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TRITERPENE GLYCOSIDES OF *Zygophyllum eichwaldii*. II. STRUCTURE OF ZYGOEICHWALOSIDE I

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Column chromatography of roots of Zygophyllum eichwaldii *C.A.M. (Zygophyllaceae) afforded the new glycoside zygoeichwaloside I. Acid hydrolysis, alkaline saponification, solvolysis, and* ^{1}H *and* ^{13}C *NMR spectroscopies using COSY, TOCSY, ROESY, HSQC, and HMBC methods established its structure as the 28-O-*β*-D-glucopyranosyl ester of pomolic acid 3-O-*β*-D-2-O-sulfonylgalactopyranoside.*

Key words: *Zygophyllum eichwaldii*, triterpene glycoside, zygoeichwaloside I, 28-O-*β*-D-glucopyranosyl ester of pomolic acid 3-O-*β*-D-2-O-sulfonylgalactopyranoside.

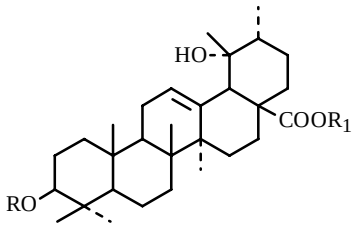
We previously reported the isolation from roots of *Zygophyllum eichwaldii* C.A.M. of glycosides C (**1**), E (**2**), and I (**3**). Glycosides C and E were known compounds, ziyu glycosides II and I, which are pomolic acid 3-O-*α*-L-arabinopyranoside and its 28-O-*β*-D-glucopyranosyl ester [2, 3].

Acid and alkaline hydrolysis of **3** indicate that it contains pomolic acid as the aglycone and glucose and galactose in the carbohydrate part, i.e., it is a bisdesmoside. Furthermore, solvolysis showed that the glycoside contains a sulfo group.

We prove the structure of I (**3**) in the present article.

The PMR spectrum of **3** contains two 1H doublets at 4.93 and 6.26 ppm that belong to anomeric H atoms and indicate that the glycoside contains two sugars (Table 2).

The formation of progenin upon alkaline saponification of **3** indicates that one of the sugar units is bonded to the CO_2H of the aglycone. This is confirmed by an absorption band in the IR spectrum at 1732 cm^{-1}, which is characteristic of an ester bond, and by signals at 6.26 and 176.33 ppm in the ^{1}H and ^{13}C NMR spectra, respectively, of this compound.



R	R_1
1: *α*-L-Ara*p*	H
2: *α*-L-Ara*p*	*β*-D-Glc*p*
3: *β*-D-2-O-SO_3H-Gal*p*	*β*-D-Glc*p*
4: *β*-D-Xyl*p*	CH_3

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TABLE 1. ^{1}H and ^{13}C NMR Spectra of Carbohydrates in **1**-**4** (C_5D_5N, δ, ppm, J, Hz, TMS = 0)

Atom	1		2		3		4*	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
	3-O-α-L-Ara*p*				3-O-β-D-Gal*p*(2-SO_3H)		3-O-β-D-Xyl*p*	
1′	107.23	4.78 ($J_{1,2}$=7.1 Hz)	107.28	4.78 ($J_{1,2}$=7.0 Hz)	104.2	4.93 ($J_{1,2}$=7.6 Hz)	107.72	4.86(d 7.6)
2′	72.63	4.45 ($J_{2,3}$=8.8 Hz)	72.63	4.45 ($J_{2,3}$=8.8 Hz)	78.69	5.41 ($J_{2,3}$=9.3 Hz)	75.57	4.05 (dd 8.5, 7.6)
3′	74.34	4.18 ($J_{3.4}$=3.3 Hz)	74.35	4.18 ($J_{3.4}$=3.3 Hz)	74.57	4.32 ($J_{3.4}$=3.2 Hz)	78.64	4.20 (t 8.5)
4′	69.23	4.34 ($J_{4.5}$<1 Hz, $J_{4.5}'$=2.2 Hz)	69.25	4.34 ($J_{4.5}$<1 Hz, $J_{4.5}'$=2.3 Hz)	69.31	4.49 ($J_{4.5}$=2 Hz)	71.27	4.26 (ddd 10.4, 8.5, 4.9)
5′	66.43	4.34, 3.84	66.45	4.38, 3.85	75.33	4.01 ($W_{1/2}$=5.8 Hz)	67.14	3.81 (dd 11.0, 10.4), 4.41(dd 11.0, 4.9
6′					61.70	4.37, 4.37		
				28-O-β-D-Glc*p*				
1′′			95.54	6.33 ($J_{1,2}$=8.3 Hz)	95.18	6.26 ($J_{1,2}$=8.2 Hz)		
2′′			73.79	4.26 ($J_{2,3}$=9.5 Hz)	73.39	4.22 ($J_{2,3}$=9.4 Hz)		
3′′			78.66	4.39 ($J_{3.4}$=9.5 Hz)	78.22	4.27 ($J_{3.4}$=9.4 Hz)		
4′′			70.96	4.49 ($J_{4.5}$=9.6 Hz)	70.70	4.30 ($J_{4.5}$=9.5 Hz)		
5′′			78.96	4.07 ($J_{5,6}$=2.8 Hz, $J_{5,6}'$=6.7 Hz)	78.46	4.04 ($J_{5,6}$=2.7 Hz, $J_{5,6}'$=6.7 Hz)		
6′′			62.06	4.50, 4.43	61.79	4.47, 4.40		

*Literature data [7].

Spectral data (^{1}H and ^{13}C NMR) of **2** and **3** (Tables 1 and 2) show that the chemical shifts and spin—spin coupling constants (SSCCs) belonging to the glucose are identical. The glucose is bonded to the CO_2H of pomolic acid in **2**. Therefore, the glucose in **3** is also localized on a carboxyl. The correlation between glucose H-1 and aglycone C-28 in the HMBC spectrum and the signal at δ 95.18 ppm (glucose C-1) in the ^{13}C NMR spectrum provide further evidence of this. The SSCC (J = 8.2 Hz) is consistent with the β-configuration of the glycoside bond.

The signals of the anomeric galactose proton (δ 4.93 ppm) and the corresponding C-1 atom (δ 104.2 ppm) in the ^{1}H and ^{13}C NMR spectra indicate that this sugar is bonded to a hydroxyl.

The ^{13}C NMR spectrum of **3** shows that only the signal for C-3 of the genin alcohols (OH-3 and OH-19) undergoes a shift to weak field (89.00 ppm). Therefore, the galactose is located on the C-3 OH of pomolic acid and has the β-configuration (J = 7.6 Hz). This is confirmed by the ROESY and HMBC spectra.

The chemical shifts in the PMR and ^{13}C NMR spectra suggest that an electronegative substituent is located on the galactose C-2. The signals for H-2 (5.41 ppm) and C-2 (78.69 ppm) occur at weak field compared with spectra of the unsubstituted galactopyranoside [4, 5]. These data indicate that the sulfo group that was observed by solvolysis replaces the hydroxyl on the galactose C-2.

Signals of the genin part were assigned based on data from two-dimensional COSY, TOCSY, ROESY, HSQC, and HMBC spectroscopies using a complex analysis of these spectra, which agree with those previously published (Table 1) [6, 7].

Thus, **3**, which we call zygoeichwaloside I, is a new compound, the structure of which can be described as the 28-O-β-D-glucopyranosyl ester of pomolic acid 3-O-β-D-2-O-sulfonylgalactopyranoside.

TABLE 2. ^{1}H and ^{13}C NMR of Aglycones in **1**-**4** (C_5D_5N, δ, ppm, TMS = 0)

Atom	1		2		3		4*	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	38.52		38.60		38.20		38.83	
2	26.38		26.40		25.83		26.72	
3	88.48	3.35	88.49	3.35	89.06	3.32	88.75	3.37 (dd 11.3,4.3)
4	39.26		39.27		38.95		39.61	
5	55.64		55.64		55.26		55.93	
6	18.34		18.40		18.00		18.64	
7	33.23		33.21		32.87		33.38	
8	40.07		40.27		39.89		40.27	
9	47.42		47.46		47.02		47.62	
10	36.72		36.71		36.31		37.02	
11	23.71		23.76		23.38		23.96	
12	127.73	5.61	128.15	5.57	127.80	5.53	128.31	5.5 (m)
13	139.65		138.97		138.61		139.45	
14	41.81		41.83		41.83		41.93	
15	29.02		28.95		28.54		29.04	
16	26.10		25.83		25.48		26.04	
17	48.00		48.35		48.20		48.61	
18	54.31	3.07	54.14	2.96	53.79	2.92	54.44	2.86 (s)
19	72.41	5.10 (OH)	72.36	5.17 (OH)	72.70	5.02 (OH)	72.59 (s)	5.25 (s)
20	42.08		41.83		41.45		42.21 (s)	
21	26.65		26.40		26.03		26.79	
22	38.22		37.44		37.03		38.15	
23	27.95	1.29	27.96	1.28	27.71	1.46	28.24	1.34
24	16.60	0.97	16.60	0.98	16.31	1.16	16.97[d]	0.93
25	15.23	0.89	15.35	0.92	14.90	0.86	15.56	0.88
26	16.89	1.10	17.12	1.21	16.72	1.15	17.03[d]	1.03
27	24.40	1.76	24.29	1.71	23.92	1.68	24.67	1.71
28	180.36		176.67		176.33		178.47	
29	26.86	1.46	26.76	1.41	26.38	1.41	26.99	1.40
30	16.48	1.14	16.39	1.08	15.99	1.07	16.68[d]	1.10
$COOCH_3$							51.54	3.74 (s)

*Literature data [7].

EXPERIMENTAL

TLC of glycosides used KSK silica-gel plates (0.005-0.043 mm) containing 10% gypsum and Silufol UV-254 (Czech Rep.) plates. Column chromatography used KSK silica gel (0.1-0.16 mm).

Sugar was chromatographed on plates impregnated with NaH_2PO_4 solution (0.3 M).

The following solvent systems were used: $CHCl_3$—CH_3OH—H_2O (40:7.5:1, 1a; 70:23:4, 1b; 65:35:8, 1c), 1-butanol—CH_3OH—H_2O (5:3:1, 2). Glycosides were detected by spraying plates with alcoholic phosphotungstic acid (15%); sugar, with *o*-toluidine salicylate with subsequent heating at 120°C for 5-10 min.

IR spectra were recorded on a Perkin—Elmer model 2000 Fourier spectrometer in KBr pellets.

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-500 instrument at working frequencies 500.13 and 125.27 MHz, respectively, in Py-d_5 at 30°C with TMS standard. Two-dimensional spectra were recorded using standard Bruker methods. Solutions were spun for 0.2 sec during recording of TOCSY and ROESY spectra. The accuracy of the ^{1}H and ^{13}C chemical shifts is 0.01 ppm; of ^{1}H/^{1}H SSCCs, 0.2 Hz.

Isolation of Glycosides. Air-dried ground roots (2.5 kg) were collected in June 1998 at Ust′yurt of Karakalpakstan Republic and exhaustively extracted with CH_3OH at 70°C with decantation after 2 h. The extraction was monitored by TLC in systems 1b and 1c. The extract was condensed in a rotary evaporator. The residue was diluted with water. The insolubles were filtered off. The filtrate was treated successively with $CHCl_3$ and 1-butanol. The solid obtained after evaporation of 1-butanol (103 g) was repeatedly chromatographed on columns using systems 1a, 1b, and 1c. *β*-Sitosterol 3-O-*β*-D-glucopyranoside and triterpene glycosides C, E, and I were isolated.

Glycoside C (1). $C_{35}H_{56}O_8$, mp 245°C (dec.), lit. mp 243-245°C, $[\alpha]_D$ +25° (*c* 0.2, DMSO) [2].
IR spectrum (KBr, ν, cm^{-1}): 3569, 2921, 1687 (CO_2H), 1461, 1388, 1219, 1237, 1139, 1072, 992, 771, 652.
^{1}H and ^{13}C NMR spectra appear in Tables 1 and 2.

Glycoside E (2). $C_{41}H_{66}O_{13}$, mp 257°C (dec.), lit. mp 256-260°C, $[\alpha]_D$ +18° (*c* 2.5, Py) [2].
IR spectrum (KBr, ν, cm^{-1}): 3407, 2938, 1734 (ester), 1648, 1457, 1389, 1227, 1138, 1073, 781, 650.
^{1}H and ^{13}C NMR spectra appear in Tables 1 and 2.

Compound I (3). $C_{42}H_{69}O_{17}S$, mp 217°C (dec.), $[\alpha]_D^{20}$ +31.9±2° (*c* 0.9, Py).
IR spectrum (KBr, ν, cm^{-1}): 3422, 2974, 1734, 1648, 1458, 1390, 1264, 1231, 1170, 1074, 835, 774, 752, 619.
^{1}H and ^{13}C NMR spectra appear in Tables 1 and 2.

Acid Hydrolysis of Glycosides 1, 2, and 3. Glycosides 1, 2, and 3 (10 mg each) were hydrolyzed by 5% aqueous methanolic (1:1) H_2SO_4 at 90°C for 6 h. The genin from the hydrolysates was extracted by $CHCl_3$. Neutralization and evaporation of the hydrolysates followed by TLC in system 2 identified by comparision with authentic samples: in C, arabinose; in E, arabinose and glucose; in I, glucose and galactose.

Saponification of 3. Compound 3 (5 mg) was dissolved in NaOH (10%, 5 mL) and heated to 95°C for 3 h. The solution was neutralized with H_2SO_4, acidified with CH_3CO_2H, and extracted with butanol. TLC in system 1b of the water-washed butanol extract detected progenin.

Solvolysis of 3. Glycoside (10 mg) was dissolved in dioxane—pyridine (1:1) and heated at 95-100°C for 5 h. The reaction mixture was diluted with water. The precipitate was separated. Sulfate was detected by $Ba(OH)_2$ in the filtrate (after concentration).

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