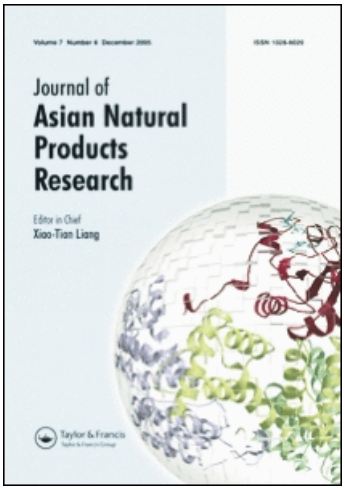

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27-Nor-triterpenoid glycosides from the barks of *Zygophyllum fabago* L.

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From the bark of *Zygophyllum fabago* L., a new 27-nor-triterpenoid glycoside, 3-*O*-β-D-glucopyranosyl-pyrocincholate (**1**), together with five known compounds, 3-*O*-6-deoxy-β-D-glucopyranosyl-pyrocincholate (**2**), quinovic acid (**3**), 3-*O*-6-deoxy-β-D-glucopyranosyl-quinovic acid (**4**), 3-*O*-β-D-glucopyranosyl- quinovic acid (**5**) and 3-*O*-6-deoxy-β-D-glucopyranosyl-cincholic acid (**6**), were isolated and their structures elucidated on the basis of spectroscopic data. Compounds **1**, **2** and **3** showed some anti-tumour activities by MTT assay.

Keywords: *Zygophyllum fabago* L; Zygophyllaceae; 27-Nor-triterpenoid glycoside; 3-*O*-β-D-Glucopyranosyl-pyrocincholate; Anti-tumour activity

1. Introduction

Zygophyllum fabago L. belongs to the Zygophyllaceae family and is mainly distributed in the Gansu province and Xinjiang autonomous region of China. It is used as an antitussive, expectorant, and anti-inflammatory agent and for relief of pain [1]. In the present work, two 27-nor-triterpenoid glycosides, 3-*O*-β-D-glucopyranosyl-pyrocincholate (**1**) and 3-*O*-6-deoxy-β-D-glucopyranosyl-pyrocincholate (**2**), which were shown to possess the uncommon aglycone that has been isolated only from *Adina rubella* [2], *Isertia haenkeana* [3] and *Mitragyna inermis* [4] so far, were isolated from the ethanolic extract of the barks of this plant, and **1** is a new compound. In addition, another four known compounds were isolated. Compounds **1**, **2** and **3** were found to inhibit effectively the proliferation of the human Eca-109 cell line.

2. Results and discussion

Compound **1** was isolated as white powder and gave positive results to Liebermann–Burchard and Molish tests. Its molecular formula was determined as $C_{35}H_{56}O_8$ by HRFAB-MS at *m/z*

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627.3860 $[M + Na]^+$, corresponding to eight degrees of unsaturation. The ^1H NMR spectrum showed six tertiary methyl groups (δ 1.33, 1.00, 0.97, 0.97, 0.94 and 0.93) and no olefinic proton resonances. The ^{13}C NMR spectrum of the aglycone showed 29 carbon signals including two quaternary olefinic carbons (δ 130.82 and 137.00) and one carboxyl carbon (δ 180.38), which showed a nor-triterpenoid skeleton. A comparison between the ^{13}C NMR spectral data of **1** and inermiside II [4] revealed that the carbon signals of the aglycone of the two molecules were almost identical, suggesting the aglycone was pyrocincholic acid. Its ^1H NMR and ^{13}C NMR spectral data (tables 1 and 2) showed that **1** has one sugar moiety. The ^1H NMR spectrum of **1** exhibited an anomeric proton of the sugar at δ 4.95 (1H, d, $J = 8.0$ Hz). The ^{13}C NMR spectrum showed an anomeric carbon at δ 107.12, and other sugar carbons at δ 76.00, 78.51, 72.00, 78.95 and 63.20, which showed that the sugar was a β-glucose [5]. Acid hydrolysis of **1** gave glucose. The HMBC correlation between H-1′ of glucose at δ 4.95 and C-3 of the aglycone at δ 89.25 revealed that glucose was attached at C-3 of the aglycone. The HMBC spectrum (figure 2) showed the correlations for H-26/C-14, H-18/C-13, H-18/C-14 and H-18/C-28. The above structural elucidation of **1** was further supported by its $^1H-^1H$ COSY, HMQC and HMBC data. From these results, the structure of **1** (figure 1) was established as 3-*O*-β-D-glucopyranosyl-pyrocincholate.

Table 1. ^{13}C NMR (100 Hz) spectral data of compound **1** in pyridine-d_5.

Position	δ_C
1	38.51
2	26.87
3	89.25
4	39.74
5	55.81
6	18.87
7	39.74
8	38.09
9	56.52
10	37.29
11	18.22
12	32.32
13	130.82
14	137.00
15	21.32
16	24.32
17	45.38
18	40.01
19	41.85
20	30.96
21	34.74
22	31.90
23	28.33
24	16.87
25	16.75
26	20.92
27	–
28	180.38
29	32.64
30	25.27
1′	107.12
2′	76.00
3′	78.51
4′	72.00
5′	78.95
6′	63.20

Table 2. ^{1}H NMR (400 Hz) spectral data of compounds **1** and **2** in pyridine-d_5.

Position	δ_H *(J in Hz)*	
	1	***2***
1	0.82 (*m*), 1.55 (*t*-like)	0.82 (*m*), 1.54 (*t*-like)
2	1.86 (overlap), 2.28 (overlap)	1.84 (overlap), 2.27 (overlap)
3	3.42 (*dd*, 4.4 Hz, 11.6 Hz)	3.40 (*dd*, 4.4 Hz, 11.6 Hz)
5	0.76 (br *d*, 11.6 Hz)	0.75 (br *d*, 11.6 Hz)
6	1.28 (overlap), 1.52 (overlap)	1.28 (overlap), 1.50 (overlap)
7	1.15 (overlap), 1.80 (overlap)	1.14 (overlap), 1.80 (overlap)
9	1.01 (*t*-like)	1.01 (*t*-like)
11	1.44 (overlap), 1.55 (overlap)	1.43 (overlap), 1.53 (overlap)
12	2.04 (overlap), 2.35 (overlap)	2.04 (overlap), 2.34 (overlap)
15	2.19 (overlap), 2.47 (*m*)	2.19 (overlap), 2.46 (*m*)
16	2.04 (*m*)	2.03 (*m*)
18	2.84 (*dd*, 4.0 Hz, 12.0 Hz)	2.84 (*dd*, 4.0 Hz, 12.0 Hz)
19	1.23 (overlap), 1.68 (*dd*-like)	1.23 (overlap), 1.67 (*dd*-like)
21	1.43 (*m*)	1.41 (*m*)
22	1.77 (*m*), 2.14 (overlap)	1.75 (*m*), 2.11 (overlap)
23	1.33 (*s*)	1.33 (*s*)
24	0.97 (*s*)	0.98 (*s*)
25	0.73 (*s*)	0.77 (*s*)
26	0.97 (*s*)	0.99 (*s*)
29	0.94 (*s*)	0.95 (*s*)
30	1.00 (*s*)	1.01 (*s*)
1′	4.95 (*d*, 8.0 Hz)	4.87 (*d*, 7.6 Hz)
2′	4.04 (*t*, 8.4 Hz)	4.03 (*t*, 8.0 Hz)
3′	3.98 (*m*)	4.14 (*t*, 8.8 Hz)
4′	4.22 (overlap)	3.73 (*t*, 8.8 Hz)
5′	4.23 (overlap)	3.81 (*m*)
6′	4.57 (*dd*, 2.4 Hz, 12 Hz) 4.40 (*dd*, 5.2 Hz,11.6 Hz)	1.66 (*d*, 6.0 Hz)

Compound **2** was isolated as white powder. By comparison with the spectral data of inermiside II [4], the structure of **2** (figure 1) was concluded to be 3-*O*-6-deoxy-β-D-glucopyranosyl-pyrocincholate. This is the first report of 27-nor-triterpenoid glycosides from the Zygophyllaceae family (figures 3 and 4).

In the case of bioactivities of compounds **1**–**6**, anti-tumour activities of them were expressed as inhibition rate. As determined by MTT assay, the inhibition rates of compounds **1**, **2** and **3** in 50 μg/ml for the human ECA-109 oesophageal carcinoma cell were 79.19, 80.27 and 54.10%, respectively. The results suggested that compounds **1**, **2** and **3** can inhibit effectively the proliferation of the human ECA-109 cell.

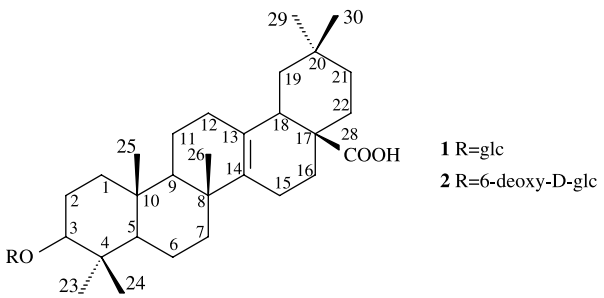


Figure 1. Structures of compounds **1** and **2**.

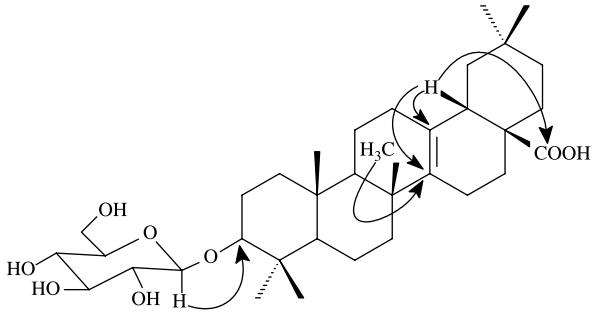


Figure 2. Key HMBC correlations of compound **1**.

3. Experimental

3.1 General experimental procedures

Melting points were determined using a Fisher Johns apparatus and are uncorrected. IR spectra were obtained in KBr disks on a Perkin–Elmer 983G spectrophotometer. NMR spectra were recorded on an INOVA 400 spectrometer. EI-MS, ESI-MS and HRFAB-MS were recorded on a Micromass ZabSpec spectrometer. TLC employed precoated Silica gel plates (5–7 μm, Qingdao Haiyang). For column chromatography, silica gel (H, 200–300 mesh, Qingdao Haiyang) and Sephadex LH-20 (Pharmacia) were used.

3.2 Plant material

The barks of *Zygophyllum fabago* L. were collected from Wulumuqi, Xinjiang Autonomous Region of China in March 2004, and identified by Professor Guo-Qiang Li of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China, where a voucher sample has been deposited.

3.3 Extraction and isolation

The air-dried, powdered barks (14 kg) of the plant material were extracted with 75% EtOH (each 65 L × 3) under reflux. The resultant extract was combined and evaporated under reduced pressure to give concentrated extracts (1200 g). The latter was subsequently suspended in water and partitioned successively with $CHCl_3$, EtOAc and n-butanol. The n-butanol part (200 g) was subjected to column chromatography by a combination of D_{101} macroporous resin, eluted gradiently with H_2O and EtOH. The fraction eluted with 75% EtOH (35 g) was subjected to a silica gel column with a $CHCl_3/CH_3OH$ gradient system

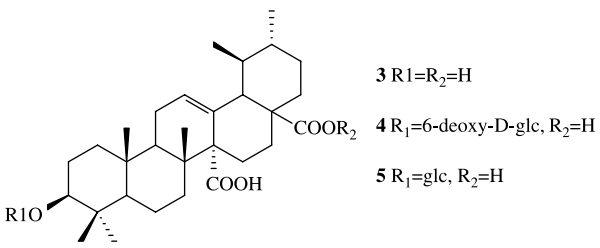
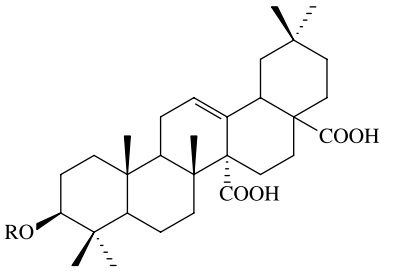


Figure 3. Structures of compounds **3**, **4** and **5**.



6 R=6-deoxy-D-glc

Figure 4. Structure of compound **6**.

(1:0–0:1), affording 20 fractions. Fraction 3 (2 g) was purified by repeated silica gel column chromatography [$CHCl_3/CH_3OH$ (98:2–90:10)] to afford compound **3** (200 mg). Fractions 15–17 were combined (4 g) and separated on Sephadex LH-20 columns with CH_3OH. Subsequently, further purification on an ODS column with CH_3OH/H_2O (5:5–8:2) provided compounds **1** (80 mg), **2** (90 mg), **4** (66 mg) and **6** (115 mg). The fraction eluted with 50% EtOH (65 g) was subjected to a silica gel column with a $CHCl_3/CH_3OH$ gradient system (1:0–0:1), affording 27 fractions. Fractions 19 and 20 were combined (3 g) and separated on Sephadex LH-20 columns with CH_3OH. Subsequently, further purification on an ODS column with CH_3OH/H_2O (4:6–8:2) provided compound **5** (80 mg).

3.3.1 Compound 1 (3-*O*-β-D-glucopyranosyl-pyrocincholate). White powder, mp 306–307°C (CH_3OH), $[\alpha]_D^{25}$ − 5.4 (*c* 0.02, CH_3OH). Give positive results to the Liebermann–Burchard and Molish tests. IR (KBr) cm^{-1}: 3442, 2944, 1701, 1633, 1459, 1385, 1077, 470. ESI-MS: *m/z* 627 $[M + Na]^+$. HRFAB-MS *m/z*: 627.3860 $[M + Na]^+$ (calcd for $C_{35}H_{56}O_8Na$, 627.3873) ^{13}C NMR and ^1H NMR spectral data, see tables 1 and 2.

3.3.2 Compound 2 (3-*O*-6-deoxy-β-D-glucopyranosyl-pyrocincholate). White powder, mp 242–243°C (CH_3OH). Give positive results to the Liebermann–Burchard and Molish tests. ESI-MS: *m/z* 611 $[M + Na]^+$. ^{13}C NMR spectral data are consistent with those in the literature [4].

3.3.3 Compound 3 (quinovic acid). White powder, give positive result to the Liebermann–Burchard test. ^{13}C NMR spectral data are consistent with those in the literature [6].

3.3.4 Compound 4 (3-*O*-6-deoxy-β-D-glucopyranosyl-quinovic acid). White powder, give positive results to the Liebermann–Burchard and Molish tests. ^{13}C NMR and ^1H NMR spectral data are consistent with those in the literature [7].

3.3.5 Compound 5 (3-*O*-β-D-glucopyranosyl-quinovic acid). White powder, give positive results to the Liebermann–Burchard and Molish tests. ^{13}C NMR spectral data are consistent with those in the literature [8].

3.3.6 Compound 6 (3-*O*-6-deoxy-β-D-glucopyranosyl-cincholic acid). White powder, give positive results to the Liebermann–Burchard and Molish tests. ^{13}C NMR spectral data are consistent with those in the literature [9].

3.4 Acid hydrolysis of compound 1

Compound **1** was applied on silica gel G HPTLC plates and left in an HCl atmosphere at 75°C for 5 h. HCl vapour was eliminated under hot ventilation and authentic sugar was then applied to the plates. The chromatoplates were developed using EtOAc/CH_3OH/HOAc/H_2O (12:3:3:2) and $CHCl_3$/CH_3OH/H_2O (7:3:0.4) successively, and spots were detected by spraying with EtOH/conc.H_2SO_4/anisaldehyde (17:2:1) followed by heating. The sugar was identified: D-glucose [10].

3.5 Anti-tumour bioassays

To evaluate the anti-proliferative effect of compounds on the human ECA-109 oesophageal carcinoma cell lines, the MTT colorimetric assay was performed. The amount of formazan was determined by photometer at 570 nm. Cells were plated into 96-well flat-bottomed cultured plates at a concentration 5×10^4 cells per well in complete RPMI 1640 culture medium. Seventy-two hours after plating, the medium containing foetal calf serum was removed and test solutions were given to cells in various final concentrations such as 1 and 50 μg/ml. After incubation with drugs for 24 h, MTT solution was added to the wells and plates were incubated at 37°C for 4 h. Results were expressed as percentage of the absorbance in control cells compared to that in the drug-treated cells.

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