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A CYCLOARTANE TRITERPENE 3β, 16β DIGLUCOSIDE FROM *ASTRAGALUS TRIGONUS* AND ITS NON NATURAL 6-HYDROXY EPIMER

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Key Word Index—*Astragalus trigonus*; Fabaceae; roots; cycloartane triterpene glucoside; 3β, 6α, 16β trihydroxy 9, 19 cyclolanost–24–ene 3β, 16β diglucopyranoside; 3β, 6β, 16β trihydroxy 9, 19 cyclolanost–24–ene 3β, 16β diglucopyranoside.

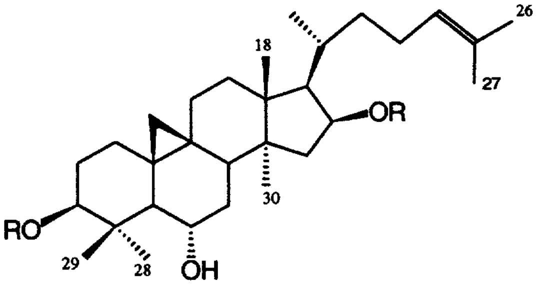
Abstract—The detailed investigation of *Astralagus trigonus* DC roots, led to the isolation of a new cycloartan-3β, 16β diglucoside, whose structure was elucidated by extensive use of 1D- and 2D- high field (600 MHz) NMR techniques. The $NaBH_4$ reduction of the natural 6-oxo 3β, 16β diglucoside, previously isolated from the same plant, afforded the 6-epi-derivative of the new compound. The conformational preference of both these compounds has been investigated by high field ^1H NMR spectroscopy and molecular modeling techniques. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The roots of *Astrlagaus* species represent a very old and well-known traditional medicine. The traditional uses include the treatment of conditions such as diabetes, leukemia and uterine cancer, and this has prompted investigations on the active constituents of the plant.

A number of cycloartane-type saponins have been isolated from *Astragalus* species, some of them have been reported to be responsible for the hypotensive and cardiotonic effects of the plants [1–3]; some have shown interesting antitumor and antiviral properties [4, 5]. Recently interest has been directed towards *Astragalus* saponins in relation to their potential immunostimulatory activity. As a part of our studies on *Astragalus* species endemic to Egypt [4–11], different parts of *A. trigonus* DC. have been chemically investigated.

From the aerial parts of *A. trigonus*, the major 6-oxo cycloartane 3β, 16β-diglucoside was isolated previously [6]. The early investigation of *A. trigonus* roots led to the isolation of Astragaloside I and II, Trigonoside I, II and III (cycloastragenol derived oligosaccharides) [10]; azukisaponin V (a pentacyclic triterpenoid oligosaccharide) [10] and 3β, 6α,16β trihydroxy, 9β,19 cycloanost-24-oxo, 25-ene, 3β,16β diglucopyranoside [11]. In this paper we describe the isolation and structure elucidation of a new cycloartane saponin, 3β,6α,16β trihydroxy 9,19 cyclolanost-24-ene 3β,16β diglucopyranoside (**1**), along with the preparation of its 6-epi derivative (**2**). We also consider their conformational features which could be related to the biological activities.



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RESULTS AND DISCUSSION

The *n*-butanol counter extract from *A. trigonus* roots, first fractionated by column chromatography, gave four major compounds identified as astragaloside II, trigonoside II and trigonoside III [10],

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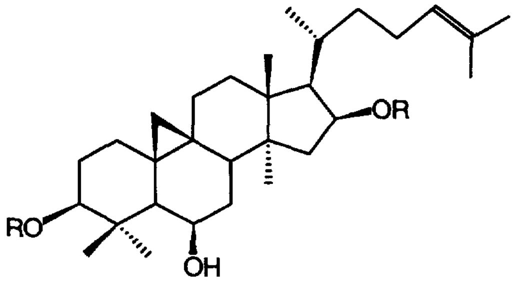

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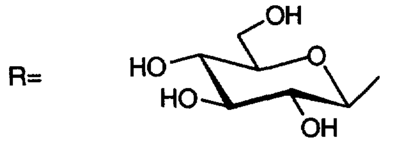


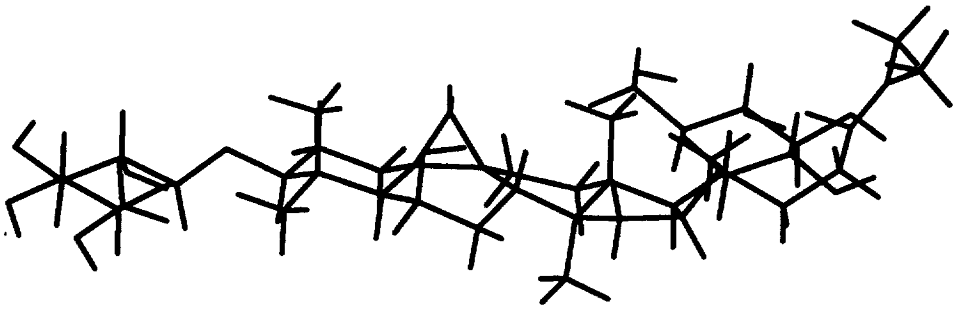
azukisaponin V [11]. A fifth component appears to be constituted of a complicated mixture of at least four products. The attempt to purify this mixture by enzymatic hydrolysis (performed with a β-glucuronidase from almonds) further complicated the mixture, presumably due to the presence of different aglycones and sugar moieties. A partial separation could be obtained by countercurrent chromatography which allowed us to isolate two pure products; one of them has been identified as $3\beta,6\alpha,16\beta$ trihydroxy 9β,19 cyclolanost-24-oxo, 25-ene, $3\beta,16\beta$ diglucopyranoside [11].

The other component (**1**) showed a pseudo-molecular ion at m/z 805 $[M+Na]^+$, corresponding to the molecular formula $C_{42}H_{70}O_{13}$. From a preliminary observation of its ^1H NMR and ^{13}C NMR spectra, the main features of a cycloartane derived glycoside were evident: two high field protons appearing as doublets ($J = 4.5$ Hz) at δ 0.45 and δ 0.21 (H-19α and H-19β); seven methyl groups, six of them appearing as singlets and one doublet, representing C-18, C-28,

Table 1. ^1H NMR chemical shifts of **1** (after exchange with D_2O) as determined by E-COSY and ROESY experiments [600 MHz, pyridine-d_5, δ in ppm from TMS]

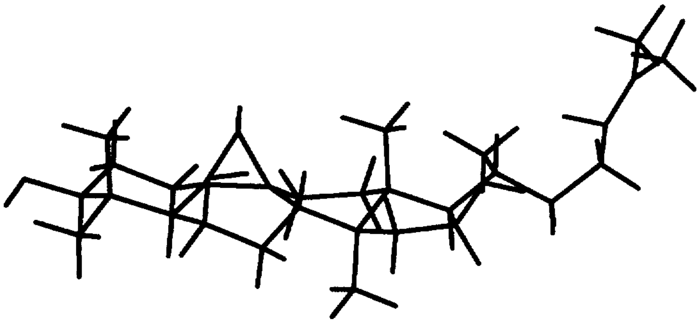
H	δ(ppm)	*m*	*J*(Hz)	Significant cross-peak correlations in the ROESY spectrum[a]
1ax	1.53	*ddd*	14.0, 13.0, 4.6	3ax(*w*), 2eq(*w*),
1eq	1.11	*ddd*	14.0, 4.6, 4.0	
2eq	2.47	*m*	$J_{A-B} = 13.6$	3ax(*m*), 1ax(*w*)
2ax	1.94	*ddt*	1.36, 5.8, 4.6	19A(*w*)
3ax	3.65	*dd*	11.2, 5.8	2eq(*m*), 28(*m*), 5ax(*m*)
5ax	1.67	*d*	9.5	3ax(*m*), 7α(*m*)
6β	3.63	*ddd*	9.5, 9.0, 4.1	7β(*m*), 29(*m*), 19B(*m*)
7β	1.69	*m*	$J_{A-B} = 12.1$	6β(*m*)
7α	1.58	*dt*	12.1, 9.0, 9.0	5ax(*m*), 30(*m*)
8β	1.70	*m*		19B(*m*)
11eq	1.89	*dd*	14.3, 6.4	12(*w*)
11ax	1.14	*dd*	14.3, 8.9	19A(*m*)
12	1.61(2H)	*m*		11eq(*w*)
15α	2.24	*dd*	14.2, 7.7	16α(*m*), 30(*m*)
15β	1.98	*dd*	14.2, 5.1	7β(*w*)
16α	4.38	*ddd*	7.7, 7.3, 5.1	15α(*m*), 22B(*w*), 17α(*m*), 30(*w*)
17α	1.87	*dd*	11.0, 7.3	16α(*m*), 30(*s*)
18	1.20	*s*		20(*m*), 15β(*m*), 8β(*s*)
19B	0.45	*d*	4.5	6(*m*), 8β(*m*), 29(*m*)
19A	0.21		4.5	11ax(*m*)
20	2.31	*dddq*	11.0, 8.0, 7.2, 5.3	24(*w*), 18(*m*), 21(*m*)
21	1.01	*d*	7.2	17α(*w*), 12(*m*)
22B	2.02	*m*	$J_{A-B} = 13.0$	16α(*w*), 20(*w*)
22A	1.27	*m*		23A(*w*), 17α(*w*)
23B	2.35	*m*	$J_{A-B} = 14.0$	
23A	2.29	*m*		
24	5.52	*dt*	7.7, 1.8	22B(*w*), 26(*m*), 22A(*w*)
26	1.66	*d*	1.8	24(*m*)
27	1.70	*d*	1.8	
28	2.00	*s*		3ax(*m*)
29	1.33	*s*		28(*m*), 2ax(*m*)
30	0.97	*s*		16α(*w*)

*Intensities of the cross peaks are defined as: weak (*w*), medium (*m*) and strong (*s*).

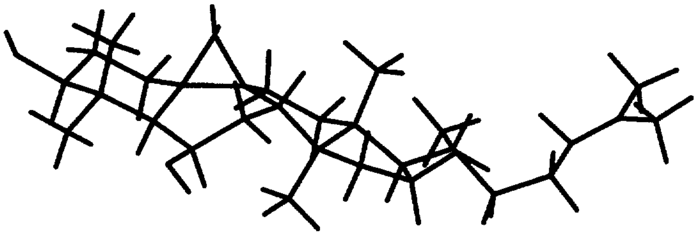


Conformer C_1

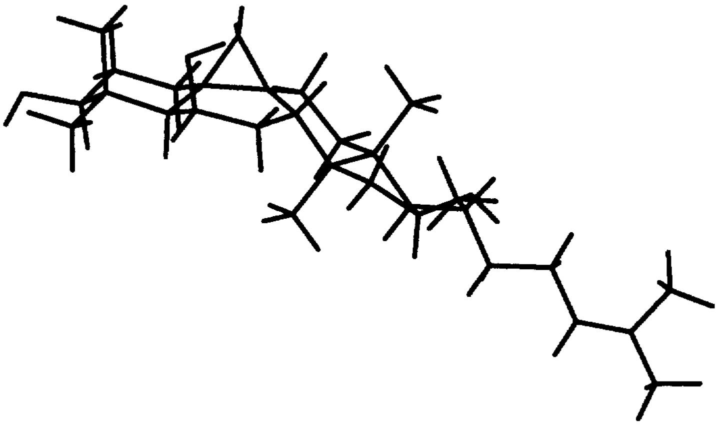
Fig. 1. Most stable conformer of compound 2.



Conformer C_2



Conformer C_3



Conformer C_4

Fig. 2. Low energy conformers of compound 2. Only the aglycone moiety has been reported.

C-29, C-30 (all singlets), C-26 and C-27 (deshielded broad singlets) and C-21 (doublet) in an unsaturated cycloartane terpenoid. The tetrahydrofuran ring typical of cyclorastragenol was absent but two monosaccharidic units were present.

Due to the small amount of material available (15 mg), the structure of compound **1** was established by a combination of 1D- and 2D-NMR techniques operating at 600 MHz, following the strategy reported in Ref. [12]. The 1D TOCSY experiments [13], by irradiating at δ 4.92 and δ 4.65, identified the spin systems of two glucose units; the E-COSY experiment [14], together with 1D and 2D TOCSY experiments [13, 15] allowed us to identify the isolated spin systems, even for overlapped protons, thus locating the three hydroxyl groups in positions 3β,6α,16β of the aglycone, and defining the sequence H-15-H-26(27) as shown in the formula. Confirmation of the structure of the side chain was provided by HMQC [16] and HMBC experiments [17]. Two carbons at δ 126.86 (*d*) and δ 130.01 (*s*) represented an internal double bond; the carbon at δ 126.86 was linked to the proton at δ 5.52 (*bt*, $J = 7$ Hz). The HMBC spectrum gave information about the location of the double bond: both the olefinic carbons correlated to two methyls at δ 1.70 (*d*, $J = 1.8$ Hz) and δ 1.66 (*d*, $J = 1.8$ Hz); the carbon resonances of these two carbons were at δ 17.93 and δ 25.94, respectively. The proton at δ 5.52 correlates to one of these methyls at δ 25.94. All proton resonances and coupling constants derived from the E-COSY experiment are shown in Table 1. The β-D-glucopyranosyl units were bonded to C-3 and C-16 as revealed by the HMCB experiment and confirmed by the ROESY spectrum [18].

The agreement of the NMR experiments, together with almost superimposable carbon signals (except for slight differences among triplets near 35 ppm) of the cycloartane skeleton, in comparison with the recently isolated 3β,6α,16β trihydroxy 9,19 cyclolanost-24-oxo, 25-ene, 3β,16β-diglucopyranoside [11], allowed us to assign **1** the structure 3β,6α,16β trihydroxy 9,19 cyclolanost-24-ene 3β,16β diglucopyranoside.

Sodium borohydride reduction of the natural 6-oxo 3β,16β diglucoside, previously isolated from the same plant [6], gave compound **2**. A series of NMR experiments were run at 600 MHz, following the path described for compound **1**. All the experiments were in accord in assigning **2** the structure of 3β,6β,16β trihydroxy 9,19 cyclolanost-24-ene 3β,16β diglucopyranoside. As expected, the hydride attack from the less hindered face, only allowed the formation of the 6-hydroxy epimeric form of **1**.

Some peculiarities found in the spectra are worth noting. Unusual high frequency resonances of one proton of the cyclopropane ring (δ 1.46, H-19β) and of the proton at the B-C ring junction (δ 2.46, H-8) were clearly visible. They could be attributed to both configurational and conformational changes.

The conformational preference of compound **2** and, by comparison, of compound **1**, has been investigated by ^{1}H NMR and molecular modeling techniques. The conformational analysis evidenced the presence, for compound **2**, of four stable conformers (C_1–C_4), which differ for less than 10 kcal mol^{-1}, mainly characterized by a different conformation of the A, B, C-rings in the aglycone moiety.[1]

In the most stable conformer C_1, ring A in a chair conformation, whereas rings B and C are half-chair (Fig. 1). A half-boat conformation of the ring C is only 2.95 kcal mol^{-1} less stable (conformer C_2) (Fig. 2). Chair inversion of ring B leads to a conformer C_3 which is slightly higher in energy with respect to C_1 (8.8 kcal ml^{-1}) (Fig. 2). Chair inversion in ring B does not appreciably affect the conformation of ring A. It does, however, influence the conformation of ring C, leading to a more "bent" topography with respect to conformer C_2. A bent topography is also found in conformer C_4, which is less stable (9.9 kcal mol^{-1}) with respect to C_1, characterized by a chair conformation of the rings A and C.

The relatively small energy differences calculated for the different conformers suggest conformational equilibria in solution, without excluding completely any of the conformers. In order to achieve more information on the conformer prevailing in solution, a comparison between the theoretical and NMR results was undertaken. The distance between non-vicinal hydrogens was the geometrical parameter selected for the correlation. Inspection of the distances between non-vicinal hydrogens revealed that the observed NOE effects are more compatible with the conformers C_2 and/or C_1 in solution. In particular the distances which discriminate between the conformation of the ring B in C_1/C_2 and C_3 are: the H_5–H_7 distance, which is 2.3 Å in C_1 and 3.86 Å in C_3, the former in agreement with a medium NOE as experimental by observed: the H_6–H_{28} distance which is 2.1 Å in C_1 and 3.23 in C_3, the former in agreement with a strong NOE as experimentally observed. The presence of the conformations C_1/C_2 in solution is further confirmed by the distance between $H_{19\beta}$ and the oxygen at C_6 which is ≈ 2.7 Å in conformers C_1, C_2, C_4 and ≈ 4.3 Å in C_3, the former in agreement with the deshielding effect experimentally observed for $H_{19\beta}$ in the ^{1}H NMR spectrum. However, conformer C_4 can be excluded on the basis of the distances $H_{7\alpha}$–H_{30} and $H_{6\alpha}$–H_{30} of 2.2 Å and 2.18 Å respectively, which should produce a NOE effect, not observed experimentally.

The analysis performed on compound **1** showed that inversion of configuration at C-6 passing from compound **2** to compound **1** does not significantly

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[1] The conformation of the rings A–C may be determinant for the interaction of cycloartenol derivatives with phospholipids in membranes (Block, K., *CRC Cat. Rev. Biochem.*, 1983, **4**, 47). In particular a α-flat topography appears to be essential for a good interaction (Milon, A., Nakatani, Y., Kitzinger, J. P. and Ourisson, G., *Helv. Chim. Acta*, 1989, **72**, 1.)

affect the conformational preference. As the calculated energy difference between the various conformers in the isolated molecule is relatively small, a conformational equilibrium in solution cannot be excluded as well as preference for one conformer or another depending on the medium conditions. This result could be of great relevance in view of a possible biological activity of these cycloartenols.

EXPERIMENTAL

General

High-speed counter-current chromatography (HSCCC) was performed on a CCC 1000 counter-current chromatograph (PharmaTech Research Corp., Baltimore, U.S.A.), equipped with a three multilayer coiled column (total vol. 350 ml) rotating at 1032 rpm, a SSI liquid chromatography pump (model 300), a PTRC speed controller and an injection valve (Rheodyne). Samples were filtered on Millex-HV filters (0.45 μm Millipore) before the injection. FAB MS spectra (positive mode) were obtained on a VG 7070 mass spectrometer using NBA as matrix. NMR: All spectra were measured on samples of about 10 mg dissolved in 750 μl of pyridine-d_5, in 5 mm tubes. Spectra were registered in phase sensitive mode at 28° on a Varian Unity 600 spectrometer, operating at 599.919 MHz for ^{1}H and at 150.858 for ^{13}C, equipped with a triple resonance indirect detection probe (^{1}H{^{13}C,^{15}N}), a waveform generator on both the observing and the decoupling channel and running the Varian software Vnmr 4.3a. The 1D experiments were run with the States-Haberkon method [19] and the 2D-ones with a collection of 2 sets of data (Hypercomplex method). The 1D and 2D spectral widths used were 6000 Hz for ^{1}H and 15,300 Hz for ^{13}C. All spectra were referenced to TMS through solvent signals. The DQF-COSY [20] spectra were acquired with 2048 points in F2, 512 complex increments in F1, 16 scans per increment and a final data matrix of 4 k × 2 k points. The TOCSY spectra [15] were acquired

Table 2. ^{1}H NMR chemical shifts of **2** (after exchange with D_2O) as determined by E-COSY and ROESY experiments [600 MHz, pyridine-d_5, δ in ppm from internal TMS]

H	δ(ppm)	*m*	*J*(Hz)	Significant cross-peak correlations in the ROESY spectrum
1ax	1.50	*ddd*	13.7, 12.7, 5.1	2eq (*w*), 5ax (*m*)
1eq	1.11	*ddd*	12.7, 5.1, 4.4	2ax(*w*), 19A(*w*)
2eq	2.39	*m*		3ax(*w*)
2ax	2.03	*m*		
3ax	3.53	*dd*	11.6, 5.1	2eq(*w*), 28(*m*), 1ax(*w*), 5ax(*s*)
5ax	1.20	*d*	4.6	28(*m*), 1ax(*m*), 7α(*m*)
6α	4.48	*ddd*	8.0, 4.5, 3.9	28(*s*), 7β(*m*), 7α(*w*), 5ax(*s*)
7β	1.59	*ddd*	12.8, 8.0, 4.5	8β(*w*), 6α(*w*)
7α	1.31	*ddd*	13.3, 12.0, 3.9	5ax(*m*), 30(*m*)
8β	2.46	*dd*	13.3, 4.5	15β(*m*), 7β(*w*), 19B(*m*), 18(*m*)
11eq	2.10	*ddd*	14.2, 10.7, 7.2	30(*w*), 12ax(*w*)
11ax	1.03	*dd*	14.2, 10.3	19A(*w*)
12eq	1.72	*ddd*	14.2, 10.3, 7.2	18(*w*), 21(*w*)
12ax	1.62	*dd*	14.2, 10.7	
15α	2.29	*dd*	14.0, 7.8	16α(*w*), 30(*m*)
15β	2.02	*dd*	14.0, 5.0	8β(*m*)
16α	4.39	*ddd*	7.5, 7.8, 5.0	15α(*w*), 17α(*m*), 30(*w*)
17α	1.88	*dd*	11.2, 7.5	16α(*m*), 30(*m*)
18	1.29	*s*		8β(*m*), 20(*m*), 15β(*m*), 12eq(*w*), 21(*m*)
19B	1.46	*d*	4.2	8β(*m*)
19A	0.42	*d*	4.2	2ax(*w*), 18(*w*), 11ax(*w*)
20	2.26	*m*		18(*m*)
21	0.99	*d*	7.0	12eq(*w*), 20(*w*)
22B	1.98	*m*	J_{A-B} = 14.5	16α(*m*)
22A	1.25	*m*		23A(*w*), 17α(*w*)
23B	2.34	*m*	J_{A-B} = 14.0	
23A	2.27	*m*		
24	5.49	*bt*	7.4	26(*m*)
26	1.64	*s*		
27	1.68	*s*		
28	1.64	*s*		5ax(*m*)
29	1.67	*s*		2ax(*m*), 19B(*w*)
30	0.97	*s*		17α(*m*)

[a] Intensities of the cross peaks are defined as: weak (*w*), medium (*m*) and strong (*s*).

Table 3. ^{1}H and ^{13}C NMR assignments (δ in ppm, J(Hz), significant ^{n}H–C as determined from a HMBC experiment) of the sugar moieties of compounds **1** and **2**. Coupling constants have been assigned through 1D TOCSY and E-COSY experiments

	1	Carbon	Proton	*m*	*J*(Hz)	Significant ^{n}H–C carbon connections
3-O-Glc	1′	106.70	4.92	*d*	7.7	C-3
	2′	75.52	4.03	*dd*	9.4, 7.7	
	3′	78.38	4.20	*t*	9.0	
	4′	71.49	4.17	*m*		
	5′	77.79	3.91	*ddd*	11.0, 2.9, 5.5	
	6′A	62.67	4.50	*dd*	12.1, 2.9	
	6′B		4.33	*dd*	12.1, 5.5	
16-O-Glc	1″	106.53	4.65	*d*	7.6	C-16
	2″	75.28	3.99	*dd*	7.6, 9.6	
	3″	78.33	4.15	*t*	8.6	
	4″	71.49	4.19	*t*	8.4	
	5″	77.74	3.85	*ddd*	9.9, 5.5, 3.3	
	6″A	62.64	4.40	*dd*	12.1, 3.3	
	6″B		4.27	*dd*	12.1, 5.5	
	2	Carbon	Proton	*m*	*J*(Hz)	Significant ^{n}H–C carbon connections
3-O-Glc	1′	105.56	4.98	*d*	7.4	C-3
	2′	75.47	4.05	*dd*	10.4, 7.4	
	3′	78.42	4.25	*t*	9.0	
	4′	71.58	4.21	*dd*	10.2, 8.4	
	5′	78.02	3.99	*ddd*	10.2, 5.8, 3.6	
	6′A	62.71	4.56	*dd*	11.6, 3.6	
	6′B		4.38	*dd*	11.6, 5.8	
16-O-Glc	1″	106.56	4.67	*d*	7.9	C-16
	2″	75.28	3.90	*t*	7.9	
	3″	78.17	4.14	*m*		
	4″	71.82	4.13	*m*		
	5″	77.73	3.87	*ddd*	9.6, 5.6, 3.6	
	6″A	62.90	4.44	*dd*	12.1, 3.6	
	6″B		4.23	*dd*	12.1, 5.6	

with a 80 ms mixing time, a MLEV-17 [21] spin-lock field of 10 kHz flanked by two 2 ms trim pulses, 1024 points in F2, 256 complex increments in F1, 8 scans per increment and a final data matrix of 2 k × 1 k points. The ROESY spectra [18] were acquired with a 400 ms mixing time, a MLEV-17 spin-lock field of 3 kHz obtained with small flip-angle pulses (30°), 1024 points in F2, 256 complex increments in F1, 8 scans per increment and a final data matrix of 2 k × 1k points. The E-COSY spectra [14] were acquired with 4096 points in F2, 1024 complex increments in F1, 32 scans per increment and a final data matrix of 8 k × 4 k points. The HMQC [16] spectra were acquired with a nulling time of 300 ms. 1024 data points in F2, 256 complex increments in F1, 8 scans per increment, a final data matrix of 2 k × 1 k points and a MPF7 [22] waveform generator-based ^{13}C-decoupling sequence during the acquisition. The HMBC spectra [17] were acquired with 1024 points in F2, 256 complex increments in F1, 32 scans per increment and a final data matrix of 2 k × 1 k points. For each sample one HMBC spectrum was optimized for a $^{n}J_{\mathrm{C-H}}$ of 8 Hz and another one for $^{n}J_{\mathrm{C-H}}$ of 4 Hz, with $n \div 2$–4. All 2D spectra were transformed with a cosine squared weighting function in both dimensions, except for the HMBC ones where a sinebell function was applied in F2 and a cosine squared in F1, together with a mixed mode display of the spectra (magnitude mode in F2 and phase sensitive mode in F1). Selective excitation spectra 1D–TOCSY [13] were acquired using waveform generator-based BURP shaped pulses [23], mixing times ranging from 80–150 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2 ms trim pulse. The repetition rates for all kind of spectra were about 1.5 s.

Plant material

Astragalus trigonus DC. was collected from El-Agami, west of Alexandria, Egypt, in April 1991. The plant was previously identified in the Department of Botany, Faculty of Science, Cairo University. Voucher specimens are also deposited in the herbarium of Faculty of Science, University of Alexandria, Egypt.

Extraction and isolation

The air-dried powdered roots of *A. trigonus* DC. (2 kg) were extracted with 95% EtOH. The extract was

Table 4. ^{13}C NMR assignments for compound **1** and ^{B}C–H connectivities as determined by the HMBC experiments

Carbon	**1**	*m*	Connected protons
1	32.56	*t*	0.21 (19A)
2	30.37	*t*	
3	89.17	*d*	5.01 (Glc-1′), 2.00 (28), 1.33 (29)
4	42.71	*s*	3.65 (3ax), 2.00 (28), 1.69 (5ax), 1.33 (29)
5	54.10	*d*	2.00 (28), 1.33 (29), 0.46 (19B), 0.21 (19A)
6	67.99	*d*	
7	38.50	*t*	
8	46.98	*d*	1.21 (18), 0.97 (30), 0.46 (19B), 0.21 (19A)
9	21.27	*s*	0.21 (19A)
10	29.34	*s*	1.69 (5ax), 0.46 (19B), 0.21 (19A)
11	26.36	*t*	0.21 (19A)
12	32.90	*t*	1.21(18)
13	45.76	*s*	4.38 (16α), 2.24, (15α), 1.87 (17α), 1.20 (18), 0.97 (30)
14	46.95	*s*	
15	48.16	*t*	0.97 (30)
16	82.57	*d*	4.71 (Glc-1″)
17	57.01	*d*	2.24 (15α), 1.19 (18), 1.01 (21)
18	19.12	*q*	1.87 (17α)
19	30.29	*t*	
20	30.44	*d*	
21	17.93	*q*	
22	37.06	*t*	1.01 (21)
23	25.78	*t*	
24	126.86	*d*	1.70 (27), 1.66 (26)
25	130.01	*s*	1.70 (27), 1.66 (26)
26	25.94	*q*	
27	17.93	*q*	1.66 (26)
28	29.08	*q*	1.33 (29)
29	16.84	*q*	2.00 (28)
30	20.40	*q*	

Table 5. ^{13}C NMR assignments for compound **2** and ^{B}C–H connectivities as determined by the HMBC experiments

Carbon	**2**	*m*	Connected protons
1	32.73	*t*	1.20 (5ax), 0.42(19A)
2	29.70	*t*	
3	89.05	*d*	4.98 (Glc-1′), 1.64 (28), 1.67 (29), 1.20 (5ax), 2.39 (2eq)
4	41.63	*s*	3.53 (3ax), 1.64 (28), 1.67 (29), 1.20 (5ax), 2.39 (2eq)
5	49.58	*d*	1.67 (29), 1.64 (28), 1.46 (19B), 0.42 (19A)
6	65.80	*d*	1.20 (5ax), 1.59 (7β), 2.46, (8β)
7	34.86	*t*	
8	49.58	*d*	1.67 (29), 1.64 (28), 1.46 (19B)
9	19.20	*s*	1.46 (19B), 2.46 (8β)
10	24.20	*s*	1.20 (5ax), 1.46 (19B), 0.42 (19A), 2.46 (8β)
11	26.06	*t*	0.42 (19A)
12	32.91	*t*	1.88 (17α)
13	45.90	*s*	2.46 (8β), 2.29, (15α), 1.88 (17α), 4.39 (16α)
14	46.70	*s*	2.46 (8β), 2.29 (15α)
15	48.67	*t*	0.97 (30), 2.46 (8β)
16	82.21	*d*	4.67 (Glc-1″), 1.88 (17α)
17	57.05	*d*	2.29 (15α), 1.28 (18), 0.99 (21), 4.39 (16α)
18	19.67	*q*	1.88 (17α)
19	32.60	*t*	2.46 (8β)
20	30.00	*d*	1.88 (17α), 0.99(21)
21	17.51	*q*	
22	36.75	*t*	1.88 (17α)
23	25.34	*t*	
24	126.66	*d*	1.68 (27), 1.64 (26)
25	129.89	*s*	1.68 (27), 1.64 (26)
26	25.59	*q*	1.68 (27)
27	17.51	*q*	1.64 (26)
28	24.64	*q*	1.67 (29), 3.53 (3ax)
29	16.69	*q*	3.53 (3ax)
30	20.64	*q*	2.46 (8β), 2.29 (15α)

concd to 200 ml which was added slowly with continuous stirring to H_2O (1 l), left 5 h at room temp. then filtered from precipitated resin. The filtrate was partitioned into petrol, Et_2O, $CHCl_3$, EtOAc and *n*BuOH, successively. The *n*-BuOH soluble fraction (30 g) was chromatographed on silica gel previously wetted with EtOAc. Elution with EtOAc–MeOH yielded 103 mg of a complex mixture which was submitted to HSCCC using a solvent system $CHCl_3$–MeOH–H_2O 5 : 5 : 3, aq. phase as mobile phase, flow rate 1 ml min.$^{-1}$). Fractions (1 ml) were collected according to their composition, affording 7 mg of 3β,6α,16β trihydroxy 9,19 cyclolanost-24 oxo, 25-ene, 3β,16β-diglucopyranoside [11] and 15 mg of **1**.

The elution with increasing amounts of MeOH gave astragaloside II, trigonoside II, trigonoside III [10] and azukisaponon V [11].

*3β,6α,16β trihydroxy 9β,19 cyclolanost-*24*-ene, 3β,16β diglucopyranoside* (**1**). mp 166° (from Et_2O–MeOH); $[\alpha]_D^{25} = -31$ (*c* 1.35, MeOH). FAB MS, *m/z*: 805 $[M+Na]^+$. ^{1}H NMR see Tables 1 and 3; ^{13}C NMR see Table 2.

3β,16β Trihydroxy 6-oxo 9,19 cyclolanost-24-ene 3β,16β diglucopyranoside (20 mg) was treated with excess $NaBH_4$ in EtOH (20 mg in 2 ml), at room temp for 2 h. The soln was evaporated to dryness, diluted with slightly acid brine and extracted with *n*-BuOH. After solvent evaporation, 18 mg of **2** were obtained.

3β,6β,16β trihydroxy 9,19 *cyclolanost*-24-*ene* 3β,16β *diglucopyranoside* (**2**). mp 238–9° (*d*)(from EtOAc–MeOH); $[\alpha]_D^{25} = +27$(*c* 0.54, MeOH). FAB MS *m/z*: 805 $[M+Na]^+$. ^{1}H NMR see Tables 1 and 3; ^{13}CNMR see Table 2.

Molecular modeling. The following strategy was applied for the modelling procedure for compound **2**.

(i) The tridimensional structure of the aglycone was built using a general 3D builder [24]. Several conformers were built and the optimization procedure was performed on each of them.

(2) A search for the rotational minima of the free bonds (through a Monte-Carlo Metropolis procedure)[24] was then performed, via rigid bond rotations.
(3) The minimum energy conformation obtained in point 2 for each conformer was then optimized using a MO semiempirical program [25, 26](AM1).
(4) Starting from the aglycone optimized conformations, the 3β,16β-O-di-glucoside was built, using the 3D builder and treated as described in points 2–3 for the aglycone.

In order to achieve a more complete description of the conformational space of rings B (in particular with respect to the configuration at C-6) and C the dihedral angles $C_5C_6C_7C_8$ and $C_{13}C_{12}C_{11}C_9$ in the most stable conformer were rotated from 60° to $-60°$, with a 20° increment. The semiempirical program AM1 was used at this step.

Calculation and molecular graphics were performed on a IBM Risc/System/6000 (model 520).

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