# TRITERPENE GLYCOSIDES FROM *Astragalus*. STRUCTURE OF CYCLOUNIFOLIOSIDE B FROM *Astragalus unifoliolatus*

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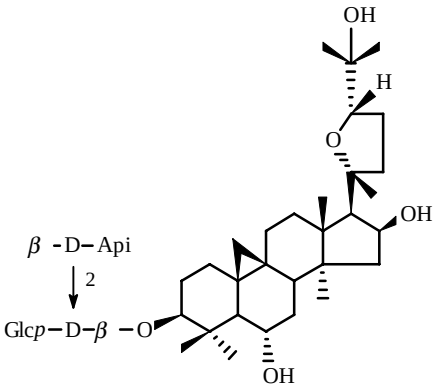
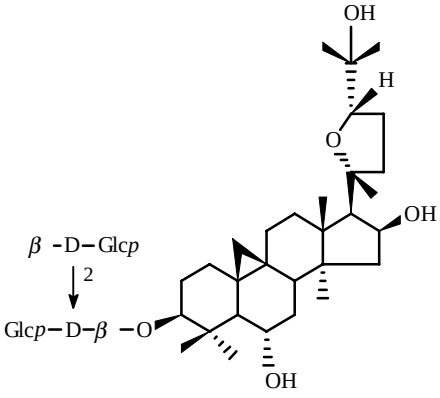
*The previously known astrailienin A and the new cycloartane glycoside cyclounifolioside B with structure cyclosiversigenin 3-O-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranoside were isolated from* Astragalus unifoliolatus *Bunge. The structures of these compounds were established using chemical transformations and two-dimensional spectra (ROESY, HMBC, HSQC, TOCSY, COSY).*

**Key words:** *Astragalus unifoliolatus* Bunge, triterpene glycoside, cycloartane.

In continuation of the study of triterpene glycosides of the cycloartane series from *Astragalus* species [1], we investigated the content of these compounds in the aerial part and roots of *Astragalus unifoliolatus* Bunge [2]. The known compound astrailienin A (cycloaraloside C) (**1**) [3, 4] was isolated from the roots; the new cycloartane glycoside cyclounifolioside B (**2**), from the aerial part. Results of the structure determination of these glycosides are presented here.




**1**




**2**

**Astrailienin A (1).** $^{13}$C NMR spectrum of **1** contains 41 signals, of which 7 belong to $CH_3$; 12 to $CH_2$, 14 to CH, and 8 to quaternary C atoms. The proton spectrum contains two signals with chemical shifts 0.21 and 0.51 ppm characteristic of cycloartane triterpenoids and was solved using the two-dimensional methods COSY and TOCSY.

Analysis of the spectra has shown that the carbohydrate part consists of two sugar units, one of which is glucose; the other, a furanose sugar, the protons of which form three isolated AB (AX) spin systems. Such a proton spectrum is typical of an apiose where the spin systems mentioned above belong to H-1 and H-2 protons and those of the O–$CH_2$ groups on C-4 and C-5. The assignment of these signals was confirmed by analyzing the HSQC and HMBC spectra. As supposed, one of the AX systems belongs to an anomeric proton at 6.53 ppm ($^{13}$C, 111.24 ppm) and an H-2 proton at 4.93 ppm ($^{13}$C, 78.73 ppm). The two remaining AX systems belong to protons of two $CH_2$ groups. The C-3 signal is found by analyzing the HMBC spectrum

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owing to the presence in it of correlations for C-5 and H-4.

The HSQC spectrum has shown that the glucose C-2 signal is found at weaker field than that in the unsubstituted unit. Furthermore, analysis of the $^{13}C$ and $^{1}H$ chemical shifts indicated that the aglycone is cyclosiversigenin (**3**) substituted at 3-OH [1]. The sequence of sugar bonding was found by analyzing the ROESY and HMBC spectra. The ROESY spectrum exhibits at the chemical shift of the anomeric glucose proton both intra-sugar correlations (H-1—H-2, H-1—H-3, and H-1—H-5) and a transglycoside correlation of H-1 with H-3 of the aglycone.

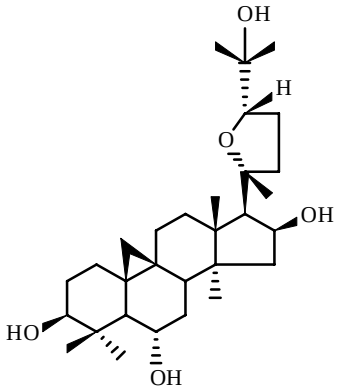
The anomeric proton of the apiose shows no correlations with the glucose ring protons. However, the ROESY spectrum shows coupling with protons of the aglycone methyls $CH_3$-29 and $CH_3$-30. Its nature could be found by analyzing the HMBC spectrum, which showed a correlation of glucose H-2 and apiose C-1. Therefore, the disaccharide $\beta$-D-Api(1→2)-$\beta$-D-Glc*p* is bonded to C-3 of the aglycone.

The appearance of a correlation peak between H-2 and H-5 in the ROESY spectrum is very important to determining the absolute configuration of the apiose. It indicates that they are *cis* to each other in the five-membered ring.

Thus, **1** has the structure cyclosiversigenin 3-O-$\beta$-[D-apiofuranosyl(1→2)]$\beta$-D-glycopyranoside. It should be noted that the compound has the same structure as that which was isolated previously and characterized by two independent groups, i.e., astrailienine A from roots of *Astragalus iliensis* [3] and cycloaraloside C from roots of *Astragalus amarus* Pall. [4].

**Cyclounifolioside B (2).** The $^{1}H$ NMR spectrum of **2** has 1H doublets at strong field (0.22 and 0.56 ppm) that are split into an AB system, which unambiguously assigns them to methylene H of a cyclopropane ring, and clearly resolved resonances of seven methyls. A band at 2935 $cm^{-1}$ in the IR spectrum also confirms the presence of a three-membered ring. C atoms C-9, C-10, and C-19 of the cyclopropane ring resonate in the $^{13}C$ NMR spectrum at 21.02, 29.42, and 30.21 ppm, respectively. This indicates that **2** is a cycloartane-type triterpene.

Acid hydrolysis of **2** produced the genin, which was identified using spectral and literature data as cyclosiversigenin (**3**) [1]. Paper chromatography of the hydrolysate detected D-glucose by comparison with authentic samples. Analysis of the chemical shifts of C atoms in the $^{13}C$ NMR spectra of **2** and **3** showed that the OH on C-3 was affected by glycosylation. The signal of anomeric C-1″ shifted to strong field. This indicates that this D-glucopyranose is bonded to C-2′ of the D-glucopyranose that is bonded to the OH on C-3 of the aglycone [5, 6].




**3**

The $^{1}H$ NMR spectrum of **2** contains signals of two anomeric protons that resonate at 4.99 and 5.42 ppm. Two anomeric C atoms resonate at 105.05 and 106.12 ppm in the $^{13}C$ NMR spectrum. The spectrum was solved using two-dimensional spectroscopy (COSY, TOCSY, and HSQC). It has been found that both monosaccharides are $\beta$-D-glucopyranoses. The signal for C-2 of one of these occurs at weaker field than that of the unsubstituted unit.

The $^{1}H$ and $^{13}C$ chemical shifts have shown that the aglycone is **3** (20R,24S-epoxycycloartan-3$\beta$,6$\alpha$,16$\beta$,25-tetraol) [1] substituted at the 3-OH. The sequence of units and their bonding to C-3 of the aglycone were determined based on ROESY and HMBC spectra. Thus, the anomeric proton of unit A at 5.42 ppm correlates with H-2 of unit B (4.30 ppm). Atom C-2 of unit B (83.61 ppm) and its anomeric proton (4.99 ppm) correlate with H-3 (3.58 ppm) and C-3 (88.93 ppm) of the aglycone in the ROESY and HMBC spectra, respectively. These data confirm that **2** is a bioside.

Thus, the structure of **2** is determined as 20R,24S-epoxycycloartan-3$\beta$,6$\alpha$,16$\beta$,25-tetraol 3-O-[$\beta$-D-glucopyranosyl-(1→2)]-$\beta$-D-glucopyranoside.

TABLE 1. Chemical Shifts of $^1H$ and $^{13}C$ NMR Signals of Cyclounifolioside B (**2**) (δ, ppm, 0 = TMS, $C_5D_5N$)

| Atom | Chemical shifts | | Atom | Chemical shifts | |
|---|---|---|---|---|---|
| | $^{13}C$ | $^1H$ | | $^{13}C$ | $^1H$ |
| 1 | 32.38 | 1.51 1.11 | | *β*-D-Glc*p*-(1→2)Glc*p* | |
| 2 | 30.62 | 2.43 1.94 | 1 | 106.12 | 5.42 |
| 3 | 88.93 | 3.58 | 2 | 77.05 | 4.13 |
| 4 | 42.76 | - | 3 | 78.15 | 4.24 |
| 5 | 53.97 | 1.66 | 4 | 71.88 | 4.30 |
| 6 | 67.80 | 3.73 | 5 | 78.08 | 3.95 |
| 7 | 38.51 | 1.82 1.64 | 6 | 62.95 | 4.50; 4.44 |
| 8 | 46.81 | 1.95 | | →2)-*β*-D-Glc*p*(1→3)Agl | |
| 9 | 21.02 | - | 1 | 105.05 | 4.99 |
| 10 | 29.42 | - | 2 | 83.61 | 4.30 |
| 11 | 26.28 | 1.90 1.21 | 3 | 78.42 | 4.33 |
| 12 | 33.47 | 1.68 1.60 | 4 | 71.67 | 4.18 |
| 13 | 45.10 | - | 5 | 77.95 | 3.88 |
| 14 | 46.19 | - | 6 | 62.89 | 4.53; 4.37 |
| 15 | 46.66 | 2.13 1.77 | | | |
| 16 | 73.47 | 5.03 5.65 (-OH) | | | |
| 17 | 58.40 | 2.54 | | | |
| 18 | 21.44 | 1.43 | | | |
| 19 | 30.21 | 0.56 0.22 | | | |
| 20 | 87.28 | - | | | |
| 21 | 28.59 | 1.32 | | | |
| 22 | 34.96 | 3.10 1.68 | | | |
| 23 | 26.47 | 2.31 2.05 | | | |
| 24 | 81.75 | 3.89 | | | |
| 25 | 71.28 | - | | | |
| 26 | 28.21 | 1.58 | | | |
| 27 | 27.15 | 1.30 | | | |
| 28 | 20.16 | 1.02 | | | |
| 29 | 28.87 | 1.96 | | | |
| 30 | 16.59 | 1.44 | | | |

## EXPERIMENTAL

Silica gel containing 10% gypsum and Silufol plates were used for TLC; silica gel (KSK, 0.1-0.08 and 0.16-0.1 mm), for column chromatography. Cycloartanes and their derivatives were detected on TLC using methanolic phosphotungstic acid (20%) with heating at 120°C for 5-10 min. IR spectra were recorded on a Perkin—Elmer System 2000 FT-IR in KBr pellets; NMR spectra, in a Bruker DRX-500 spectrometer for glycosides in deuteropyridine at 30°C with TMS internal standard. Two dimensional spectra were recorded using standard Bruker methods. The delay time for recording TOCSY and ROESY spectra was 0.2 sec.

Paper chromatography was performed on FN-11 paper.

The following solvent systems were used: $CHCl_3$—$CH_3OH$—$H_2O$ (70:23:3, 1), $CHCl_3$—$CH_3OH$ (25:1, 2), *n*-butanol—$C_5H_5N$—$H_2O$ (6:4:3, 3).

**Isolation of Astrailienin A (1).** Air-dried and ground roots (2.2 kg) of *A. unifoliolatus* Bunge were collected in May 1998 at Berdakh collective farm in Amudar′inskii Region of the Karakalpakstan Republic. The starting material was extracted with methanol (5×5 L). The methanol extract was condensed and diluted with water. The remaining methanol was distilled in a rotary evaporator. Polar and slightly polar cycloartanes were separated by extracting the aqueous remainder with ethylacetate and then butanol. The solvents were evaporated in vacuum to afford butanol (12.93 g) and ethylacetate (30 g)

fractions. The butanol fraction was chromatographed over a column using system 1 to afford **1** (50 mg, 0.0023%), $C_{41}H_{68}O_{14}$, mp 228-230°C (methanol). IR spectrum (KBr, ν, $cm^{-1}$): 2939 ($CH_2$ of cycloartane ring), 3478 (OH).

PMR spectrum (δ, ppm, $C_5D_5N$, TMS): 1.50, 1.08 (H-1), 2.42, 1.87 (H-2), 3.58 (H-3), 1.66 (H-5), 3.74 (H-6), 1.82, 1.63 (H-7), 1.90 (H-8), 1.87, 1.17 (H-11), 1.66, 1.58 (H-12), 2.12, 1.73 (H-15), 5.03, 5.65 (H-16), 2.55 (H-17), 1.42 (H-18), 0.21, 0.54 (H-19), 1.32 (H-21), 3.10, 1.66 (H-22), 2.32, 2.09 (H-23), 3.88 (H-24), 1.58 (H-26), 1.30 (H-27), 1.02 (H-28), 2.00 (H-29), 1.42 (H-30); β-D-Api: 6.53 (H-1), 4.93 (H-2), 4.76, 4.41 (H-4), 4.32, 4.26 (H-5); β-D-Glc: 4.98 (H-1), 4.19 (H-2), 4.24 (H-3), 4.12 (H-4), 3.86 (H-5), 4.51, 4.33 (H-6).

$^{13}C$ NMR spectrum: 32.74 (C-1), 30.29 (C-2), 88.94 (C-3), 42.68 (C-4), 54.08 (C-5), 67.98 (C-6), 38.62 (C-7), 46.97 (C-8), 20.92 (C-9), 29.48 (C-10), 26.24 (C-11), 33.45 (C-12), 45.08 (C-13), 46.18 (C-14), 46.71 (C-15), 73.47 (C-16), 58.41 (C-17), 21.51 (C-18), 30.46 (C-19), 87.28 (C-20), 28.58 (C-21), 34.96 (C-22), 26.46 (C-23), 81.76 (C-24), 71.27 (C-25), 28.20 (C-26), 27.15 (C-27), 20.18 (C-28), 28.86 (C-29), 16.65 (C-30); (β-D-Api(→2) 111.24 (C-1′), 78.23 (C-2′), 80.66 (C-3′), 75.66 (C-4′), 66.24 (C-5′); (→2)β-D-Glc*p*(1→3)Agl); 105.57 (C-1″), 79.51 (C-2″), 78.82 (C-3″), 72.09 (C-4″), 77.96 (C-5″), 62.98 (C-6″).

**Isolation of Cyclounifolioside B (2).** The dried aerial part of *A. unifoliolatus* Bunge was extracted with methanol (5×6 L). Workup by the aforementioned method afforded butanol (42 g) and ethylacetate (130.63 g) fractions. Chromatographic separation of the butanol fraction over a column with elution by system 1 isolated **2** (400 mg, 0.018%), $C_{42}H_{70}O_{15}$, mp 210-215°C. IR spectrum (KBr, ν, $cm^{-1}$): 2935 ($CH_2$ of cyclopropane ring), 3487 (OH). $^1H$ and $^{13}C$ NMR are listed in Table 1.

**Acid Hydrolysis.** Compound **2** (100 mg) was hydrolyzed in methanolic $H_2SO_4$ (15 mL, 0.25%) at 70°C for 4 h. The reaction mixture was cooled and diluted with water (25 mL). The methanol was distilled. The precipitate was filtered off, washed with water, dried, and chromatographed over a silica-gel column with elution by system 2 to afford **3** (23 mg), $C_{30}H_{50}O_5$, mp 240-242°C (methanol). IR spectrum (KBr, ν, $cm^{-1}$): 3396 (OH), 3037 (cyclopropane).

The hydrolysate was neutralized with $BaCO_3$ and evaporated. Paper chromatography using system 3 detected D-glucose by comparison with authentic samples.

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