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New pomolic acid triterpene glycosides from *Zygophyllum eichwaldii*

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Received July 5, 2007, accepted August 3, 2007

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Pharmazie 62: 957–959 (2007)
doi: 10.1691/ph.2007.12.7685

Two new bisdesmosidic triterpenoid saponins, 3-*O*-[α-L-2-*O*-sulphonylarabinopyranosyl]-pomolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygoeichwaloside H) and 3-*O*-[β-D-2-*O*-sulphonylglucopyranosyl]-pomolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygoeichwaloside K) were isolated from the roots of *Zygophyllum eichwaldii*. The structures were established primarily on the basis of NMR spectroscopy and chemical transformations.

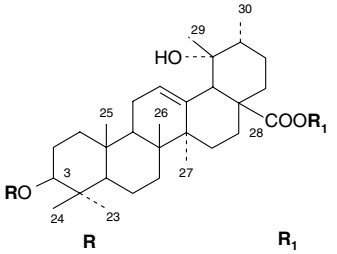
As a part of our continuing phytochemical research on plants of the genus *Zygophyllum* which are used in the traditional medicine of Asian countries (Sasmakov et al. 2001; 2003), this paper deals with the isolation and structural elucidation of two new triterpenoid saponins from *Zygophyllum eichwaldii*.

Separation of triterpene-containing fractions of the methanolic extract of the roots of *Zygophyllum eichwaldii* on a silica gel column (gradient chloroform, methanol and water) led to the isolation of two triterpenes (**1** and **2**). Saponin **1** was obtained as an amorphous white powder. IR absorptions at 3410, 1734 and 1648 cm^{-1} indicated the presence of hydroxyl (OH), ester carbonyl (C=O), and double bond (C=C) functionalities. The olefinic resonances of the aglycone at δ 128.05 and 138.97, corresponding to quaternary and methine carbons suggested the urs-12-ene skeleton with a hydroxyl group at C-19 (Inada et al. 1987; Ouyang et al. 1997). The ^{13}C NMR spectral data of **1** were consistent with pomolic acid as the aglycone (Sasmakov et al. 2001). 41 different signals in the ^{13}C NMR spectrum supported that **1** has a bisdesmosidic structure (Table). This is confirmed by availability of hydrogen anomeric atoms at δ 5.10 and δ 6.32 in the ^{1}H NMR spectra. Acidic hydrolysis of the glycoside yielded pomolic acid as aglycone and arabinopyranose and glucopyranose as sugar parts. The ^{13}C NMR spectrum of **1** contained signals at δ 89.41 attributable to C-3 and showing that the hydroxyl group at this carbon is glycosylated. The signals of C-1′ carbon atom arabinose at δ 103.60 and H-1′ proton at δ 5.10 showed that the arabinose is located at C-3 of the aglycone. The downfield shifts of the H-2′ and C-2′ signals of arabinose compared with those of Ziyu-glycoside I (**3**) (Table) (Sasmakov et al. 2001; Yosioka et al. 1970) indicated that the sulphate group was in position C-2′ of the arabinose. The presence of the $-SO_3H$ group was con-

Table: ^{13}C NMR spectral data for triterpene glycosides 1–4

Atom	1	2	3	4
1	38.60	38.20	38.60	38.20
2	26.28	25.84	26.40	25.83
3	89.41	89.10	88.49	89.06
4	39.47	38.95	39.27	38.95
5	55.82	55.30	55.64	55.26
6	18.40	18.10	18.40	18.00
7	33.21	32.87	33.21	32.87
8	40.27	39.89	40.27	39.89
9	47.46	47.08	47.46	47.02
10	36.72	36.31	36.71	36.31
11	23.76	23.38	23.76	23.38
12	128.05	127.82	128.15	127.80
13	138.97	138.62	138.97	138.61
14	41.83	41.83	41.83	41.83
15	28.95	28.55	28.95	28.54
16	25.83	25.48	25.83	25.48
17	48.35	48.21	48.35	48.20
18	54.14	53.80	54.14	53.79
19	72.40	72.70	72.36	72.70
20	41.80	41.54	41.83	41.45
21	26.40	26.10	26.40	26.03
22	37.45	37.23	37.44	37.03
23	27.90	27.72	27.96	27.71
24	16.60	16.31	16.60	16.31
25	15.30	14.90	15.35	14.90
26	17.10	16.72	17.12	16.72
27	24.30	23.96	24.29	23.92
28	176.60	176.38	176.67	176.33
29	26.76	26.34	26.76	26.38
30	16.39	15.95	16.39	15.99
1′	103.60	104.30	107.28	104.2
2′	77.68	79.56	72.63	78.69
3′	73.09	78.30	74.35	74.57
4′	67.58	70.57	69.25	69.31
5′	68.90	77.81	66.45	75.33
6′		68.20		61.70
1″	95.50	95.20	95.54	95.18
2″	73.69	73.40	73.79	73.39
3″	78.66	78.25	78.66	78.22
4″	70.96	70.75	70.96	70.70
5″	78.96	78.56	78.96	78.46
6″	62.06	61.72	62.06	61.79

firmed by solvolysis. The cross peaks of the ^{3}J long range couplings between H-18/C-28, H-1″ of glucose/C-28 enabled us to determine the resonance of C-28 and the glucosidations in this position. In addition, the shifts observed on the carbons of the β-D-glucose unit, particularly the values at δ 95.50 of the anomeric carbon were in agreement with a site of glucosylation at the 28-carboxyl group. Consequently, the structure of compound **1** was established as 3-*O*-[α-L-2-O-sulphonylarabinopyranosyl]-pomolic acid-28-*O*-[β-D-glucopyranosyl] ester, for which the trivial name zygoeichwaloside H is proposed.



	R	R_1
1	α–L-Arap(2-O-SO_3H)	β-D-Glcp
2	β–D-Glcp(2-O-SO_3H)	β-D-Glcp
3	α–L-Arap	β-D-Glcp
4	β–D-Galp(2-O-SO_3H)	β-D-Glcp

The ^{13}C NMR resonance of the aglycone of compound **2** matched well with the aglycone of **1** indicating the same aglycone for both compounds. The occurrence of two anomeric signals at δ 4.82 and 6.25 in the ^{1}H NMR spectrum of **2** showed the presence of two sugar units. Acidic hydrolysis of the glycoside gave glucopyranose as sugar component. The existence of a glucose ester at position C-28 was established by comparison of ^{1}H and ^{13}C signals of **2** and zygoeichwaloside I (**4**) (Sasmakov et al. 2001) (Table). The ^{13}C NMR spectrum of **2** exhibited significant glycosidation shifts for C-3 (δ 89.10) of the aglycone. The signals at δ 104.30 and δ 5.10 in ^{13}C and ^{1}H NMR spectrum showed that the one of them glucose is located at C-3 of aglycone. The downfield shifts of the H-2′ and C-2′ glucose signals of **2** indicate that the sulphate group is in position C-2′ of the glucose. The presence of the sulphate group was confirmed by solvolysis. Therefore, glycoside **2** was determined to be 3-*O*-[β-D-2-*O*-sulphonyl-glucopyranosyl]-pomolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygoeichwaloside K).

Experimental

1. General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 instrument at working frequencies 500.13 and 125.27 MHz, respectively, in C_5D_5N and CD_3OD at 30 °C with TMS standard. Two-dimensional spectra were measured using standard methods of Bruker. The accuracy of the ^{1}H and ^{13}C chemical shifts were 0.01 ppm; of ^{1}H/^{1}H spin-spin coupling constants, 0.2 Hz. IR spectra were recorded on a Perkin-Elmer System 2000 FT IR Fourier spectrometer in KBr pellets; CC: silica gel (0.063–0.16 mm); TLC: silica gel (0.025 mm) and Merck TLC-plates precoated with $Si_{60}F_{254}$ or $Si_{60}RP18F_{254}$. Sugars were chromatographed on plates impregnated with 0.3 M solution of NaH_2PO_4. Glycosides and sugars were detected by sprinkling the plates with 15% ethanolic solution of wolfram-phosphoric acid and o-toluidine-salicilate accordingly.

2. Isolation

The conditions of extraction and fractionisation have been published (Sasmakov et al. 2001; 2003). The fractions enriched with glycosides **1** and **2** were rechromatographed on a column with silica gel using the mobile phase $CHCl_3$–MeOH–H_2O (70 : 23 : 4 to 65 : 28 : 5) and yielded 21 mg **1** and 24 mg **2**.

3. Acid hydrolysis

The appropriate triterpene glycosides **1–2** (5 mg) were dissolved in 5 ml 5% H_2SO_4 and refluxed for 3.5 h at 100 °C. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The aqueous layer was neutralized with $BaCO_3$ and sugars were identified by TLC (n-BuOH–MeOH–H_2O, 5 : 3 : 1) and spraying with O-toluidine-salicilate.

4. Solvolysis

The solutions of **1** and **2** in a 1 : 1 mixture of dioxane and pyridine were heated in a stoppered reaction vial at 120 °C for 4 h. The mixture was diluted with H_2O and extracted with n-BuOH.

5. Compound 1

IR (KBr, ν, cm^{-1}): 3410, 2939, 1734, 1648, 1460, 1389, 1238, 1227, 1142, 1072, 836, 773, 649. ^{1}H NMR (C_5D_5N): 3.35 (H-3), 5.57 (H-12), 2.96 (H18), 5.16 (HO-19), 1.29, 0.96, 0.90, 1.20, 1.71, 1.41, 1.08 (3 H, s, 23, 24, 25, 26, 27, 29, 30- CH_3). 3-*O*-α-L-Arap (2-SO_3H): 5.10 (H-1), 5.40 (H-2), 4.54 (H-3), 4.36 (H-4), 4.30, 3.80 (H_2-5).
28-*O*-β-D-Glcp: 6.32 (H-1), 4.26 (H-2), 4.38 (H-3), 4.49 (H-4), 4.06 (H-5), 4.50, 4.43 (H_2-6). ^{13}C NMR spectra: see Table.
$C_{41}H_{66}O_{16}S$

6. Compound 2

M.p. 212–214 °C (MeOH). $[\alpha]_D^{22} + 32.3 \pm 2°$ (c 0.4; C_5H_5N).
IR (KBr, ν, cm^{-1}): 3418, 2939, 1732, 1648, 1460, 1389, 1238, 1221, 1142, 1072, 836, 773, 649. ^{1}H NMR (C_5D_5N): 3.32 (H-3), 5.53 (H-12), 2.92 (H-18), 5.04 (HO-19), 1.44, 1.16, 0.86, 1.12, 1.70, 1.41, 1.06 (3 H, s, 23, 24, 25, 26, 27, 29, 30- CH_3). 3-*O*-β-D-Glcp (2-SO_3H): 4.82 (H-1), 5.02 (H-2), 4.38 (H-3), 4.16 (H-4), 3.90 (H-5), 4.28, 4.48 (H_2-6). 28-*O*-β-D-

Glcp: 6.25 (H-1), 4.23 (H-2), 4.27 (H-3), 4.29 (H-4), 4.03 (H-5), 4.47, 4.40 (H_2-6). ^{13}C NMR spectra: see Table.
$C_{42}H_{69}O_{17}S$

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