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TRITERPENOID SAPONIN FROM *ZYGOPHYLLUM DUMOSUM*

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تم فصل مركب صابونيني جديد من نبات زيجوفيلم دوموزيم من مجزئ الكحول البيوتيلي باستخدام عمود السليكا جيل ثم عمود من السيفادكس LH-20 ثم استخدام كروماتوجرافيا الضغط العالى. وتم التعرف علي التركيب الكيميائي باستخدام الرنين النووي المغناطيسي للهيدروجين والكربون ١٣ ذات البعد والبعدين مع طيف الكتلة عند ١٠٢٩ الذي دل على وجود: تراكساست ٢٠ (٢١ إن ٢٨ حمض أويك) كشق أجليكوني وتم تحديد النبضات الرأسية والأفقية للهيدروجين. ومن وجود أربعة من البروتونات الأنومورية دل على وجود أربعة وحدات سكرية ، ثلاثة منهم مرتبطين برابطة جليكوزيدية وواحدة برابطة جليكوإستيرية. وباستخدام التقنية الحديثة للرنين المغناطيسى وتحديد الإنحراف الضوئى لكل من الزيلوز والجلوكوز والذى يحرف الضوء المستقطب ناحية اليمين ، والرامنوز والأرابينوز والذى يحرف الضوء المستقطب ناحية اليسار. كما تم تحديد موقع الاتصال بين الوحدات السكرية والشق الأجليكونى بواسطة HMBC. كما تم دراسة الرنين النووى المغناطيسى للمركبات ودلالات ذلك على التركيب وقد دل على أن تركيب الصابونين هو:
3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-taraxast-20(21)-en-28-oic acid-28-O-[2-*O*-R-β-D-glucopyranosyl].

The new triterpenoid saponin, 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-taraxast-20(21)-en-28-oic acid-28-O-[2-O-R-β-D-glucopyranosyl] ester has been isolated from the roots of Zygophyllum dumosum. The structure was established primarily by NMR spectroscopy. All NMR signals were assigned by ^{1}H-^{1}H COSY, HMQC, HMBC, ROESY and TOCSY experiments.

INTRODUCTION

Leaves, stems and fruits of *Zygophyllum coccineum* are used in the Egyptian folk medicine as part of a drug against rheumatism, gout, asthma and hypertension. This was the motivation to investigate the natural products of different *Zygophyllum* species, grown in Egypt. Previous investigations on *Z. coccineum*, *Z. album*, *Z. dumosum* and *Z. decumbens* showed the occurrence of saponins with quinovic acid,[1] arjunolic acid, 30-norarjunolic acid and 29-hydroxyoleanolic acid[2] as aglycones. In this report the isolation and structure elucidation of the new saponin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-taraxast-20(21)-en-28-oic acid-28-*O*-[2-*O*-R-β-D-glucopyranosyl] ester (**1**) from the roots of *Zygophyllum dumosum* Boiss. is described.

EXPERIMENTAL

General

Mp: uncorr.; negative ion MS: MAT 8500 (Finnigan), matrix glycerol. NMR: 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C), reverse probehead, δ in ppm, solvent CD_3OD, CD_3OD signals were used as int. standard (^{1}H: 3.30, ^{13}C: 49.0), temp. 290 K, TOCSY: phase-sensitive using TPPI, mixing time 134.3 msec (80 MLEV-17 cycles plus 2 trim pulses of 2.5 msec each), HMQC: phase-sensitive using TPPI, BIRD sequence, GARP decoupled, HMBC: using TPPI, delay to achieve long range couplings: 71 msec ($J_{C,H}$= 14 Hz).

CC: silica gel (0.063-0.2 mm); TLC: silica gel (0.25 and 1 mm precoated plates 60 F_{254}, Merck, 0.25 mm precoated plastic sheets SIL G/UV_{254} Macherey-Nagel), the spots were sprayed with 10% H_2SO_4 in MeOH, 'triterpene reagent' (1% soln. of vanillin in 50% H_3PO_4), 'sugar reagent' (4% ethanolic aniline-4%

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ethanolic diphenylamine-H_3PO_4, 5:5:1 v/v) and phosphomolybdic acid reagent (Aldrich). For the prep. HPLC a Knauer HPLC system equipped with a variable wavelength monitor together with Spherisorb ODS II (250 x 8 mm, 5 μm, Bischoff) prepacked column was used. GLC (He at 50 kPa; 3 min 80°, 80-120° with 3° min^{-1}, 120-170° with 0.5° min^{-1} 170-280° with 5° min^{-1}) was carried out on a Fisons GC 8000 instrument using a fused silica capillary column coated with DB 1 phase (30 m x 0.32 mm, J&W).

Isolation

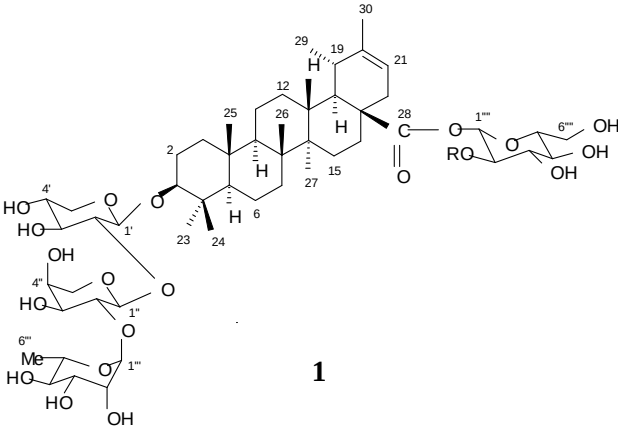
Z. dumosum was collected in 1991 in the North of Sinai and identified by Dr. M. El-Gebaly from the National Research Centre (NRC) Cairo. A voucher specimen of the plant is deposited at the Herbarium of the NRC, Department of Chemotaxonomy. Dried powder of the roots of *Zygophyllum dumosum* (4.0 kg) was exhaustively extracted with 80% MeOH. After removal of the solvent by evaporation, the residue was successively partitioned between H_2O-petrol, H_2O-EtOAc and H_2O-n-BuOH. The butanolic fr. was evaporated under red. pres. at 50° to obtain a crude saponin mixture (25 g). CC (10 g) on silica gel eluting with $CHCl_3$-MeOH-H_2O with increasing amounts of MeOH and H_2O. yielded fr. I (15 mg) which was further purified on Sephadex LH-20 eluting with MeOH-H_2O 85:15 v/v (8 mg). Prep. HPLC (isocratic, 32% acetonitrile in H_2O, 205 nm, 3.0 ml/min) gave pure saponin **1** (4.0 mg).

(R)-2-Butylglycosides

A sample (ca. 250 μg) of zygophyloside L (**1**) was hydrolysed with 0.5 ml 5% HCl for at least 3 h at 80°. After evaporation of the acid under red. pres., 0.5 ml (*R*)-2-BuOH was added, dried HCl gas was bubbled through the solution for 30 s and the reaction mixture was heated for 3 h at 80° under N_2 in a sealed vessel. Trimethylsilylation was performed with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. (*R*)-2-butyl-L-Ara: R_t 39.41, (*R*)-2-butyl-D-Ara: R_t 38.43, (*R*)-2-butyl-L-Xyl: R_t 39.23, (*R*)-2-butyl-D-Xyl: R_t 38.11, (*R*)-2-butyl-L-Glc: R_t 81.85, (*R*)-2-butyl-D-Glc: R_t 82.23, (*R*)-2-butyl-L-Rhamn: R_t 52.34. Identification of the sugars were done by comparison of the R_t values and co-injection with the appropriate standard. Consequently it was shown for zygophyloside L (**1**) that arabinose and rhamnose belong to the L-series, glucose and xylose to the D-series.

Spectroscopic data

3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-taraxast-20(21)-en-28-oic acid-28-*O*-[2-O-R-β-D-glucopyranosyl] ester (**1**): amorphous powder, m.p. 260-264°. LSI-MS negative ion mode m/z (rel. int.): 1029 (10). ^{1}H-NMR and ^{13}C-NMR: Table I.



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RESULTS AND DISCUSSION

The n-BuOH extract of the roots of *Z. dumosum* was obtained as desribed in the experimental section and was subjected to column chromatography on silica gel using $CHCl_3$, $CHCl_3$-MeOH and $CHCl_3$-MeOH-H_2O with increasing amounts of MeOH and H_2O. Further purification was achieved by column chromatography on Sephadex LH-20 followed by preparative HPLC on Spherisorb ODS II.

The ^{1}H- and ^{13}C NMR spectra of **1** showed the presence of taraxast-20(21)-en-28-oic acid as aglycone. 23-Hydroxytaraxast-20(21)-en-28-oic acid is the aglycone of two saponins from *Fagonia indica*.[3] Total assignment of ^{1}H and ^{13}C NMR spectra of pseudotaraxasterol was described before by Reynolds.[4] In the case of taraxast-20(21)-en-28-oic acid the 28-methyl group of pseudotaraxasterol is oxidized to a carboxyl group. The signals of the axial and equatorial oriented protons of the aglycone of **1** were assigned by ROESY experiments. Four anomeric ^{1}H/^{13}C signals at δ 4.42 (*d*, $^3J_{1',2'}$ = 7.4 Hz)/ 105.8, 4.97 (*d*, $^3J_{1'',2''}$ = 4.2 Hz)/ 101.5,

Table I: ^{1}H and ^{13}C NMR spectral data for saponin **1** in CD_3OD.

C	^{1}H ax/eq	^{13}C		^{1}H	^{13}C
1	0.97/1.69	40.3	Xyl		
2	1.69/1.99	27.2	1‘	4.42 *d* 7.4 Hz	105.8
3	3.16	91.0	2‘	3.38	79.7
4		40.4	3‘	3.56	78.0
5	0.72 *d* 11.1 Hz	57.3	4‘	3.46	73.7
6	1.36/1.51	19.3	5‘	3.39 ax, 3.75 eq	63.4
7	1.32/1.50	35.2	Ara		
8		42.2	1‘‘	4.97 *d* 4.2 Hz	101.5
9	1.36	52.2	2‘‘	3.87 *dd* 4.2 6.6 Hz	75.9
10		38.0	3‘‘	3.78	72.6
11	1.32/1.56	22.8	4‘‘	3.78	67.7
12	1.16/1.72	28.8	5‘‘	3.42 ax, 3.93 eq	63.4
13	2.38	40.4	Rham		
14		42.9	1‘‘‘	4.99 *d* 1.3 Hz	102.3
15	1.20/1.29	30.8	2‘‘‘	3.83	72.4
16	1.36/2.26	33.3	3‘‘‘	3.67	72.1
17		49.8	4‘‘‘	3.38	73.9
18	1.19 *dd* 9.1 Hz	50.6	5‘‘‘	3.83	70.3
19	2.09	38.4	6‘‘‘	1.26 *d* 6.2 Hz	18.2
20		143.9	*28-Glc*		
21	5.23 *d* 7.0 Hz	118.5	1‘‘‘‘	5.47 *d* 8.0 Hz	92.9
22	1.80/2.30	38.0	2‘‘‘‘	4.16	79.9
23	1.04 *s*	28.4	3‘‘‘‘	3.72	77.5
24	0.82 *s*	16.9	4‘‘‘‘	3.49	71.0
25	0.87 *s*	17.0	5‘‘‘‘	3.37	78.3
26	0.97 *s*	16.5	6‘‘‘‘	3.69/3.79	62.2
27	0.97 *s*	15.3			
28		175.7			
29	1.00 *d* 6.6 Hz	23.9			
30	1.59 *s*	22.0			

4.99 (*d*, $^{3}J_{1''',2'''}$ = 1.3 Hz) / 102.3 and 5.47 (*d*, $^{3}J_{1'''',2''''}$ = 8.0 Hz) / 92.9 indicated the presence of four saccharide units, three bonded as glycosides and one bonded as glycosylester (δ 5.47/92.9). By use of $^{1}H,^{1}H$ COSY-45 and TOCSY experiments and the determination of the D-form for xylose, glucose and the L-form for arabinose and rhamnose (as described in the experimental part) the individual saccharides were identified as D-xylopyranose, D-glucopyranose, L-arabinopyranose and L-rhamnopyranose. The coupling constants of the anomeric proton signals of the D-xylopyranose and D-glucopyranose moiety of saponin **1** (^{3}J = 7.4 and 8.0 Hz) are in agreement with a β-configuration. The coupling constants $^{3}J_{1'',2''}$ and $^{3}J_{2'',3''}$ of the α-L-arabinopyranose moiety are only 4.2 and 6.6 Hz and the ^{13}C chemical shifts of the L-arabinose of **1** are different from arabinofuranoside moiety[5] indicating to the α configuration. Further evidence supporting a $^{4}C_1$ conformation of α-L-arabinopyranoside in **1** was obtained from the ROESY cross peak H-$1''_{ax}$ → H-$5''_{ax}$ which can not be observed for the $^{1}C_4$ conformation. The coupling constants $^{3}J_{1'',2''}$ = 4.2 Hz and $^{3}J_{2'',3''}$ = 6.6 Hz indicate an equilibrium between the $^{4}C_1$ and $^{1}C_4$ with $^{4}C_1$ conformer in excess.[6] The linkage of xylose and glucose to the aglycone was deduced by means of HMBC spectra. The cross peaks of the ^{3}J long range couplings between H-1’ xylose → C-3 aglycone and H-1’’’’

glucosylester → C-28 aglycone indicated the points of linkage to the sapogenin. The HMBC cross peaks between C-2' xylose → H-1'' arabinose, C-2'' arabinose → H-1''' rhamnose prove the interglycosidic linkage of arabinose at position C-2' xylose and rhamnose at position C-2'' arabinose. The ROESY cross peaks between H-3 aglycone → H-1' xylose, H-2' xylose → H-1'' arabinose and H-2'' arabinose → H-1''' rhamnose are in agreement with the linkage of the trisaccharide unit α-L-rhamnopyranosyl-(1→2)-α-L-arabino-pyranosyl-(1→2)-β-D-xylopyranose at position 3 of the sapogenin. The substitution in position 2'''' of the glucosylester could be established by the downfield shifts of the H-2'''' (Δδ + 0.85) and C-2'''' (Δδ + 6.1) signals compared with those of the nonsubstituted glucosylester of Zygophyloside K (2). The moiety R could be $-SO_3H$ but this assumption could not be proved by LSI-MS.

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