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CYCLOARTANE TRITERPENE GLYCOSIDES FROM *ASTRAGALUS SIEBERI*§

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Key Word Index—*Astragalus sieberi*; Leguminosae; structural elucidation; 1D- and 2D- gradient enhanced NMR techniques; 20(*S*),24(*R*)-epoxy-9β,19-cyclolanostan-3β,6α,16β,25-tetrol glycosides; sieberoside I and II.

Abstract—Two new cycloartane saponins were isolated from the aerial parts of *Astragalus sieberi*. The structures were elucidated by 1D- and 2D- gradient-enhanced NMR analyses and enzymatic hydrolysis as 20(*S*),24(*R*)-epoxy-9β,19-cyclolanostan-3β,6α,16β,25-tetrol-3-*O*-β-D-glucopyranoside and 20(*S*),24(*R*)-epoxy-9β,19-cyclolanostan-3β,6α,16β,25-tetrol-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

INTRODUCTION

Astragalus species are used in Chinese traditional medicine as an antiperspirant, antihypertensive, diuretic and tonic [1]. Recently many *Astragalus* species have attracted interest because of their cytotoxic constituents [2–4]. In previous publications, phytochemical studies of Egyptian *Astragalus* species resulted in the isolation and structure elucidation of a number of new cycloartane triterpene glycosides [5–11]. Cycloartane saponins isolated from genus *Astragalus* exhibited a wide range of biological properties, including cardiotonic, analgesic, sedative, hepatoprotective, antiviral and immunostimulant activities [5, 12–17]. In this paper we report the isolation and identification of two new cycloartane triterpene glycosides from the aerial parts of *Astragalus sieberi* DC.

RESULTS AND DISCUSSION

An ethanolic extract of the aerial parts of *Astragalus sieberi* was partitioned into ethyl acetate and *n*-butanol fractions. The compounds were purified on silica gel, leading to the isolation of two saponins **1** and **2**.

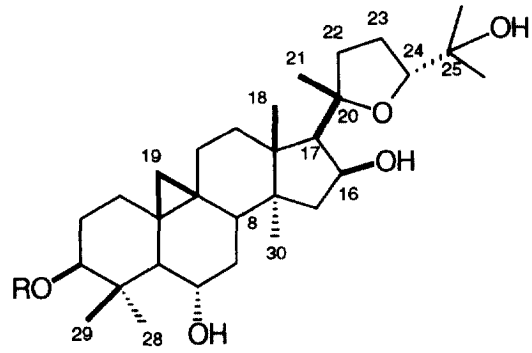
Compound **1**, $C_{36}H_{60}O_{10}$, showed a quasi molecular peak at *m/z* 651 $[M-H]^-$ in the FAB-mass spectrum, while compound **2**, $C_{42}H_{70}O_{15}$, showed a molecular peak at *m/z* 837 $[M+Na]^+$. The ^1H NMR and ^{13}C NMR spectra of the sugar parts identified the presence of a β-glucopyranose unit in compound **1**, while compound **2**, which differs of 162 mass units from **1**, contains the disaccharide sophorose (β-glucopyranosyl(1→2)-β-D-glucopyranose).

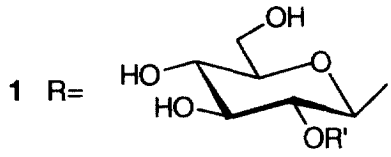
The two compounds **1** and **2** showed superimposable resonances both into the ^1H NMR and ^{13}C NMR spectra, with respect to the terpenoidic part of the two molecules. Signals characteristic of an oxygenated cycloartane triterpenoid could be observed.

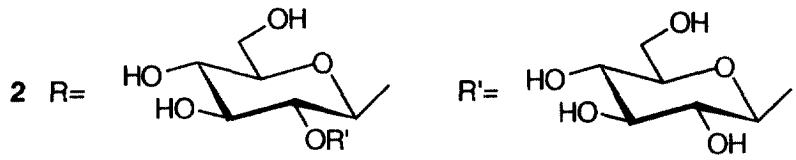
Chemical shifts assignments, coupling constants, sugar connections and position of bonding to the aglycone have been made possible by the combination of 1D- and 2D- gradient-enhanced NMR techniques ^1H-^{13}C GHSQC [18], ^1H-^{13}C GHMBC [19], besides the DQF-COSY [20], E-COSY [21], ROESY [22], 1D- and 2D- TOCSY [23, 24] experiments at 600 MHz (see Experimental). The spectra of compound **2** was fully interpreted through the use of the cited NMR techniques; the resonances in the spectra of **1** were attributed consequently.

Compound **2** showed signals of two cyclopropane protons resonating as doublets at δ 0.56 and 0.21 (connected to a carbon atom resonating at δ 30.4); seven methyl groups as singlets were found at δ 1.96 (correspondent carbon at δ 29.0), 1.65 (carbon resonance at δ 21.2), 1.50 (carbon at δ 28.3), 1.42 (carbon at δ 16.7), 1.33 (δ 26.5), 1.27 (δ 27.0), 1.00 (δ 20.6). Four protons on oxygenated carbons were present,

* Author to whom correspondence should be addressed.
§ This paper is dedicated to the memory of Prof. G. Jommi.







3 R= H

resonating as doublets at δ 4.81 (correspondent carbon at δ 73.0), 3.94 (δ 85.1), 3.74 (δ 67.9) and 3.57 (δ 89.1). The interpretation of the NMR (chemical shifts and coupling constants) and mass spectral data is compatible with the structure of a 20,24-epoxycycloartane-3β,6α,16β,25-tetrol.

Cycloastragenol [20(*R*),24(*S*)-epoxy-9β,19-cyclolanostan-3β,6α,16β,25-tetrol] has been reported to be the epoxycycloartane tetrol commonly found in *Astragalus* species endemic to Egypt [5, 7, 8, 10, 11] and mostly characterizing the *Astragalus* genus [13]. A direct comparison with a pure sample of cycloastragenol 3-*O*-β-D-glucopyranoside was precluded, but the NMR properties of **2** showed differences in the proton and carbon resonances of the tetrahydrofuran ring, with respect to the corresponding part of all the cycloastragenol derived saponins we had so far isolated and characterized from *Astragalus* species [5, 7, 8, 10, 11]. Both configurational or conformational changes could be adduced to explain the differences into the NMR spectra.

Compound **1** was enzymatically hydrolized and the aglycone (**3**) purified and characterized. Its ^{1}H NMR and ^{13}C NMR spectra were superimposable with the corresponding terpenoid part of the saponins **1** and **2**. Conformational preferences of compounds **1** and **2** with respect to the previously isolated cycloastragenol saponins were so far excluded. The physico-chemical (melting point and optical rotation) and spectroscopic properties of **3** again differed from those of cycloastragenol.

Slight spectroscopic NMR differences between compound **3** and cycloastragenol were observed in the side chain resonances while ring A, B, C and D proton and carbon resonances were almost superimposable [10, 25]. Starting from the C-24 resonance (δ 85.0), which has been attributed after the assignment of the other doublets of the molecule, the spin sequence H-24–H-23–H-22 has been assigned through GHSQC, TOCSY and E-COSY experiments. The C-20 resonance (δ 86.5) was confirmed through the long range coupling with H-17ax, CH_3-21 and H-22α. The α- and β-protons were assigned through a ROESY experiment which also allowed identification of spatial coup-

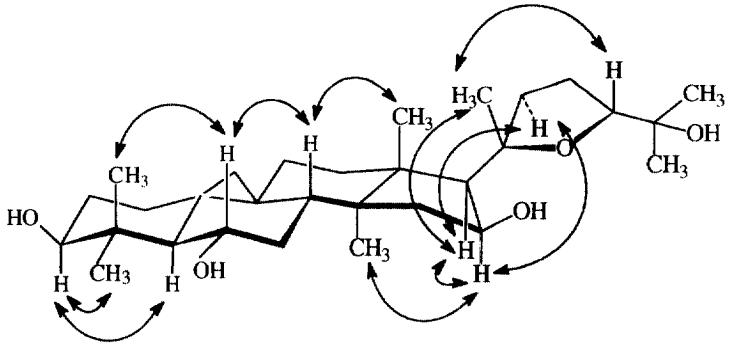


Fig. 1. Significant NOE couplings observed into the ROESY spectrum of compound (3).

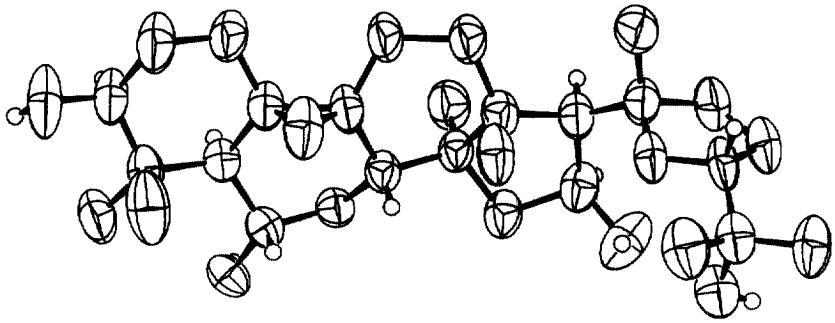


Fig. 2. ORTEP-II (34) plot of (3). H atoms of the methyl and methylene groups are omitted for clarity. Atomic displacement ellipsoids were drawn at 50% of probability level. H atoms not to scale.

lings among protons as depicted in Fig. 1. These couplings were also observed in the cycloastragenol series of saponins and are so far not diagnostic of the absolute stereochemistry of the tetrahydrofuran ring [10, 25].

The absolute stereochemistry of **3** was defined by submitting the compound to X-rays diffraction analysis, which permitted assignment of the structure as 20(*S*),24(*R*)-epoxy-9β,19-cyclolanostan-3β,6α,16β,25-tetrol. This genin was characterized by Russian authors as cyclogalagenin [26]. Any confusion between the 20(*R*), 24(*S*) [cycloastragenol series] and the 20(*S*), 24(*R*) [cycloastragenin series] epoxycyclolanostanols was overcome after assignment of the absolute stereochemistry of cyclogalagenin by X-ray analysis [26] but no NMR data had been described for this terpenoid.

From these considerations, compound **1** was assigned the structure of cyclogalagenin 3-*O*-β-D-glucopyranoside, which we have named sieberoside I, and **2** was assigned the structure of cyclogalagenin 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside, which we have named sieberoside II.

EXPERIMENTAL

Plant material

The aerial parts of *A. sieberi* were collected in March 1995 from 80 km South of El Arish. The plant was previously identified by the late Prof. V. Tackholm (Faculty of Science, Cairo University). A voucher specimen is deposited in the Herbarium of the Faculty of Science, University of Alexandria, Alexandria, Egypt.

General methods

FAB-MS were obtained on a VG 7070 mass spectrometer using glycerol or 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 three-channel Varian Unity-600 spectrometer, operating at 599.919 MHz for ^{1}H and at 150.858 for ^{13}C, employing an actively shielded z gradient and a pulsed field gradient (pfg) accessory, equipped with a pfg triple resonance indirect detection probe (^{1}H {^{13}C,^{15}N}), a waveform generator on all three channels and running the Varian Software Vnmr 5.1B. The 1D experiments were run with the States-Haberkon method [27] and the 2D-ones with collection of 2 sets of data (Hypercomplex [28] or States-TPPI [29] methods), except for the HMBC ones with only one set of data. The 1D and 2D spectral widths used were 6000 Hz (10 ppm) for ^{1}H, 15,300 Hz (100 ppm) for ^{1}H-^{13}C HSQC, 35,000 Hz (230 ppm) for ^{1}H-^{13}C HMBC. The ^{1}H and ^{13}C spectra were referenced to TMS through internal signals: for ^{1}H the residual H^{2,6} of pyridine-d_5 at 8.71 ppm and for ^{13}C the C^{2,6} of

pyridine-d_5 at 149.9 ppm. 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 4k × 2k points. The TOCSY [24] spectra were acquired with an 80 ms mixing time, a MLEV-17 [30] 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 2k × 1k points. The ROESY [22] spectra were acquired with a 300 ms mixing time, a MLEV-17 spin-lock field of 3 kHz obtained with small flip-angle pulses (30°), 2048 points in F2, 256 ÷ 512 complex increments in F1, 8 ÷ 32 scans per increment and a final data matrix of 2k × 1k points. The E-COSY [21] spectra were acquired with 4096 points in F2, 1024 complex increments in F1, 32 scans per increment and a final data matrix of 8k × 4k points. The Gradient-Enhanced ^{1}H-^{13}C HSQC [18] spectra were acquired with a 4: −1 gradient ratio (controlled with 2:0.5 ms gradient duration at ca 20 G cm^{-1}), spectral editing (CH_2 negative, CH/CH_3 positive), 2048 points in F2, 256 complex increments in F1, 1–2 scans per increment, a final data matrix of 2k × 1k points and a MPF7 [31] waveform generator-based ^{13}C-decoupling sequence during the acquisition. The Gradient-Enhanced ^{1}H-^{13}C HMBC [19] spectra were acquired with a 2:2: −1 gradient ratio (controlled with 10:10: −5 G cm^{-1} gradient strength for a duration of 2 ms), 2048 points in F2, 300 × 512 complex increments in F1, 8 scans per increment and a final data matrix of 2k × 2k points. For each sample one HMBC spectrum was optimized for a $^nJ_{C-H}$ of 8 Hz and another one for a $^nJ_{C-H}$ of 4 Hz, with $n = 2 \div 4$. All 2D spectra were in phase sensitive mode and transformed with a cosine squared weight-

Table 1. ^{1}H NMR chemical shifts of compound **3** as determined by E-COSY and ROESY experiments [600 MHz, pyridine-d_5, δ in ppm from internal TMS]

H	δ (ppm)	J (Hz)	Significant cross-peak correlations in the ROESY spectrum
1ax	1.65 *m*	13.5	28
1eq	1.26 *ddd*	13.5, 4.4, 4.3	19A, 11eq
2eq	2.04 *ddd*	12.0, 4.3, 6.6	3ax
2ax	1.95 *m*	12.0	19A
3ax	3.66 *ddd*	11.5, 7.8, 6.6	1ax, 2eq, 5ax
5ax	1.73 *d*	9.5	3ax
6ax	3.80 *tdd*	9.5, 7.4, 6.1	19B, 29, 7ax, 8ax
7ax	1.81 *m*		30
7eq	1.67 *m*	9.5	
8ax	2.00 *dd*	7.9, 4.6	19B, 18
11eq	2.00 *ddd*	12.9, 6.0, 5.4	1eq
11ax	1.26 *ddd*	12.9, 4.8	19A
12eq	1.85 *ddd*	13.0, 9.6, 6.0	17ax
12ax	1.73 *ddd*	13.0, 5.4, 4.8	30, 11ax, 21
15α	2.14 *dd*	13.2, 7.7	16ax, 30
15β	1.81 *dd*	13.2, 5.8	18
16ax	4.82 *tdd*	7.7, 7.9, 5.5	22α, 17ax, 15α, 30
17ax	2.25 *d*	7.7	16ax, 30, 21
18	1.68 *s*		8ax, 15α
19B	0.63 *d*	5.0	6ax, 8ax, 29
19A	0.34 *d*	5.0	29, 2eq
21	1.36 *s*		17ax
22α	2.50 *ddd*	12.0, 8.0, 7.6	
22β	1.69 *ddd*	12.0, 4.7, 4.1	
23α	2.24 *dddd*	12.0, 7.6, 6.3, 4.7	24β, 12ax, 27 or 26
23β	1.90 *ddt*	12.0, 8.0, 7.5	
24β	3.96 *dd*	7.5, 6.3	22β, 23α, 23β, 26, 27, 21
26	1.51* *s*		24β
27	1.29* *s*		24β
28	1.89 *s*		3ax
29	1.35 *s*		19B
30	1.01 *s*		16ax, 12ax, 17ax
16OH	5.87 *d*	5.5	
3OH	5.68 *d*	7.8	
6OH	5.21 *d*	6.1	

* Interchangeable values.

ing function in both dimensions, except for the HMBC ones which were in magnitude mode and transformed with a sinebell weighting function in both dimensions. Selective excitation spectra, 1D-TOCSY [23], were acquired using waveform generator-based BURP [32] shaped pulses with 90° flip-angle, mixing times ranging from 80 to 150 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2 ms trim pulse. The repetition rates for all the previous kind of spectra were 1.5÷2 s. All the shaped pulses were created with the software Pbox 5.2 (Pandora's Box, by E. Kupce and R. Freeman) available in the Varian Software Users Library.

Extraction and isolation

The air-dried powdered aerial parts of *Astragalus sieberi* (500 g) were extracted by maceration with 95% EtOH. The extract was concentrated to 100 ml which was added slowly, with continuous stirring to hot water (250 ml), left for 5 h, then filtered from precipitated resin. The filtrate was partitioned into petrol, Et_2O, $CHCl_3$, EtOAc and *n*-BuOH, successively. The EtOAc soluble fraction (12 g) was chromatographed on silica gel column, eluted with $CHCl_3$–MeOH mixtures and yielded **1** (sieberoside I) (80 mg, 85:15). The *n*-BuOH soluble fraction (8 g) was chromatographed on silica gel column. Elution with EtOAc–MeOH mixtures yielded **2** (sieberoside II) (30 mg, 4:1).

Compound 1 (Sieberoside I). White needles from MeOH; mp 177°; $[\alpha]_D^{25} = +29.9$ (pyridine, *c* 0.62). FAB MS (glycerol, negative mode) *m/z*: 651 $[M-H]^-$, 489 $[M-162-H]^-$. ^1H NMR: δ 4.76 (H-16ax), 3.95 (H-24β), 3.74 (H-6ax), 3.65 (H-3ax), 2.47 (H-22α+H-2eq), 2.22 (H-17ax), 2.00 (CH_3-28), 1.63 (CH_3-18), 1.51 (CH_3-26 or 27), 1.35 (CH_3-29), 1.30 (CH_3-21), 1.28 (CH_3-27 or 26), 1.10 (H-1eq), 0.98 (CH_3-30), 0.54 (H-19B), 0.22 (H-19A). ^{13}C NMR see Table 2. Sugar part see Table 3.

Compound 2 (Sieberoside II). Pale yellow needles from MeOH; mp 250°; $[\alpha]_D^{25} = -82$ (MeOH, *c* 0.5). FAB MS (NBA, positive) *m/z*: 837 $[M+Na]^+$. ^1H

Table 2. ^{13}C NMR assignments for compounds **1**–**3** and ^BC—H connectivities as determined by the GHMBC experiments

Carbon	**1** δ (ppm)	**2**	**3**	Connected protons
1	32.3	32.5	32.5 *t*	2eq, 2ax, 5, 19B, 19A
2	30.5	30.4	31.3 *t*	
3	88.9	89.1	78.3 *d*	2eq, 28, 5, 29, 1′ (only in **1** and **2**)
4	42.4	42.9	42.2 *s*	2eq, 28, 5, 29
5	53.8	54.2	53.9 *d*	28, 29, 19B, 19A
6	67.9	67.9	68.3 *d*	8, 7eq
7	38.9	38.6	38.6 *t*	8, 5
8	46.6	46.9	47.0 *d*	18, 30, 19B, 19A
9	20.8	21.2	20.6 *s*	8, 11ax, 19B, 19A
10	29.3	29.5	29.4 *s*	11eq, 5, 19B, 19A
11	26.4	26.6	26.3 *t*	17, 19B, 19A
12	33.5	33.9	33.6 *t*	17, 18
13	46.3	46.6	46.5 *s*	16, 17, 15eq, 7ax
14	46.6	47.0	46.1 *s*	8
15	48.6	49.0	48.9 *t*	8, 30, 16OH
16	72.7	73.0	72.9 *d*	17, 15ax, 16OH
17	56.2	56.6	56.4 *d*	15eq, 18, 21
18	21.0	21.2	21.0 *q*	11eq
19	30.0	30.4	30.8 *t*	8, 5
20	86.5	86.8	86.5 *s*	22α, 17, 21
21	26.1	26.5	26.1 *q*	
22	37.3	37.7	37.3 *t*	21
23	24.1	24.5	24.1 *t*	22α
24	85.5	85.1	85.0 *d*	23α, 22β, 26, 27
25	70.2	70.4	70.0 *s*	24, 23α, 26, 27
26	27.8*	28.3*	27.9* *q*	24, 27
27	26.6*	27.0*	26.8* *q*	
28	28.8	29.0	29.0 *q*	3, 29
29	16.5	16.7	15.9 *q*	3, 28, 5
30	20.3	20.6	20.2 *q*	15eq

* Interchangeable values.

Table 3. ^1H and ^{13}C NMR assignments (in ppm, J (Hz), significant ^nH—C as determined from a HMBC experiment) of the sugar moieties of compounds **1** and **2**

	Carbon	Proton	J (Hz)	Significant ^nH—C carbon connections
Compound **1**				
3-*O*-Glu				
1′	106.7	4.96 *d*	7.4	C-3
2′	75.6	4.05 *dd*	9.4, 7.4	
3′	78.4	4.22 *t*	9.4	
4′	71.5	4.18 *m*		
5′	77.9	3.94 *m*		
6′	62.7	4.52A *dd*	12.0, 3.3	
6′		4.38B *dd*	12.0, 5.6	
Compound **2**				
3-*O*-Glu				
1′	105.1	4.97 *d*	8.1	C-3
2′	83.7	4.24 *t*	9.0	
3′	78.5	4.26 *m*		
4′	71.7	4.16 *m*		
5′	78.1	3.86 *m*		
6′	63.0	4.52A *dd*	10.2, 7.0	
		4.35B *dd*	10.2, 3.3	
2′-*O*-Glu				
1″	106.2	5.38 *d*	8.1	C-2′
2″	77.2	4.12 *t*	9.1	
3″	78.2	4.22 *t*	7.9	
4″	72.0	4.29 *m*		
5″	78.3	3.94 *m*		
6″	63.0	4.48A *dd*	10.5, 7.7	
		4.42B *dd*	10.5, 3.0	

NMR: δ 4.81 (H-16ax), 3.94 (H-24β), 3.74 (H-6ax), 3.57 (H-3ax), 2.49 (H-22α), 2.42 (H-2eq), 2.24 (H-17ax), 2.23 (H-23β), 2.13 (H-15eq), 1.97 (H-8ax), 1.96 (CH_3-28), 1.94 (H-2ax), 1.90 (H-11eq), 1.90 (H-23α), 1.82 (H-12ax), 1.80 (H-15ax), 1.79 (H-7eq), 1.70 (H-12eq), 1.68 (H-22β), 1.67 (H-5ax), 1.65 (CH_3-18), 1.62 (H-7ax), 1.51 (H-1ax), 1.50 (CH_3-26 or 27), 1.42 (CH_3-29), 1.33 (CH_3-21), 1.27 (CH_3-27 or 26), 1.22 (H-11ax), 1.10 (H-1eq), 1.00 (CH_3-30), 0.56 (H-19B), 0.21 (H-19A). ^{13}C NMR see Table 2. Sugar part see Table 3.

Compound 3 (cyclogalagenin). Compound **3** was obtained through enzymatic hydrolysis from sieberoside I (**1**) (130 mg), which was treated with β-glucuronidase from *Helix pomatia* (art. 1.04114.0002 Merck, 4 ml) at 38° for 7 days. The mixture was extracted with $CHCl_3$–MeOH (9:1) and purified on silica gel (5 g), eluted with $CHCl_3$–MeOH 93:7, 48 mg of **3** was obtained and crystallized from EtOAc (X-rays) or MeOAc. mp 200–1°; $[\alpha]_D^{25} = +38$ (MeOH, *c* 0.66); CIMS *m/z*: 491 $[M+H]^+$; ^1H and ^{13}C NMR see Tables 1 and 2.

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