugar structures and sequences as those of **1**. From its ^{13}C NMR, there was a characteristic signal due to carbonyl carbon at δ_c 167.2 (C-18), implied the aglycone of **2** was 2-hydroxystauntogenin (Tai et al., 2006; Zhu et al., 1999), whose corresponding resonances were almost identical,