TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS. Vlll. ASKENDOSiDE C FROM *Astragalu8 taschkendiau8*

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A new glycoside of the cycloartane series $-$ askendoside $C -$ has been isolated from the roots of the plant *Astragalus taschkendicus* Bge., and its structure has been established on the basis of chemical transformations and spectral characteristics as $24R-cycloartane-3B$, 6α , 168 , 24 , 25 -pentaol $3-[0-\alpha-L-arabinopyranosyl (1+2)-8-D-xy1$ opyranoside].

We have previously reported the structures of two isoprenoids of the cycloartane series -- cycloasgenin A and cycloasgenin C, isolated from the plant *Astragalus taschkendicus* Bge. (family Leguminosae) [i, 2]. In the present paper we give a proof of the structure of substance E, of glycosidic nature $[1]$, which we have called askendoside C (I).

It was found by the GLC method [3, 4] that the molecule of askendoside C contains D-xylose and L-arabinose residues in a ratio of $1:1$.

The presence in the PME spectra of compound (I) under investigation of two one-proton doublets at 0.20 and 0.50 ppm (Table 1), and also an absorption band at 3040 cm^{-1} in the IR spectrum [6] permitted the new glycoside to be assigned to derivatives of the cycloartane series [5]. This conclusion was confirmed by the fact that the methanolysis of glycoside (I) led to the formation of a genin which was identified as cycloasgenin C (II) [2].

The Smith degradation [7] of askendoside C gave compounds (III) and (IV). The physicochemical constants and spectral properties of product (IV) showed its identity as $166,245$ epoxy-25-norcycloartane-3B,6a,24-triol [2].

Product (III) had a molecular mass $(M⁺ 460)$ 28 units greater than product (IV) $(M⁺ 432)$. This permits the assumption that product (III) was the 24-ether of compound (IV). In actual fact, the PMR spectrum of substance (III) showed the signals of the anomeric proton at C-24 $(4.6$ ppm) and of a proton at C-16 ($\sqrt{4.1}$ ppm) and in the range of 3.1-3.7 ppm a multiplet corresponding to four proton units. At 1.13 ppm the partially masked resonance lines of the methyl group of an ethoxy residue appeared.

The acetylation of (III) led to the diacetate (X) . As was to be expected, in the spectrum of substance (X) the signals of the H-3 and H-6 protons were shifted downfield and they resonated at 4.5 and 4.6 ppm, respectively. One-proton quartets of nonequivalent methylene protons of an ethyl group were observed at 3.3 and 3.7 ppm.

It must be mentioned that substance (III) was an artifact and, in all probability, was formed in the process of treating the products of Smith degradation of askendoside C. It could be obtained as the result of the reaction of the hemiacetal (IV) with ethanol present in the chloroform. To confirm this, we converted compound (IV) into the ethoxy acetal (III) under the action of an ethanolic solution of sulfuric acid.

The formation of substances (iII) and (IV) on the Smith degradation of glycoside (I) shows that in askenoside C (I) the hydroxy groups at C-24 and C-25 are not linked to sugars.

To determine the position of attachment of the carbohydrate residues, we performed the Hakomori methylation of glycoside (I) [8]. From the reaction products we isolated the nona-O-methyl derivative (VI) $(M⁺ 882)$ and the octa-O-methyl derivative (VII) $(M⁺ 868)$ of

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askendoside C. It was shown with the aid of GLC and TLC that the carbohydrate moieties of the methyl ethers (VI) and (VII) were identical and consisted of two components. It must be assumed that in the octa-O-methyl ether (VII) the hydroxy group that remained free was present in the aglycone part of the molecule. It was apparently the hydroxy group at C-6, as was shown by a considerable paramagnetic shift of the signal of one of the methyl groups in the PMR spectrum of (VII) recorded in pyridine (Table 1) $[2]$.

3,4-Di-O-methyl-D-xylopyranose and 2,3,4-tri-O-methyl-L-arabinopyranose were isolated from a hydrolysate of the nona-O-methyl-ether (VI) and were identified by GLC [9]. Thus, askendoside C (I) contains L-arabinose as the terminal sugar, this being attached to the D-xylose residue by a $1 \rightarrow 2$ bond, and it is a monodesmosidic glycoside. This was confirmed by the partial hydrolysis of glycoside (I) . This formed a single progenin - cycloasgenin C D-xylopyranoside (V).

As was to be expected, the acid hydrolysis of the octa-O-methyl ether (VII) led to the tri-O-methyl ether of cycloasgenin C (IX). The PMR spectrum of compound (IX) had one-proton signals at 3.24 and 3.49 ppm. We may note that the resonance signals of protons in the geminal positions to the hydroxy groups at $C-3$ and $C-6$ appeared in the PMR spectrum of compound (IV) at 3.25 and 3.50 ppm, respectively. The good agreement of the figures given shows that in compound (IX) the hydroxy groups at C-3 and C-6 had remained free and, consequently, it was the 16,24,25-trimethyl ether of cycloasgenin C. In actual fact, in the PMR

spectrum of the diacetate (XI), the $H=3$ and $H=6$ signals had undergone a paramagnetic shift and appeared at 4.52 and 4.67 ppm, respectively, which practically coincide with the analogous values of the spectrum of the pentaacetate of cycloasgenin C [2].

The acid hydrolysis of the nona-O-methyl ether (VI) formed the tetramethyl ether of eycloasgenin C (VIII) (M^+ 548). In the PMR spectrum of compound (VIII), a one-proton multiplet appeared at 3.2 ppm, corresponding in the value of its chemical shift to the proton geminal to the hydroxy group at $C-3$. As was to be expected, the H-6 signal underwent a diamagnetic shift and appeared at 2.88 ppm, being superposed on the H-24 resonance lines. These facts define (VIII) as the 6,16,24,25-tetra-O-methyl ether of eyeloasgenin C.

In combination, the experimental facts given indicate that the carbohydrate chain in askendoside C is attached to the genin through the hydroxy group at $C-3$.

In the PMR spectrum of the nona-O-methyl ether (VI), the anomeric protons resonate at 4.30 and 4.57 ppm in the form of doublets with spin-spin coupling constants $3J = 7.5$ Hz, which shows the C1 conformations of the monosaccharide rings and, consequently, the β configuration of the glycosidic center of the D-xylose residue and the α configuration of the L-arabinose residue [i0, Ii]. A calculation of molecular rotation differences confirmed this conclusion [12].

Thus, askendoside C has the structure of $24R$ -cycloartane-38, 6 α , 16 β , 24, 25-pentaol $3-0-[0-a-L-arabinopyranosyl-(1 \rightarrow 2)-8-D-xylopyranoside]$.

EXPERIMENTAL

For General Observations, see [I]. The following solvent systems were used: i) chloroform—methanol (10:1); 2) benzene—ethyl acetate (1:1); 3) benzene—ethyl acetate (5:1); 4) chloroform-acetone (15:2); 5) chloroform-methanol (15:1); 6) chloroform-methanol-water $(70:23:4); 7)$ benzene-chloroform-ethyl acetate $(5:1:1); 8)$ chloroform-ethyl acetate $(1:1);$ 9) butanol-methanol-water (5:3:1). For column chromatography we used type L silica gel with a grain size of $50-100$ μ m.

The sugars and their derivatives were detected in TLC by spraying with o-toluidine salicylate followed by heating at 100-110°C for 2-5 min.

Ga~liquid chromatography was performed on a Chrom-5 chromatograph. Monosaecharides were analyzed in the form of the trimethylsilyl derivatives of the methyl glycosides [3, 4]. A column (3.7 m × 3 mm) containing Chromaton N-AW impregnated with 5% of the silicone phase SE-30 was used, the temperature of the thermostat was 190°C and the carrier gas was helium at a rate of flow of 45 ml/min.

The methyl glycosides obtained by boiling the methyl ethers of the monosaecharides in a 5% methanolic solution of HCl $(4 h)$ were chromatographed on a column $(1.2 m \times 3 mm)$ containing 20% of poly(butan-l,4-diyl suceinate) on Celite. The temperature of the thermostat was 180°C and the rate of flow of helium 45 ml/min. The retention times (T_{rel}) for the methylated

methyl glycosides were calculated in relation to the retention time of methyl 2,3,4,6 tetra-O-methyl-D-glucopyranoside [9].

PMR spectra were recorded in CDC1₃ or C₅D₅N on JNM-4H-100/100 MHz and Varian XL-100-15 and XL-200 instruments (δ , ppm, $O - HMDS$).

For the isolation of the isoprenoids of *Astragalus taschkendicus* Bge., see [I, 2].

Askendoside C (I) - substance E [1], $C_{40}H_{68}O_{13}$, mp 197-198°C (from methanol),

 $[a]_{D}^{23}+27,3 \pm 2^{\circ}$ (c 1,1; methanol), $[a]_{D}^{22}+2,1\pm 2^{\circ}$ (c 1,9; pyridine). v_{max}^{KBr} , cm⁻¹: 3470-3340 (OH), 3040 (CH2 of a cyclopropane ring).

Cycloasgenin C (II) and Askendoside C (I). A solution of 2 g of glycoside (I) in 200 ml of a 0.5% methanolic solution of sulfuric acid was boiled on the water bath for i h. The reaction mixture was diluted with a twofold volume of water, and the methanol was evaporated off. The precipitate that deposited was filtered and was chromatographed on a column with elution by system i. This gave 786 mg of the genin (II), which was identified from its spectral characteristics and physical constants as cyeloasgenin C, mp 244-2460C (from acetone, $\lceil \alpha \rceil^2 + 34 \pm 2^{\circ}$ (c 1,2; methanol), $\lceil \alpha \rceil^2 + 43.6 \pm 2^{\circ}$ (c 1,1; pyridine) $\lceil 2 \rceil$.

The filtrate was evaporated to a volume of 100 ml, and then 2 ml of concentrated sulfuric acid was added and the mixture was heated at 100°C for 6 h. The reaction mixture was neutralized with ARA-8p anion-exchange resin and then D-xylose and L-arabinose were identified by TLC (system 9), in the presence of markers. According to GLC results, the ratio of the sugars was 1.00:0.91.

The Nona-O-Methyl Ether (VI) and the Octa-O-methyl Ether (VII) of Askendoside C from (1) . In small portions, 1 g of sodium hydride was added to a solution of 1.177 g of the glycoside (I) in i00 ml of dimethyl sulfoxide, and the mixture was stirred at room temperature for 1.5 h. Then 12 ml of methyl iodide was added to it dropwise and stirring was continued for another 3 h. The reaction mixture was poured into 200 ml of 2% sodium hyposulfite solution, and then 150 ml of water was added and the products were extracted with chloroform. The chloroform extract was washed with water and was dried over anhydrous sodium sulfate. After the solvents had been distilled off, the dry residue was methylated similarly twice more. The reaction products were chromatographed on a column with elution by system 2. This led to the isolation in amorphous form of 180 mg of the ether (VI), $C_{49}H_{86}O_{13}$, $[\alpha]_{D}^{28}+51.9\pm2^{\circ}$ (c 0,77; methanol) the IR spectrum of which lacked absorption due to hydroxy groups. M⁺ 882. Continuing the washing of the column with the same mixture of solvents gave 748 mg of the amorphous octa-O-methyl ether (VII), $C_{48}H_{84}O_{13}, [a]_{D}^{\infty}+30.6+2^{\circ}$ (c1,18 ; methanol). $v_{\text{max}}^{\text{Nujol}}$, cm⁻¹: 3500 (OH). M⁺ 868.

 $24R-Cycloartane-3\beta$,6 α ,16 β , 24,25-pentaol 6,16,24,25-Tetramethyl Ether (VIII) from (VI).

A solution of 110 mg of the nona-O-methyl ether (IV) in 20 ml of methanol containing 0.5% sulfuric acid was boiled on the water bath for 1.5 h. Then the reaction mixture was diluted with a threefold volume of water, the methanol was evaporated off, and the precipitate that deposited was filtered off and chromatographed on a column with elution by system 3. This gave 20 mg of compound (VIII), $C_{34}H_{60}O_5$, mp 84-85°C (from methanol),

 $[a]_{D}^{23}+100 \pm 2^{\circ}$ (c 0,16 ; benzene); v_{max}^{KBT} , cm⁻¹: 3440-3390 (OH), 3050 (CH₂ of a cyclopropane ring). Mass spectrum, m/z (%): M^+ 548 (18.2), 530 (50.0), 516 (36.4), 498 (56.8), 475 (31.8), 459 (33.0), 443 (46.6), 425 (18.2), 411 (50.0), 393 (18.2), 379 (31.8), 361 (18.2), 256 (52.3), 160 (75.0), 123 (I00).

 $24R-Cycloartane-3\beta$, 6α , 16β , 24 , $25-pentao1$ 16 , 24 , $25-Trimethyl$ Ether (IX) from (VII).

A solution of 422 mg of the octa-O-methyl ether (VII) in 50 ml of a 0.5% methanolic solution of sulfuric acid was boiled on the water bath for 1.5 h. The reaction mixture was diluted with water to 200 ml and the methanol was evaporated off. The precipitate that deposited wasfiltered off and chromatographed on a column with elution by system 2. This gave 90mg of compound (IX), $C_{33}H_{58}O_5$, mp 122-123°C (benzene-ethyl acetate $(1:1)$), $[z]_{D}^{25}+88\pm2^{\circ}$ (c0,5; methanol). $\chi^{\rm KBr}_{\rm max}$, cm $^{-1}$ 3500-3400 (OH), 3040 (CH₂ of a cyclopropane ring). Mass spectrum, m/z (%): M + 534 (1.5), 516 (33.3), 498 (28.9) 486 (17.8), 483 (16.7), 469 (13.3), 461 (23.3), 445 (44.4), 429 (46.7), 411 (57.8), 397 (44.4), 393 (25.6), 379 (64,4), 361 (33.3), 311 (27.8), 271 (33.3), 227 (46.7), 213 (57.8), 201 (84.4), 161 (95.6), 159 (i00).

24R-Cycloartane-3B,6~,16B,24,25-pentaol 16,24,25-Trimethyl Ether 3,6-Diacetate (XI) from (IX). The trimethyl ether (IX) (45 mg) was acetylated with 1 ml of acetic anhydride in 1 ml of pyridine at room temperature for 3 days. After the solvents had been distilled off, the residue was chromatographed on a column, with elution by system 7. This gave 30 mg of compound (XI), $C_{3,7}H_{6,2}O_7$, mp 144-145°C (from ethanol), $[x]_{D}^{30}+83,3\pm2^{\circ}(c0,36;$ methanol). $v_{\text{max}}^{\text{KBr}}$, cm⁻¹: 3060 (CH₂ of a cyclopropane ring), 1740, 1255 (ester group). Mass spectrum, m/z (%): M⁺ 618 (0.3), 603 (0.5), 601 (0.2), 586 (2.6), 571 (7.7), 558 (15.4), 545 (17.9), 529 (29.1), 513 (12.8), 498 (30.8),486 (18.8), 453 (44.4), 421 (43.6), 393 (44.4), 379 (9.4), 361 (44.4), 353 (10.3), 335 (11.9), 319 (26.5), 295 (23.9), 293 (18.8), 287 (35.0), 279 (16.2) , 255 (35.0) , 201 (100) .

Identification of the Methylated Sugars. The filtrates from the two preceding experiments on the hydrolysis of compounds (VI) and (VII) were evaporated to a volume of 25 ml and the residual aqueous solutions were heated on the water bath for 7 h and were then

neutralized with type ARA-8p anion-exchange resin. TLC in systems 4 and 5 showed that the filtrates each contained the same two components.

The filtrate obtained in the hydrolysis of the nona-O-methyl ether (VI) was separated from the anlon-exchange resin and evaporated to dryness, giving 33 g of a syrupy mass. The methylated carbohydrates were ehromatographed on a column with el ution by system 4. First 7 mg of an individual compound having two peaks in GLC was isolated. The intensities and retention times $(T_{rad}: 1.36$ and 1.65) of the latter coincided with those for 3,4-di-Omethyl-D-xylopyranose [9].

On continuing the elution of the column with the same solvent system, 5 mg of a methylated sugar revealing a single peak on GLC was isolated. The intensity and retention time ($C_{r,0}$:1.03) of this peak coincided with the corresponding indices of 2,3,4-tri-O-methyl-L-arablnopyranose [9].

The carbohydrate moiety of the octa-O-methyl ether (VIII) was identified similarly as 3,4-di-O-methyl-D-xylopyranose and 2,3,4-tri-O-methyl-L-arabinopyranose.

Partial Hydr01ysis of Askendoside C (I). A solution of 1 g of glycoside (I) in I00 ml of a $0.5%$ methanolic solution of sulfuric acid was boiled on the water bath for 30 min. The reaction mixture was diluted with a threefold volume of water, and the methanol was evaporated off. The precipitate that deposited was filtered off and, after drying, it was chromatographed on a column with elutlon by system 6. This gave 270 mg of cycloasgenin C (II), mp 244-246°C (from acetone).

Continued elutlon of the column gave i00 mg of cyeloasgenin C 3-O-B-D-xylopyranoside (V), C₃₅H₆₀O₉, mp 252-254°C (from methanol), $[x]_{D}^{22}+34.9 \pm 2^{\circ}$ (c 0.63; pyridine). $v_{\text{max}}^{\text{KBT}}$, cm⁻¹: 3495, 3385, 3235 (OH), 30'60 (CH2 of a cyclopropane ring).

Smith Degradation of Askendoside C. With constant stirring, a solution of 2.1 g of sodium periodate in 20 ml of water was added to a solution of 440 mg of glycoside (I) in 60 ml of methanol, and the mixture was left at room temperature for 2 days. Then it was poured into i00 ml of water, and i0 ml of ethylene glycol was added to decompose the excess of oxidant. After evaporation of the methanol, the reaction products were extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in 70 ml of methanol, and the same volume of water was added. This solution was treated with 1.8 g of sodium tetrahydroborate and the resulting reaction mixture was left at room temperature for 2 days, Then it was acidified with 10% sulfuric acid to pH 2 and was left at the same temperature for another 2 days. After this, i00 ml of water was added and the methanol was distilled off. The residual aqueous solution was shaken with chloroform. The chloroform extract was washed with water to neutrality and was dried over anhydrous sodium sulfate. The residue obtained after the distillation of the chloroform was chromatographed on a column with elution by system 8. Fractions similar in qualitative composition were combined.

~6B~245-Epoxy-25-noreycloartane-3B,6~24-triol 24-Ethy ! Ether (III) from (I). The fractions eluted first yielded 100 mg of substance (III), $\rm{C_{29}H_{48}O_{4}}$, mp 196-197°C (from ethyl acetate), $[z]_{D}^{22}+31.5\pm2^{\circ}(c\;1.46\,;\;\text{method})$. $\frac{N\text{D}}{max}$, cm⁻⁺: 3520-3450 (OH), 3037 (CH₂ of a cyclopropane ring). Mass spectrum, m/z (%): M⁺ 460 (5.5), 442 (86.6), 427 (75.0), 424 (83.3), 409 (27.5), 399 (31.3), 383 (45.0), 381 (52.5), 363 (42.5), 311 (43.8), 293 (25.0), 271 (48.8), 257 (31.3), 253 (35.0), 246 (76.3), 241 (32.5), 239 (36.3), 231 (87.5), 213 (87.5), 201 (93.8), 187 (93.8), 161 (i00).

 $166,245-Epoxy-25-norcycloartane-3\beta,6\alpha,24-triol (IV) from (I).$ The material from the following fractions, after recrystallization from ethyl acetate, gave 60 mg of product (IV) with mp 203-204°C, $[\alpha]_D^{\infty}+30.3\pm2^{\circ}$ (c 0,50 ; methanol). Substance (IV) was identified from its spectral characteristics and physicochemical constants as 168, 245-epoxy-25-norcycloartane- $38,6\alpha,24$ -triol $[2]$.

16B, 245-Epoxy-25-norcycloartane-3B, 6a, 24-triol 24-Ethyl Ether 3, 6-Diacetate (X) from (III). Substance (III) (35 mg) was acetylated with 1 ml of acetic anhydride in 2 ml of pyridine at room temperature for 12 h. The reaction products were poured into water. This gave 30 mg of the amorphous diacetate (X), $\rm C_{33}H_{z0}O_{s1}$ [a] $^{22}_{\rm s} + 77.7 \pm 2^{\circ}$ (c0.54; methanol).

v^{err}, cm⁻¹: 1740, 1250 (ester grouping). Mass spectrum, m/z (%): M 544 (0.9), 529 (0.5),
max 526 (1.0), 498 (21.7), 484 (I00), 469 (34.8), 424 (82.6), 409 (82.6), 380 (34.8), 364 (34.8), 336 (26.1).

 $166,245$ -Epoxy-25-norcycloartane-38,6 α ,24-triol 24-Ethyl Ether (III) from (IV). A solution of 40 mg of the triol (IV) in 6 ml of ethanol containing 1% of sulfuric acid was left at room temperature for 24 h, Then 30 ml of water was added to the reaction mixture and the reaction products were extracted with chloroform. After the usual working up and evaporation of the chloroform extract, 32 mg of a product with mp 196-197°C (from ethyl acetate) was isolated; $[\alpha]_{D}^{24} + 30.9 \pm 2^{\circ}$ (c 1.3; methanol). This substance was shown to be identical with compound (III) obtained from the products of the Smith degradation of askendoside C, their IR spectra and R_f values in TLC also being identical.

SUMMARY

A new glycoside -- askendoside $C -$ has been isolated from the roots of the plant *Astragalus taschkendicus* Bge.; it is 24R-cycloartane-3~,6~,168,24,25-pentaol 3-O-[O-~-L $arabinopy ranosyl-(1 \rightarrow 2) - \beta - D-cylopy ranoside].$

LITERATURE CITED

- i. M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, M. R. Yagudaev, and N. K. Abubakirov, Khim. Prir. Soedin., 572 (1981).
- 2. M. I. Isaev, M. B. Gorovits, N. D. Abdullaev, and N. K. Abubakirov, Khim. Prir. Soedin., 458 (1982).
- 3. G. Wulff, J. Chromatogr,, 18, 285 (1965).
- 4. T. T. Gorovits, Khim. Prir. Soedin., 263 (1970).
- 5. R. B. Boar and C. R. Romer, Phytochemistry, 14, 143 (1975).
- 6. K. Nakanishi, Infrared Absorption Spectroscopy, Holden-Day, San Francisco (1962).
- 7. M. Abdel-Akher, J. K. Hamilton, R. Montgomery, and F. Smith, J. Am. Chem. Soc., 74, 4970 (1952).
- 8. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
- 9. Y. O. Aspinall, J. Chem. Soc., 1676 (1963).
- 10. J. M. Van der Veen, J. Org. Chem., 28, 564 (1963).
- 11. T. Konoshima, H. Fukushima, H. Inui, K. Sato, and T. Sawada, Phytochemistry, 20, 139 (1981).
- 12. W. Klyne, Biochem. J., 47, xli (1950).