Triterpenoid Saponins from *Astragalus trigonus*

Kamel H. Shaker[a], Mirko Bernhardt[a], M. Hani A. Elgamal[b, deceased] and Karlheinz Seifert[a,*]

[a] University of Bayreuth, Organic Chemistry I/2, NW II, D-95440 Bayreuth, Germany. Fax: 49-921-555358. E-mail: karlheinz.seifert@uni-bayreuth.de

[b] National Research Centre, Laboratory of Natural Products, Dokki-Cairo, Egypt

* Author for correspondence and reprint requests

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One new, 3-*O*-{[[-α-L-rhamnopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl}-3β,22α,24-trihydroxyolean-12-ene and two known triterpenoid saponins, 3-*O*-{[α-L-rhamnopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl}-3β,22β,24-trihydroxyolean-12-ene (azukisaponin V) and 3,16-di-*O*-β-D-glucopyranosyl-3β,6α,16β-trihydroxycycloart-24-ene have been isolated from *Astragalus trigonus.* The structures were determined primarily by NMR spectroscopy. The assignment of NMR signals was performed by means of ^{1}H-^{1}H COSY, NOESY, ROESY, TOCSY, HMQC and HMBC experiments.

Introduction

Astragalus trigonus DC is a spiny shrub indigenous to Egypt and belongs to the family Fabaceae of the order Leguminales (Tackholm, 1974). Extracts from various *Astragalus* species represent common drugs in traditional medicine, mainly used as a remedy for the treatment of nephritis, diabetes, leukemia and uterine cancer (Hartwell, 1970; McCracken *et al.*, 1970). Cycloartane triterpene glycosides which have been isolated from several Egyptian *Astragalus* species showed antitumor activity against some human tumor cell lines and anti-HIV activity (Abdallah *et al.*, 1993). As part of our continuing search for triterpenoid saponins (Shaker *et al.*, 1999, Shaker *et al.*, 2000) we investigated the plant constituents of *Astragalus trigonus.*

Previously 3,16-di-*O*-β-D-glucopyranosyl-3β,16β-dihydroxycycloart-24-en-6-one has been isolated from the aerial parts of *A. trigonus* (El-Sebakhy and Waterman, 1985). Additionally, 6 cycloartane glycosides, trigonoside I-III, astragaloside I-II and 3,16-di-*O*-β-D-glucopyranosyl-3β,6α,16β-trihydroxycycloart-24-ene have been obtained from the roots of *A. trigonus* (Gariboldi *et al.*, 1995; Verotta *et al.*, 1998). In this report we describe the isolation and structure determination of one new and two known triterpenoid saponins.

Results and Discussion

The butanol extract of the whole plants of *A. trigonus* was obtained as described in the experimental section. The crude saponins were subjected to column chromatography on silica gel to be eluted successively with $CHCl_3$, $CHCl_3$-MeOH and $CHCl_3$-MeOH-H_2O with increasing amounts of MeOH and H_2O. Three saponins have been isolated after further purification by column chromatography on Sephadex LH-20 and RP-18 material.

The LSI mass spectrum of **1** exhibited $[M-1]^-$ ion at *m/z* 1103. The fragment ion at *m/z* 957 $[M-1-146]^-$ was a proof for the elimination of a desoxyhexose. The fragment ion at *m/z* 795 $[M-1-146-162]^-$ showed the loss of a desoxyhexose plus hexose moiety. The $[M-1]^-$ ion together with ^{1}H and ^{13}C NMR data allowed us to propose the molecular formula $C_{54}H_{88}O_{23}$.

The ^{1}H and ^{13}C NMR spectra of **1** (Fig. 1) showed the presence of 3β,22α,24-trihydroxyolean-12-ene as aglycone. The signals of the axial and equatorial oriented protons of the aglycone were assigned by ROESY experiments. The proton signal 22 (δ = 3.40) showed crosspeaks to the signal of proton 18 (δ = 2.08) and the protons of the methyl group 30 (δ = 1.02) in the ROESY-spectrum. These facts proved the axial orientation of proton 22 and accordingly the 22α-hydroxy configuration. Four anomeric proton signals at δ 4.22

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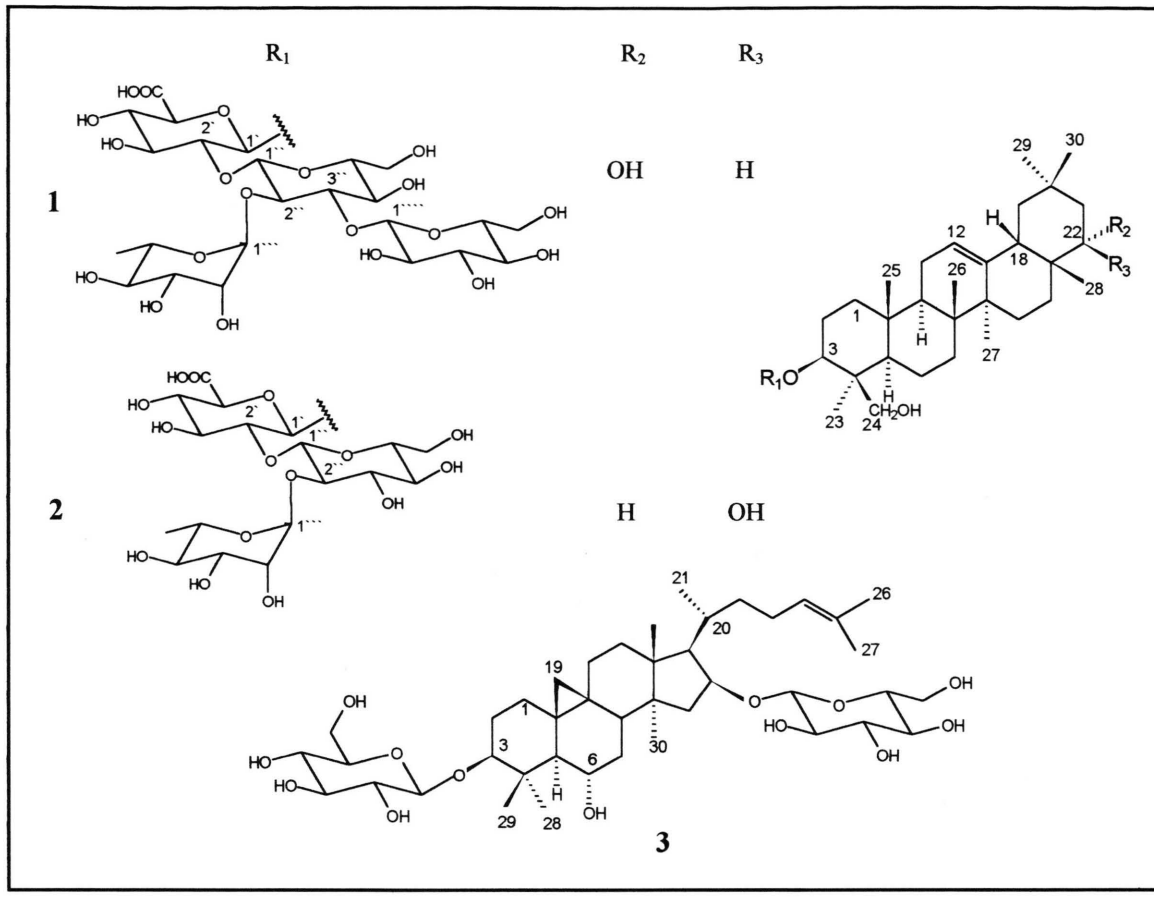

Fig. 1. Triterpenoid saponins from *Astragalus trigonus*

(J = 7.7 Hz), 4.44 (J = 7.9 Hz), 4.87 (J = 7.5 Hz) and 5.12 (J = 1.2 Hz) indicated the presence of four saccharide units, bound as glycosides. By use of ^{1}H-^{1}H COSY-45 and TOCSY spectra and the determination of the D-form for glucose, glucuronic acid and the L-form for rhamnose (as described in the experimental section) the individual saccharides were identified as two D-glucopyranoses, D-glucuronopyranose and L-rhamnopyranose. The coupling constants of the anomeric proton signals of the both glucopyranoses and the glucuronopyranose J = 7.5, 7.7 and 7.9 Hz are in agreement with a β-configuration. The linkage of saccharide units to the aglycone was determined by means of HMBC spectra. The cross peaks of the ^3J long range couplings between H-1′ glucuronic acid → C-3 aglycone indicated the point of linkage to the sapogenin. The HMBC cross peaks between H-1″ glucose → C-2′ glucuronic acid, H-1‴ glucose → C-3″ glucose and H-1‴ rhamnose → C-2″ glucose prove the interglycosidic linkages.

The triterpenoid glycoside **2** is known as azukisaponin V (Pelizzoni *et al.*, 1996) and has been isolated before together with 3,16-di-*O*-β-*D*-glucopyranosyl-3β,6α,16β-trihydroxycycloart-24-ene (**3**) from the roots of *Astragalus trigonus* (Verotta *et al.*, 1998) (See Fig. 1 for structures).

Experimental

General

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 (^1H: 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, Düren, Germany), the spots were sprayed with 'triterpene reagent' (1% vanillin in 50% H_3PO_4), 'sugar reagent' (4% ethanolic aniline-4% ethanolic diphenylamine-H_3PO_4, 5:5:1 v/v/v) and phosphomolybdic acid reagent (Aldrich). GLC (H_2 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 × 0.32 mm, J & W).

Isolation

A. trigonus was collected in 1996 nearby Alexandria Egypt and identified by Dr. M. Elgebaly 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 whole plant of *A. trigonus* (4.5 kg) was exhaustively extracted with 80% MeOH (20 l). After removal of the solvent by evaporation, the residue was successively partitioned between H_2O and *n*-BuOH. The butanolic fr. was evaporated under red. pres. at 50 °C to obtain a crude saponin mixture (16 g). CC

Table I. ^1H and ^{13}C NMR spectral data for the aglycones of saponins **1–3** in CD_3OD.
C = carbon atoms of the aglycones.

	1		**2**	**3**
C	^1H ax/eq	^{13}C	^{13}C	^{13}C
1	0.99/1.60	40.0	39.9	33.8
2	1.82/1.82	24.9	24.4	30.8
3	3.39	92.4	91.8	90.6
4	–	44.7	44.7	43.6
5	0.90	57.3	57.3	55.2
6	1.32/1.60	19.4	19.2	70.0
7	1.55/1.37	34.4	34.4	38.8
8	–	40.8	40.7	47.0
9	1.58	49.0	49.0	22.6
10	–	37.5	37.5	31.1
11	1.83/1.83	24.8	24.2	27.5
12	5.23	123.6	123.2	34.1
13	–	145.3	145.2	47.0
14	–	43.3	43.3	48.2
15	1.72/1.00	27.1	27.3	48.8
16	1.75/1.37	29.5	30.0	84.2
17	–	38.2	38.6	58.2
18	2.08 *d* 13.4 Hz	47.0	46.8	19.9
19	1.74/0.93	47.4	47.4	32.3
20	–	31.3	31.4	31.8
21	1.50/1.31	38.2	42.2	18.7
22	3.40	77.8	76.9	37.4
23	1.26	23.4	23.4	26.4
24	4.12/3.13 *d* 11.4 Hz	64.4	64.4	127.8
25	0.89	16.5	16.5	131.9
26	0.96	17.5	17.5	26.5
27	1.11	25.6	25.9	18.0
28	0.88	21.1	20.4	29.3
29	0.91	32.6	32.8	17.2
30	1.02	29.0	28.9	21.0

Table II. ^1H and ^{13}C NMR spectral data for the sugar moieties of saponins **1–3** in CD_3OD.
C = carbon atoms of the sugar moieties, GlcA = β-D-glucuronopyranose, Glc = β-D-glucopyranose, Rha = α-L-rhamnopyranose

		1	**2**	**3**
C	^1H	^{13}C	^{13}C	^{13}C
GlcA				3-O-Glc
1′	4.44 *d* 7.9 Hz	106.0	105.9	107.4
2′	3.74	78.1	78.5	76.2
3′	3.58	77.7	77.3	78.8
4′	3.42	74.3	74.2	72.2
5′	3.52	77.0	77.0	78.3
6′	–	n. d.	n. d.	63.4
Glc				16-O-Glc
1″	4.87 *d* 7.5 Hz	102.1	102.1	107.4
2″	3.63	78.4	78.3	76.2
3″	3.52	83.4	76.9	78.8
4″	3.65	71.5	71.5	72.2
5″	3.53	76.3	76.3	78.3
6″	3.66/3.72	62.2	61.8	63.4
Rha				
1‴	5.12 δ 1.2 Hz	102.3	102.3	
2‴	3.91	72.2	72.1	
3‴	3.71	72.3	72.2	
4‴	3.38	74.2	74.2	
5‴	4.09	69.6	69.5	
6‴	1.28 *d* 6.2 Hz	18.3	18.3	
Glc				
1⁗	4.22 *d* 7.7 Hz	102.1		
2⁗	3.15	75.2		
3⁗	3.33	77.3		
4⁗	3.27	71.9		
5⁗	3.21	77.7		
6⁗	3.82/3.65	62.9		

on silica gel eluting with $CHCl_3$-MeOH-H_2O with increasing amounts of MeOH and H_2O gave three frs. I (2 g), II (5 g) and III (2 g). II and III were further chromatographed by means of Sephadex LH-20 eluting with MeOH-H_2O 17:3 followed by CC on RP-18 eluting with MeOH-H_2O 13:7 to give pure saponins **1** (3.5 mg), **2** (5 mg) and **3** (5 mg).

(R)-2-Butylglycosides

A sample (ca 250 μg) of the appropriate saponin was hydrolysed with 0.5 ml 5% HCl for at least 3 h at 80 °C. After evaporation of the acid under red. pressure, 0.5 ml (*R*)-2-BuOH was added, dried HCl gas was bubbled through the soln. for 30 s and the reaction mixture was heated for 3 h at 80 °C under N_2 in a sealed vessel. Trimethylsilylation was performed with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. (*R*)-2-butyl-L-Rha: *Rt* 52.23, R_i 1854; (*R*)-2-butyl-L-Glc: R_t 81.92, R_i 2086; (*R*)-2-butyl-D-Glc: R_t 82.25, R_i 2088, (*R*)-2-butyl-D-GlcA: R_t 81.97, R_i 2085; (*R*)-2-butyl-L-GlcA: R_t 82.55, R_i 2095. Identification of the sugars were done by comparison of the R_i values and co-injection with the appropriate standard. It was shown that rhamnose belongs to the L- , glucose and glucuronic acid to the D-series.

Spectroscopic data

3-*O*-{[[-α-L-rhamnopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl}-3β,22α,24-trihydroxyolean-12-ene (**1**): ($C_{54}H_{88}O_{23}$, M_r 1104); amorphous powder; $[\alpha]_D^{22}$ +22 (MeOH; c 0.20). LSI-MS negative ion mode *m/z* (rel. int.): 1103 $[M-H]^-$ (25), 957 $[M-H-Rha]^-$ (4), 795 $[M-H-Rha-Glc]^-$ (3). For ^1H NMR and ^{13}C NMR: see Tables I and II.

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