TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS.

XIV. ASKENDOSIDE A FROM *Astragalus taschkendicus*

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A new glycoside of the cycloartane series — askendoside A — has been isolated from the roots of *Astragalus taschkendicus* Bge. (family Leguminosae), and on the basis of chemical transformation and spectral characteristics its structure has been established as 3β-[O-α-L-arabinopyranosyl-(1→2)-(3'-O-acetyl-β-D-xylopyranosyl)-oxy]-24R-cycloartane-6α,16β,24,25-tetraol.

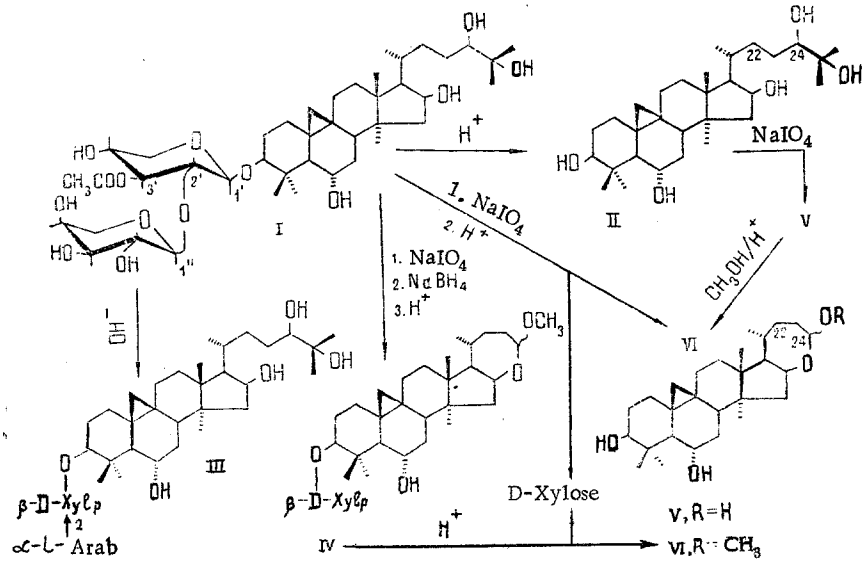
We have continued a study of the isoprenoids of the plant *Astragalus taschkendicus* Bge. (family Leguminosae) [1-5]. The present paper is devoted to a proof of the structure of compound (I), which we have called askendoside A [1].

The presence in the PMR spectrum of compound (I) (Fig. 1) of two one-proton doublets at 0.16 and 0.45 ppm interacting in the manner of an AB system has enabled us to assign askendoside A to derivatives of isoprenoids of the cycloartane series [6]. This was confirmed by the production of cycloasgenin C (II) by the acid hydrolysis of glycoside (I).

It was found with the aid of TLC that that the askendoside A molecule contains D-xylose and L-arabinose residues. According to GLC [7] the ratio of the monosaccharides is 1:1.

The IR spectrum of askendoside A has absorption bands at 1735 and 1258 cm^{-1} showing the presence of an ester grouping. In the PMR spectrum of the substance under consideration, a singlet signal corresponding to three proton units appears at 2.03 ppm. The facts given show that askendoside A contains one acetate group.

The alkaline hydrolysis of askendoside A (I) led to the formation of glycoside (III), which was found to be identical with askendoside C [3]. Askendoside A (I) was subjected to periodate oxidation followed by acid hydrolysis. This gave D-xylose and compound (VI). The formation of D-xylose shows that it is this particular sugar residue that contains the acetate group.



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Substance (VI) had a molecular mass of M^+ 466, i.e., 14 mass units greater than the derivative (V) described previously (M^+ 432). The latter was obtained by the periodate oxidation of cycloasgenin C (II) [2]. The PMR spectrum of compound (VI) showed at 3.29 ppm the three-proton singlet of an OCH_3 group. These facts permitted the assumption that compound (VI) was the 24-O-methyl ether of the triol (V). To confirm this, cycloasgenin C (II) was converted by periodate oxidation into substance (V) [2], the treatment of which with a methanolic solution of sulfuric acid led to the isolation of a compound identical with (VI).

The Smith degradation [8] of askendoside A led to a glycoside (IV) having in its PMR spectrum the signal of an OCH_3 group at 3.25 ppm. As was to be expected, D-xylose was detected in the products of the methanolysis of glycoside (IV). A compound identical with substance (VI) was isolated from the genin fraction of the hydrolysate. The facts given determine glycoside (IV) as 24-methoxy-3β-β-D-xylopyranosyloxy-25-nor-16β,25ξ-epoxycycloartan-6α-ol. As we have shown above, the acetyl group is attached to the cylopyranoside residue. In the PMR spectrum of askendoside A (I), the proton geminal to the ester group resonates at 5.54 ppm in the form of a triplet with $^3J_1 = {}^3J_2 = 7.5$ Hz. The signal of a proton at C-3' should possess splitting with a triplet nature. Consequently, the acetyl group is located in the xylopyranoside ring at this (C-3') carbon atom.

A comparative analysis of the chemical shifts of the anomeric carbon atoms and the ^{13}C spectra of the compound under investigation (I) of askendoside C (III), and of other glycosides of the cycloartane series confirmed the conclusion of the location of the acetyl group. In particular it has been shown [9, 10] that the magnitude of the chemical shift of the anomeric carbon atom (C-1') of the xylopyranoside residue attached to the aglycone at C-3 is 107.3-107.4 ppm in the spectra of cyclosiversioside E and cyclosiversioside F. In the ^{13}C NMR spectra of askendoside A (I) and askendoside C (III), the resonance signals of the anomeric carbon atoms are present (the figures for (III) are given in parentheses) at 103.9 (105.3) ppm for C-1' and 104.7 (106.2) ppm for C-1". The magnitude of the diamagnetic shift of the signal of the C-1' anomeric carbon atom in the spectrum of askendoside C (III) caused by the attachment of the terminal sugar residue at C-2' is 2 ppm (107.3 ppm − 105.3 ppm = 2 ppm). The signal under consideration undergoes an additional upfield shift by 1.4 ppm (105.3 ppm − 103.9 ppm = 1.4 ppm) on passing from askendoside C (III) to askendoside A (I). This is possible only if the O-acetyl group is located at the C-3' carbon atom.

Thus, the experimental facts given, taken all together determine askendoside A as 3β-[O-α-L-arabinopyranosyl-(1→2)-(3'-O-acetyl-β-D-xylopyranosyl)oxy]-24R-cycloartane-6α,16β,24,25-tetraol.

EXPERIMENTAL

General Observations. For general observations see [1]. The following solvent systems were used: 1) chloroform–methanol (15:1); 2) butanol–methanol–water (5:3:1); 3) ethyl acetate–acetic acid–water (6:3:2); 4) chloroform–methanol–water (70:23:4); and 5) benzene–methanol (15:1).

PMR spectra were taken on JNM-4H-100 and XL-200 spectrometers in deuteropyridine or deuterochloroform (δ, ppm; 0 − HMDS), and ^{13}C NMR spectra on a CFT-20 instrument (Varian) in deuteropyridine (δ, ppm; 0 − TMS). The isolation of the isoprenoids of *Astragalus tashkendicus* Bge. has been described previously [1, 2].

Askendoside A (I) — substance C [1], $C_{42}H_{70}O_{14}$, mp 213-214°C (from methanol), $[\alpha]_D^{25}$ 0±3° (c 0.8; methanol); ν_{max}^{KBr}, cm^{-1}: 3520-3325 (OH), 3040 (CH_2 of a cyclopropane ring); 1735, 1258 (ester group). PMR (C_5D_5N), ppm: 0.16 (1 H at C-19, d, ^2J = 4.4 Hz); 0.45 (1 H at C-19, d, ^2J = 4.4 Hz); 0.89 (3 H, s, CH_3); 0.98 (3 H, d, ^3J = 6.6 Hz, CH_3 at C-20); 1.27 (6 H, s, 2 × CH_3); 1.36 (3 H, s, CH_3); 1.38 (3 H, s, CH_3); 1.70 (3 H, s, CH_3); 2.03 (3 H, s, $OCO\underline{CH_3}$ at C-3'); 3.34 (1 H at C-3, m); 4.59 (1 H at C-16, m); 4.88 and 4.89 (2 anomeric protons, d, ^3J = 5.8 and 7.0 Hz, respectively); 5.54 (1 H at C-3', t, $^3J_1 \simeq {}^3J_2 \simeq 7.5$ Hz).

Cycloasgenin C (II) from Askendoside A (I). A solution of 50 mg of askendoside A in 15 ml of 0.5% methanolic sulfuric acid was boiled on the water bath for 1.5 h. Then the reaction mixture was diluted with water to a volume of 100 ml and the methanol was evaporated off. The precipitate that had deposited was filtered off, washed with water, and dried. Then it was chromatographed on a column with elution by system 1. This gave 19 mg of cycloasgenin C (II) with mp 244-246°C (from acetone), $[\alpha]_D^{25}$ +34 ± 2° (c 1.2; methanol), which was identical with an authentic sample [2] both according to its chromatographic mobility on TLC in various solvent systems and according to a comparison of IR spectra.

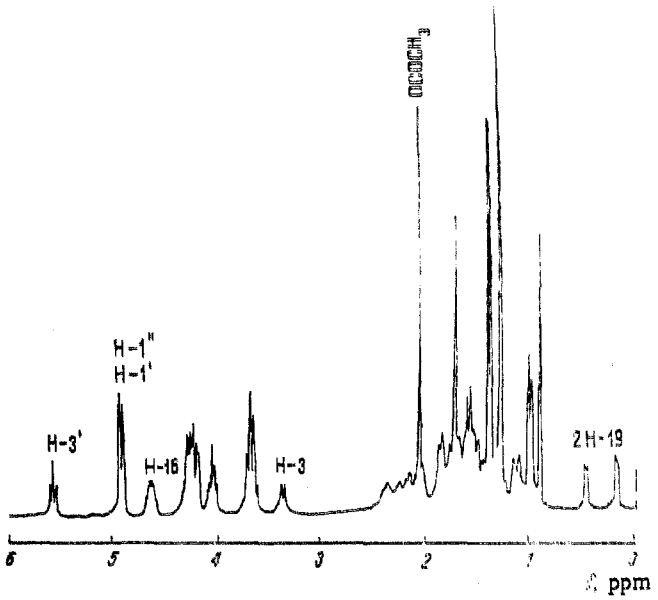

Fig. 1. PMR spectrum of askendoside A.

The filtrate was evaporated to a volume of 10 ml and was heated at 100°C for 4 h to destroy the methyl glycosides. After neutralization with ARA-8p anion-exchange resin and evaporation of the water D-xylose and L-arabinose were found in the residue by TLC in the presence of authentic samples (systems 2 and 3). GLC showed the presence of the same monosaccharides in the molecule of the glycoside (I) in a ratio of 1.00:0.86.

Askendoside C (III) from (I). A solution of 83 mg of askendoside A (I) in 20 ml of methanol was treated with 10 ml of methanol containing 200 mg of caustic soda. The reaction mixture was left at room temperature for 4 h. Then the methanolic solution was diluted with a threefold volume of water and the methanol was evaporated off. The reaction product was extracted with butanol (90 ml), and the butanolic extract was washed with water. The dry residue obtained after the evaporation of the solvents was recrystallized from methanol; this gave 57 mg of askendoside C (III) [3], with mp 197-198°C, and $[\alpha]_D^{25}$ +27.5 ± 2° (c 1.0; methanol), which was identified both from its R_f value in TLC (system 4) and by the characteristics of its IR and PMR spectra.

24-Methoxy-16β,24ξ-epoxy-25-norcycloartane-3β,6α-diol (VI) and D-Xylose from (I). A solution of 200 mg of sodium periodate in 5 ml of water was added to a solution of 84 mg of askendoside A (I) in 25 ml of methanol. The reaction mixture was left at room temperature for 24 days. After this, to decompose the excess of oxidant, 2 ml of ethylene glycol was added to the reaction mixture and it was diluted with water to a volume of 50 ml. The reaction products were extracted with chloroform (50 ml). The chloroform extract was washed with water and evaporated to dryness. The residue was dissolved in 22 ml of a 0.5% methanolic solution of sulfuric acid and was boiled in the water bath for 1.5 h. Then the reaction mixture was diluted with 50 ml of water. The precipitate that deposited was filtered off, washed with water, and dried. The residue was chromatographed on a column with elution by system 5. This gave 35 mg of product (VI), $C_{28}H_{46}O_4$, mp 180-182°C (from methanol), $[\alpha]_D^{21}$ +48 ± 2° (c 1.0; methanol). ν_{max}^{KBr}, cm^{-1}: 3500-3385 (OH), 3055 (CH_2 of a cyclopropane ring). Mass spectrum, m/z (%): M^+ 446(10.0), 431(22.0), 428(100), 413(70.0), 410(60.0), 395(42.0), 381(38.0), 369(32.0), 363(34.0), 311(34.0), 293(16.0), 271(20.0), 253(20.0), 246(38.0), 231(42.0).

PMR ($CDCl_3$); 0.30 (1 H, at C-19, d, ^2J = 4 Hz); 0.47 (1 H at C-19, d, ^2J = 4 Hz); 0.87 (3 H, d, CH_3 at C-20); 0.91 (3 H, s, CH_3); 1.08 (3H, s, CH_3); 1.20 (6 H, s, 2 × CH_3); 3.29 (3 H, s, OCH_3 at C-24); 4.52 (1 H, m, H-24).

The aqueous solution was separated into two equal parts. The first part was evaporated to a volume of 10 ml and was boiled on the water bath for 4 h. The acid solution was neutralized with type ARA-8p anion-exchange resin. After the elimination of the resin and evaporation, D-xylose was detected in the residue by TLC.

The second half was neutralized with the same type of anion-exchange resin, and D-xylose was identified by the GLC method [7].

16β,24ξ-Epoxy-25-norcycloartane-3β,6α,24-triol (V) from (II). A solution of 156 mg of sodium periodate in 6 ml of water was added to a solution of 156 mg of cycloasgenin C (II) in 10 ml of methanol, and the mixture was left at room temperature for 16 h. Then it was poured into 50 ml of water, and the resulting precipitate was filtered off, washed with water and dried. The reaction products were chromatographed on a column with elution by system 1.

This gave 130 mg of substance (V) with mp 203-204°C (from ethyl acetate); $[\alpha]_D^{24}$ +30 ± 2° (c 0.7; methanol) [2].

24-Methoxy-16β,24ξ-epoxy-25-norcycloartane-3β,6α-diol (VI) from (V). A solution of 120 mg of compound (V) in 10 ml of methanol containing 1% of sulfuric acid was left at room temperature for 48 h. Then it was poured into 10 ml of water and the reaction products were extracted with benzene. The benzene extract was washed with water and evaporated. The residue was chromatographed on a column with elution by system 5, which yielded 109 mg of a compound with mp 180-182°C (from methanol), $[\alpha]_D^{31}$ +48.9 ± 2° (c 0.98; methanol), identical with the 24-methyl ether (VI) obtained from (I).

Smith Degradation of Askendoside A (I). The oxidation of 146 mg of askendoside A in 35 ml of methanol was carried out with a solution of 600 mg of sodium periodate in 20 ml of water for 3 days. The excess of oxidant was decomposed by the addition of 5 ml of ethylene glycol. Then the solution was treated with 100 ml of water and the products were extracted with chloroform. The chloroform extract was washed with water and evaporated. The dry residue was dissolved in 50 ml of methanol and 500 mg of sodium tetrahydroborate was added in small portions to the resulting solution, which was then left at room temperature for 3 h. After this, 30 ml of 6% methanolic sulfuric acid was added to the reaction mixture and it was left at the same temperature for 1.5 h. Then it was diluted with water to 200 ml and extracted with chloroform. The chloroform extract was washed with water to neutrality. The residue obtained after the evaporation of the solvents was chromatographed on a column with elution by system 1. This led to the isolation of 60 mg of glycoside (IV), $C_{33}H_{54}O_8$, mp 201-203°C (from ethyl acetate), $[\alpha]_D^{31}$ +46.6 ± 2° (c 0.3; methanol). ν_{max}^{KBr}, cm^{-1}: 3530-3320 (OH), 3050 (CH_2 of a cyclopropane ring) PMR (C_5D_5N), ppm: 0.43 (1 H at C-19, d, ^2J = 4 Hz), 0.79 (3 H, d, CH_3 at C-20); 0.91 (3 H, s, CH_3); 1.06 (3 H, s, CH_3); 1.17 (3H, s, CH_3); 1.83 (3 H, s, CH_3); 3.25 (3 H, s, OCH_3 at C-24); 4.72 (1 H, anomeric proton of a D-xylopyranoside residue, d, ^3J = 7.5 Hz).

24-Methoxy-25-nor-16β,24ξ-epoxycycloartane-3β,6α-diol (VI) and D-Xylose from (IV). The glycoside (IV) (45 mg) was hydrolyzed with 30 ml of a 0.5% methanolic solution of sulfuric acid on the water bath for 1 h. The reaction mixture was diluted with 100 ml of water, and the methanol was evaporated off. The precipitate that deposited was filtered off, washed with water, dried, and chromatographed on a column, with elution by system 5. This gave 17 mg of a substance with mp 180-182° (from methanol), $[\alpha]_D^{31}$ +48.5 ± 2° (c 1.1; methanol); identical with the 24-methyl ether (VI) obtained from (I).

The filtrate was concentrated to a volume of 15 ml and was boiled for 4 h. The aqueous solution after neutralization with ARA-8p anion-exchange resin and the separation of the latter was evaporated to dryness. D-Xylose was detected in the residue by TLC (systems 2, 3, and 4).

SUMMARY

A new glycoside of the cycloartane series — askendoside A — has been isolated from the roots of *Astragalus taschkendicus* Bge.; it has the structure of 3β-[O-α-L-arabinopyranosyl-(1→2)-(3'-O-acetyl-β-D-xylopyranosyl)oxy]-24R-cycloartane-6α,16β,24,25-tetraol.

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GAS–LIQUID CHROMATOGRAPHY OF TRITERPENE ALCOHOLS OF THE DAMMARANE SERIES FROM THE LEAVES OF THE GENUS *Betula*

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The relative retention times have been determined and the stability has been shown of tetracyclic alcohols of the dammarane series from leaves of Far Eastern species of the genus *Betula*, and of their TMS ethers, on the stationary liquid phases OV-1 and OV-225 under GLC conditions. The conditions of obtaining the TMS ethers of the compounds under investigation, and the properties of these ethers, have been studied with the aid of GLC analysis, IR spectroscopy, and mass spectrometry.

From the unsaponifiable part of ethereal extracts of the leaves of Far Eastern species of genus *Betula*, in addition to known triterpenoids of the dammarane series, we have isolated a number of compounds of this class, have established their structure, and have shown that they are present in considerable amount [1], and this has enabled some Far Eastern species of birch to be regarded as promising sources of initial material for the synthesis of physiologically active analogs of the panaxosides.

In this connection, the necessity arose for developing a method for the qualitative and quantitative estimation of the triterpenoid composition of the raw material.

The well-known advantages of GLC showed the desirability of using it for solving the problem posed.

There is an extremely limited amount of information in the literature on the gas-chromatographic properties of triterpene alcohols of the dammarane series [2-5], and therefore the natural approach to the development of method was the study of the behavior of these compounds under the conditions of gas-chromatographic analysis.

Here we give the results for triterpene alcohols the side chains of which are closed to form tetrahydrofuran rings (Fig. 1a) differing from one another by the number (from 2 to 4), the positions (at C atoms 3, 11, 12, 17, and 25), and configurations of their hydroxy groups (Table 1, compounds (I-VIII)).

The gas-chromatographic behavior was studied on two high-temperature stationary phases (SLPs) of different polarities: OV-1 and OV-225. Each of the alcohols was recorded on the chromatogram as a single peak and they were stable under the conditions of chromatographic separation, as was shown by the complete coincidence of the mass spectra of compounds recorded by direct introduction and after passage through the chromatographic column.

It can be seen from Table 1 that the gas-chromatographic properties of the alcohols are determined mainly by the numbers of hydroxy groups, while the positions and configurations of

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