Chemistry of Natural Compounds, Vol. 32, No. 5, 1996

TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. LIV. ASKENDOSIDE G FROM *Astragalus taschkendicus*

M. I. Isaev

UDC 547.918:547.926

Chromatography of the polar fraction of a methanolic extract of the roots of Astragalus taschkendicus *Bunge (Leguminosae) has given a new triterpene glycoside of the cycloartane series, askendoside G, having the structure of 24R-cycloartane-3β,6α,16β,24,25-pentaol 3-O-[(α-L-arabinopyranosyl)-(1→2)-β-D-xylopyrano-side] 16-O-β-D-glucopyranoside.*

Continuing investigations of the cycloartane methylsteroids of plants of the *Astragalus* genus (Leguminosae), we have isolated from the roots of *Astragalus taschkendicus* yet another new glycoside, which we have called askendoside G (**1**). The determination of the structure of this glycoside is described in the present paper.

The results of a consideration of the ^{1}H NMR spectrum of the glycoside under study (**1**), which contained one-proton doublets of an *AB* system at 0.15 and 0.38 ppm, and also the presence of the signals of seven methyl groups in the high field, permitted us to assign askendoside G to the triterpene glycosides of the cycloartane series [1, 2].

Acid hydrolysis of askendoside G led to the genin (**2**), which was identified as cycloasgenin C [3]. By PC in the presence of authentic specimens we detected *D*-glucose, *D*-xylose, and *L*-arabinose in the carbohydrate fraction of the hydrolysate. The ^{1}H and ^{13}C NMR spectra of glycoside (**1**) (Table 1) showed that askendoside G contained one molecule of each monosaccharide and, consequently, was a trioside.

A comparative analysis of the ^{13}C NMR spectra of cycloasgenin C and askendoside G showed the bisdemosidic nature of the new glycoside: the C-3 and C-16 atoms in the spectrum of askendoside G had undergone downfield shifts in comparison with cycloasgenin C and resonated at 88.59 and 83.14 ppm, respectively. The signals of the corresponding atoms of cycloasgenin C were observed at 78.37 and 71.78 ppm.

The Smith degradation [4] of askendoside G led to the formation of the nor- compound (**3**). This product was identical with a tetraol that we had obtained previously from cycloasgenin C and cyclocanthogenin [5]. The formation of tetraol (**3**) from glycoside (**1**) on Smith degradation confirmed the conclusion that one of the carbohydrate chains was located at C-16 [6-8].

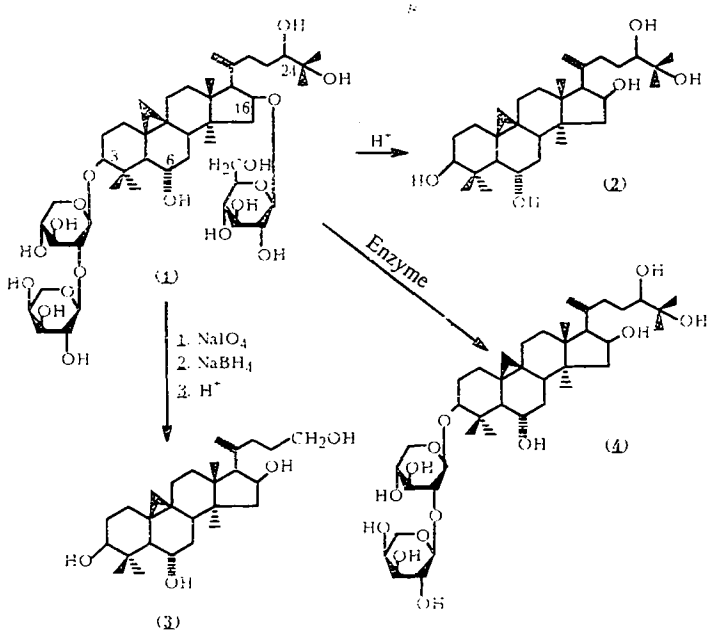
The enzymatic hydrolysis of askendoside G with the gastric juice of the grape snail (*Helix pomatia*) gave bioside (**4**), identical with askendoside C [9]. This means that the *D*-glucose was attached to the genin through the hydroxy group at C-16.

The anomeric proton of the *D*-glucose residue resonated in the form of a doublet with the SSCC ^{3}J = 8 Hz at 4.69 ppm and showed that the hexose had the pyranose form, the ^{4}C$_1$ conformation, and the β- configuration. This conclusion found confirmation in the magnitudes of the chemical shifts of the carbon atoms of the *D*-glucose residue in the ^{13}C NMR spectrum of askendoside G.

Thus, we have grounds for concluding that askendoside G has the structure of 24R-cycloartane-3β,6α,16β,24,25-pentaol 3-O-[(α-*L*-arabinopyranosyl)-(1→2)-β-*D*-xylopyranoside] 16-O-β-*D*-glucopyranoside.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 723-727, September-October, 1996. Original article submitted May 1, 1996.

 0009-3130/96/3205-0706$15.00



EXPERIMENTAL

For **general observations**, see [10]. The following solvent systems were used: 1) *n*-butyl alcohol–pyridine–water (6:4:3); 2) chloroform–methanol–water (70:23:4); 3) chloroform–methanol (10:1).

For the TLC and CC conditions, see [10]. Paper chromatography was conducted on type FN-11 paper in the descending variant using system 1. In PC, the substances were revealed by spraying with aniline phthalate followed by heating at 100-110°C for 5-10 min.

^1H and ^{13}C NMR spectra were taken on Bruker AM 400 and Tesla BS-567 A spectrometers in deuteropyridine. ^{13}C NMR spectra were recorded with complete and partial suppression of interactions with protons and also under J-modulation conditions.

Isolation and separation of the triterpenoids of *Astragalus taschkendicus* Bunge are described in [3, 11]. The fractions eluted after askendoside F and containing askendoside G were combined and rechromatographed on a column in system 2, giving 500 mg of askendoside G (yield 0.0095% of the air-dry raw material).

Askendoside G (1), $C_{46}H_{78}O_{18}$, mp 273-275° (from MeOH), $[\alpha]_D^{20}$+ 11±2° (c 0.9; C_5H_5N). IR spectrum (KBr, ν, cm^{-1}): 3348 (OH). PMR spectrum (100 MHz, C_5D_5N, δ, ppm, 0-HMDS, J, Hz): 0.15 and 0.38 (2H-19, d, ^2J = 4 Hz), 0.87 (CH_3-21, d, ^3J = 6 Hz), 0.83; 1.08; 1.30; 1.32; 1.36; 1.84 (6 × CH_3, s), 4.69 (H-1 of *D*-glucopyranose, d, ^3J = 8 Hz), 4.81 (H-1 of *D*-xylopyranose, d, ^{13}J = 7 Hz), 5.08 (H-1 of *L*-arabinopyranose, d, ^3J = 7 Hz). For the ^{13}C NMR spectrum see Table 1.

Cycloasgenin (2) from (1). Askendoside G (100 mg) was hydrolyzed with 12 ml of 0.5% methanolic sulfuric acid at the boiling point of the reaction mixture for 6 h. The solution was diluted with water, and the methanol was evaporated off. The precipitate that had deposited was filtered off, dried, and chromatographed on a column with elution by system 3. This led to the isolation of 22 mg of the genin (**2**), mp 244-246°C (from Me_2CO), $[\alpha]_D^{20}$+ 34±2° (*c* 1.0, MeOH), identified as cycloasgenin C [3]. For the ^{13}C NMR spectrum, see Table 1.

The filtrate was evaporated to a volume of 10 ml and was boiled. The residue after neutralization of the solution with type ARA-8p anion-exchange resin and evaporation was chromatographed on paper in system 1 in the presence of authentic specimens of monosaccharides. PC revealed the presence of *D*-glucose, *D*-xylose, and *L*-arabinose in the carbohydrate fraction of the hydrolysate. The ^1H and ^{13}C NMR spectra (see Table 1) showed that askendoside G contained these monosaccharides in a ratio of 1:1:1.

25-Norcycloartane-3β,6α,16β,24-tetraol (3) from (1). To 100 mg of askendoside G in 12 ml of methanol was added a solution of 200 mg of sodium periodate in 2 ml of water, and the reaction mixture was stirred at room temperature for 4 h.

TABLE 1. Chemical Shifts of the Carbon Atoms of Compounds (1), (2) and (4) (δ, ppm, 0 – TMS, C_5D_5N)

C atom	Compound		
	1	2	4
1	32.64[a]	32.81	32.45
2	29.21	31.45	29.13
3	88.59	78.37	88.44
4	42.80	42.44	42.67
5	54.01	54.01	53.98
6	67.98	68.31	67.91
7	38.40	38.62	38.33
8	46.84	47.24	46.92
9	21.29*	21.31	21.25
10	30.33[b]	30.37	30.29
11	26.30[c]	26.40	26.25
12	34.44	33.23	33.11
13	45.57	45.74	45.61
14	46.84	46.98	46.78
15	47.73	48.81	48.55
16	83.14	71.78	71.67
17	57.52	57.27	57.20
18	18.08	18.81	18.71
19	30.33[b]	29.36	29.95
20	31.97	31.64	31.51
21	19.05	19.09	18.92
22	30.33[b]	29.62	29.33
23	32.64[a]	34.84	34.78
24	80.00	80.59	80.42
25	72.76	72.69	72.68
26	25.40*	25.90*	25.81*
27	26.30*[c]	26.20*	26.01*
28	20.17	20.30	20.14
29	28.69	29.37	28.54
30	16.36	16.13	16.18
	β-*D*-Xyl_p residue		
1	105.70		105.53
2	83.66		83.47
3	77.76		77.48
4	71.04		70.25
5	66.71		66.48
	α-*L*-Ara_p residue		
1	106.74[d]		106.52
2	73.65		73.51
3	74.32		74.16
4	69.17		69.02
5	67.08		66.90
	β-*D*-Glc_p residue		
1	106.74[d]		
2	75.82		
3	78.81		
4	71.79		
5	78.13		
6	62.90		

Signals marked with the same letters are superposed on one another, while the assignment of those with asterisks is uncertain.

The excess of oxidant was destroyed with a few drops of ethylene glycol. The reaction mixture was diluted with water and was treated with chloroform. The chloroform extract was evaporated and the residue was dissolved in 15 ml of methanol. This solution was treated with 200 mg of sodium tetrahydroborate in small portions, and the mixture was left at room temperature for 2 h and was then acidified by the addition of 0.5 ml of concentrated sulfuric acid.

The acid solution was left for a day at room temperature and it was then diluted with water and extracted with chloroform. The residue after evaporation of the chloroform extract was chromatographed on a column in system 3. This gave 15 mg of the nor- product (**3**), $C_{27}H_{46}O$, mp 192-194°C (from EtOH) $[\alpha]_D^{20}$+ 43±2° (*c* 0.5, MeOH), identical with the analogous tetraol described in [5, 8].

Enzymatic Hydrolysis of Askendoside G. A solution of 25 mg of glycoside (**1**) in 25 ml of water was treated with 15 mg of freeze-dried gastric juice of the grape snail and with one drop of benzene, and the mixture was incubated at 37°C

for 30 days. After this, the solvents were evaporated off, and the residue was chromatographed on a column with elution by system 2. This led to the isolation of 12 mg of glycoside (**4**), $C_{40}H_{68}O_{13}$, mp 197-198°C (from MeOH), $[\alpha]_D^{20}$+ 27±2° (*c* 0.9; MeOH), identified as askendoside C [9]. For the ^{13}C NMR spectrum, see Table 1.

REFERENCES

1. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 431 (1985).
2. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 156 (1989).
3. M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, and N. K. Abubakirov, Khim. Prir. Soedin., 458 (1982).
4. M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, J. Am. Chem. Soc., **74**, 4970 (1952).
5. Yu. M. Fadeev, M. I. Isaev, Yu. A. Akimov, P. K. Kintya, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 817 (1987).
6. T. V. Ganenko, M. I. Isaev, V. I. Lutskii, A. A. Semenov, N. D. Abdullaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 66 (1986).
7. T. V. Ganenko, M. I. Isaev, A. S. Gromova, N. D. Abdullaev, A. A. Semenov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 341 (1986).
8. Yu. M. Fadeev, M. I. Isaev, Yu. A. Akimov, P. K. Kintya, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 73 (1988).
9. M. I. Isaev, M. B. Gorovits, T. T. Gorovits, N. D. Abdullaev, and N. K. Abubakirov, Khim. Prir. Soedin., 173 (1983).
10. M. A. Agzamova, M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 719 (1986).
11. M. I. Isaev, Khim. Prir. Soedin., 820 (1995).