dissolution results for all the capsules complied with the BP specification requiring 70% of the drug to be dissolved within 45 min. Using the test described by Moore and Flanner [10] there was also no similarity found between the mean dissolution profiles of the capsules measured with the USP and BP methods after 3 months' accelerated storage. For the USP test, the mean dissolution profile after 3 months' accelerated storage of only capsule B was comparable to that at time 0. However, for the BP test the mean dissolutions profiles for all the products after 3 months' accelerated storage were not significantly different from that at time 0.

These results show that accelerated stability testing leads to physicochemical changes in oxytetracycline capsules and that the BP dissolution method for oxytetracycline hydrochloride capsules was not able to measure the effect of these changes on the dissolution properties of the capsules. Based on previous reports [7, 8] describing bioavailability problems with oxytetracycline capsules, relying on the BP dissolution test might lead to the acceptance of clinically unacceptable and bio-inequivalent products.

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A new triterpene glycoside from *Zygophyllum eichwaldii*

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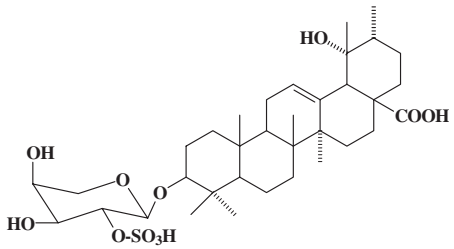
In our continuing studies on the triterpene glycosides from *Zygophyllum eichwaldii* C.A.M. (Zygophyllaceae) [1, 2] we have isolated the new glycoside zygoeichwaloside G (**1**) from the methanol extract of the roots of the plant. Here we report about isolation, structure elucidation and biological tests of the extract and of isolated compound.

Chromatography of the methanol extract on silicagel column afforded the glycoside **1** as an amorphous substance. Acidic hydrolysis of the glycoside yielded pomolic acid as aglycone and arabinopyranose as sugar component. The ^{13}C NMR spectrum (Table) contained 35 different signals suggesting that the glycoside **1** is a monoside. This is confirmed by availability of hydrogen anomeric atom at δ 5.16 and carbon atom at δ 103.59 in the ^{1}H and ^{13}C NMR spectra. The signal of carbon atom of the (COOH) group at δ 180.68 showed that it is free, and that arabinose is linked to one of the aglycone hydroxyls. The ^{13}C NMR spectrum of zygoeichwaloside G (**1**) contained signals at δ 89.41 attributable to C-3 and showing that the hydroxyl group at this carbon is glycosylated. Consequently, arabinose is located at C-3 of pomolic acid. The conclusion was confirmed by HMBC and ROESY spectra, in which the correlation was observed among anomeric protone (H-1) of arabinose and C-3 and H-3 of aglycone. The value of CSSI (5 Hz) corresponds to α-configuration of glycosidic bond.



1

The NMR data of zygoeichwaloside G are very similar to those of zygoeichwaloside C (**2**) isolated earlier [1]. Comparison of the ^{1}H and ^{13}C NMR spectra of the compounds **1** and **2** showed that the aglycone parts of these compounds concided. But there is a considerable difference between the chemical shifts of protons and carbon atom signals which belong to arabinose (Table). By COSY, TOCSY and HSQC experiments it was established that arabinose at C-2 is substituted and that the substituent does not contain carbon atoms. The substituent was electronegative enough to cause characteristic shifts

of C-1–C-3 atoms signals of the sugar moiety. By comparison of shift values with a nonsubstituted moiety it was concluded that the substitute is a SO_3H-group [2]. To the same conclusion led electrospray ionization mass spectrometry (ESIMS). The molecular ion peak $[M-H]^-$ at m/z 683.6 corrresponds to $C_{35}H_{56}O_{11}S$.

Thus the structure of zygoeichwaloside G (**1**) was shown to be the 3-*O*-α-L-(2-*O*-sulphonyl)-arabinopyranoside of 19-α-hydroxyursolic acid.

The methanol extract from the roots of *Z. eichwaldii* as well as zygoeichwaloside G were examined for antimicrobial and cytotoxic activities. Whereas the extract (0.2 mg/disc) shows remarkable activity against the gram positive bacteria *Staphylococcus aureus* (inhibition zone 11 mm) and *Bacillus subtilis* (inhibition zone 10 mm) the isolated compound is not active. Gram-negative bacteria and fungi are not influenced both by extract and glycoside. Only the highest concentration of the extract (1 mg/ml) displays cytotoxic activity against FL cells. The cell viability is reduced by 90%. The same concentration of zygoeichwaloside G and lower concentrations of both materials are not cytotoxic. The results lead to the conclusion that other compounds than zygoeichwaloside G in the extract are responsible for the observed activity or that a combination of several compounds is responsible for the biological action.

Experimental

1. General procedures

TLC was performed on Silufol UV-254 (Czech) and Merck TLC-plates precoated with $Si_{60}F_{254}$ or $Si_{60}RP18F_{254}$. Silica gel (0.1–0.16mm) was used for column chromatography. Sugars were chromatographed on plates impregnated with 0.3 M solution of NaH_2PO_4. Following mobile phases were used: 1. $CHCl_3-MeOH-H_2O$: a) 40 : 7.5 : 1 b) 70 : 23 : 4 c) 65 : 35 : 8; 2. *n*-BuOH–MeOH–H_2O 5 : 3 : 1. Glycosides and sugars were detected by sprinkling the plates with 15% ethanolic solution of wolfram-phosphoric acid and *o*-toluidine-salicilate accordingly, and heating at 120 °C during 5–10 min. ^1H and ^{13}C NMR spectra were measured in pyridine-d_5 at 30 °C on a Bruker DRX-500 spectrometer at 500.13 MHz and 125.27 MHz accordingly. 2D spectra were measured using standard methods of Bruker. Mixing at measuring TOCSY and ROESY spectra was continued for 0.2 s. The exactness of measuring the chemical shifts of ^1H and ^{13}C formed δ 0.01, CSSI $^1H/^1H$ – 0.2 Hz. Massspectra were measured on a Finnigan LCQ spectrometer.

2. Isolation of glycosides

The air-dried, powdered roots (2.5 kg) of *Z. eichwaldii* collected from Ustjurt, Republic of Karakalpakstan, were extracted with MeOH at 70 °C. The solvent was removed by rotary evaporation. The obtained sum of extractive substances was diluted with H_2O and the undissolved part was filtered. The filtrate was treated with $CHCl_3$ and *n*-BuOH. The obtained dried remainder (103 g) after removing *n*-BuOH was many times chromatographed on a column in mobile phases 1 a, b and c. 3-*O*-β-D-Glucopyranoside of β-sitosterine and triterpene glycosides C, E, I [2] and G were isolated. The fractions enriched with glycoside **1** were rechromatographed on a column with Silica gel using the mobile phase $CHCl_3-MeOH-H_2O$ 65 : 28 : 5 and yielded 20 mg amorphous substance. ^1H and ^{13}C NMR spectra are given in the Table. IR (KBr): $\nu_{max} = 3418, 2939, 1690, 1460, 1389, 1238, 1221, 1142, 1072, 836, 773, 649\ cm^{-1}$; ESIMS m/z 683.6 $[M-H]^-$, 639.5, 603.5, 471.4, 433.3, 341.6, 211.1.

3. Acidic hydrolysis

Compound **1** (5 mg) was hydrolysed in 5% H_2SO_4 at 95 °C for 6 h. After cooling the hydrolysate a genin identified as pomolic acid (19-α-hydroxyursolic acid) on TLC (mobile phase $CHCl_3-MeOH$ 25 : 1) was isolated. The sugar part was identified as arabinose by PC (*n*-BuOH–MeOH–H_2O 5 : 3 : 1).

4. Biological tests

The antimicrobial activity was determined by the disc diffusion method [3] against the following test strains: *Staphylococcus aureus* (ATTC 6538), *Bacillus subtilis* (ATTC 6051), *Pseudomonas aeruginosa* (ATTC 7853), *Escherichia coli* (ATTC 11229) and *Candida maltosa* (SBUG 700). Ampicillin and nystatin were used as positive and MeOH as negative control. An inhibition zone of 8 mm or greater was used as the criterion for designating significant antimicrobial activity.

The cytotoxicity was measured by the neutral red uptake assay [4] using FL cells, a human amniotic epithel cell line [5].

Table: ^1H and ^{13}C NMR Data of zygoeichwalosides G (1) and C (2)

Atom	**1**		**2**	
	^{13}C	^1H	^{13}C	^1H
1	38.72	1.48; 0.84	38.52	1.53; 0.96
2	26.28	2.05; 1.78	26.38	2.19; 1.92
3	89.41	3.23	88.48	3.35
4	39.47	–	39.26	–
5	55.82	0.77	55.64	0.86
6	18.65	1.47; 1.27	18.34	1.53; 1.32
7	33.54	1.55; 1.32	33.23	1.76; 1.37
8	40.37	–	40.07	–
9	47.67	1.76	47.42	1.85
10	37.00	–	36.72	–
11	24.03	2.04; 1.96	23.71	2.09; 2.09
12	128.05	5.58	127.73	5.61
13	139.99	–	139.65	–
14	42.133	–	41.81	–
15	29.31	2.32; 1.28	29.02	2.34; 1.33
16	26.44	3.14; 2.06	26.10	3.17; 2.1
17	48.33	–	48.00	–
18	54.65	3.05	54.31	3.07
19	72.75	4.96(OH)	72.41	5.10(OH)
20	42.40	1.50	42.08	1.53
21	26.97	2.10; 1.35	26.65	2.10; 1.33
22	38.52	2.16; 2.05	38.22	2.19; 2.13
23	27.19	1.30	27.95	1.29
24	16.79	1.03	16.60	0.97
25	15.46	0.82	15.23	0.89
26	17.19	1.06	16.89	1.10
27	24.71	1.73	24.40	1.76
28	180.68	–	180.36	–
29	28.35	1.44	26.86	1.46
30	16.90	1.13	16.48	1.14
α-L-Arap				
1′	103.59	5.16	107.23	4.78
2′	77.68	5.40	72.63	4.45
3′	73.08	4.55	74.34	4.18
4′	67.58	4.37	69.23	4.34
5′	68.91	4.29; 3.79	66.43	4.34; 3.84

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