TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XXX. CYCLOARALOSIDE A FROM Astragalus amarus

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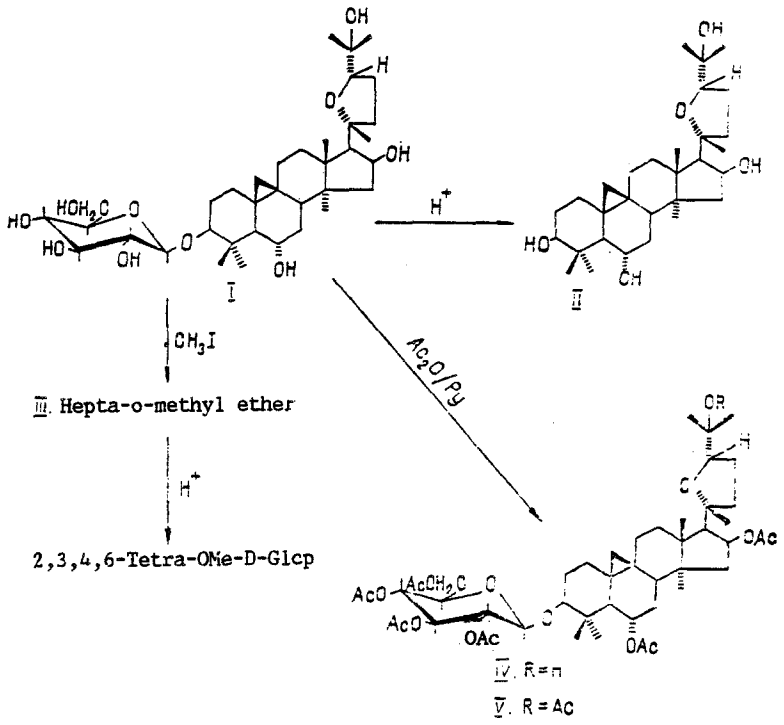
In addition to β-sitosterol, cyclosieversigenin, and β-sitosterol β-D-glucopyranoside, the roots of the plant Astragalus amarus Pall. (Leguminosae) have yielded a new triterpene glycoside of the cycloartane series – cycloaraloside A, which has the structure of 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-β-D-glucopyranoside.

In continuing investigations on cycloartane methylsteroids and their glycosides, we are studying the plant Astragalus amarus Pall. (Leguminosae). The epigeal part of this plant does not contain the desired substances. The TLC of a methanolic extract of the roots of A. amarus in various solvent systems revealed about ten compounds of sterol and triterpenoid nature. The column chromatography of an extract of the roots gave fractions containing four products of low polarity designated in order of increasing polarity as substances 1-4. The rechromatography of these fractions permitted their isolation in the individual form. Compounds 1-3 were identified as β-sitosterol [1], cyclosieversigenin [2, 3], and β-sitosterol β-D-glucopyranoside [1], respectively.



Substance 4 was a new glycoside of the cycloartane series and we have called it cycloaraloside A (I). In the present paper we consider a proof of the structure of this compound.

The PMR spectrum of glycoside (I) containing two one-proton doublets of an AB system at 0.08 and 0.42 ppm and the signals of seven methyl groups, showed that the substance under

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consideration belonged to the cycloartane series [4]. In actual fact, the acid hydrolysis of glycoside (I) led to the production of a genin which was identified as cyclosieversigen (II) [2, 3]. In a hydrolysate, D-glucose was identified by PC and GLC. GLC gave evidence in favour of the assumption that cycloaraloside A was a monoside.

The Hakomori methylation [5] of glycoside (I) gave the hepta-O-methyl ether (III). By the GLC method, a 2,3,4,6-tetra-O-methyl-D-glucopyranose residue was identified in the hepta-O-methyl ether (III).

Cycloaraloside A was acetylated with acetic anhydride in pyridine. The hexacetate (IV) and the heptaacetate (V) were isolated from the reaction products. The mass spectrum of the hexaacetate (IV) had as the maximum peak that of an ion with m/z 143, corresponding to the side chain. Consequently, the hydroxy group remaining free in the hexaacetate (IV) was present at C-25. This was also shown by the downfield shift of the signals of the two methyl group (CH_3-26 and CH_3-27) on passing from the hexaacetate (IV) (1.22 ppm) to the heptaacetate (V) (1.47 ppm).

The H-3 signal in the PMR spectra of the heptaacetate (V) and the hexaacetate (IV) was observed at 3.28 and 3.25 ppm in the form of a doublet of doublets with the SSCCs $^3J_1$ = 10 Hz and $^3J_2$ = 4 Hz, respectively. Consequently, the D-glycopyranose residue was located at C-3 [6]. In the PMR spectrum of the glycoside (I) and its derivatives (III-V) the anomeric proton of the D-glucose gave a signal in the form of a doublet with the SSCC ^3J = 7.5-9 Hz. This showed the C1 conformation of the monosaccharide residue and the β-configuration of the glycosidic bond.

Thus, cycloaraloside A has the structure of 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-β-D-glucopyranoside.

All known cyclosieversigen glycosides contain a D-xylose residue at C-3. It must be assumed that cycloaraloside A is a progenitor of a new series of cyclosieversigen glycosides.

EXPERIMENTAL

General Observations. We used the following solvent systems: 1) chloroform–methanol–water (140:14:1); 2) benzene–chloroform–ethyl acetate (5:1:1); 3) chloroform–methanol (15:1); 4) chloroform–methanol–water (70:12:1); 5) n-butyl alcohol–pyridine–water (6:4:3); 6) benzene–acetone (10:1); and 7) benzene–ethyl acetate (3:1).

PC was conducted on type FN-11 paper. The conditions for GLC, TLC, and CC are given in [6].

PMR spectra were recorded in deuteropyridine or deuterochloroform on a Tesla BS-567A instrument (δ, ppm; 0 – HMDS).

For other observations see [7].

Isolation and Separation of the Isoprenoids of Astragalus amarus. The air-dry comminuted roots (1.5 kg) of A. amarus gathered in the middle of June in Ustyurt, in the Locality of Aktumsyk (shores of the Aral Sea) were extracted with ethanol. The total extractive substances (63 g) were chromatographed on a column with elution successively by chloroform ethyl acetate, and system 1. The chloroform fractions contained substance 1. Ethyl acetate eluted substance 2. Substances 3 and 4 were eluted by system 1.

β-Sitosterol. The fractions containing compound 1 were rechromatographed on a column in system 2. This led to the isolation of 134 mg of substance 1 (0.009%, the yields here and below being calculated on the air-dry raw material), mp 131-132° (from methanol) $[\alpha]_D^{20}$ −37.5 ± 2° (c 0.7; chloroform), which was identified as β-sitosterol [1].

Cyclosieversigenin (II). The fractions containing substance 2 were rechromatographed repeatedly on a column in system 3. This led to the isolation of 50 mg of substance 2 (0.0033%) mp 239-241° (from methanol), $[\alpha]_D^{20}$ +52 ± 2° (c 1.0; methanol). Substance 2 was identified as cyclosieversigen [2, 3].

β-Sitosterol β-D-Glucopyranoside. The fractions containing substance 3 were combined and, after recrystallization from methanol, 280 mg of substance 3 was obtained (0.019%), mp 276-279°, $[\alpha]_D^{24}$ −35 ± 2° (c 1.0; pyridine), and this was identified as β-sitosterol β-D-glucopyranoside [1].

Cycloaraloside A (I). The fractions containing substance 4 were rechromatographed repeatedly in systems 1 and 4. This led to the isolation of 350 mg of cycloaraloside A (0.023%), $C_{36}H_{60}O_{10}$, mp 240-242°C (from system 1), $[\alpha]_D^{25}$ + 33 ± 2° (c 1.15; methanol). ν_{max}^{KBr}, cm^{-1}: 3580-3205 (OH), 3050 (CH_2 of a cyclopropane ring). PMR spectrum (C_5D_5N): 0.08 and 0.42 (2H-19, d, ^2J = 4 Hz), 0.87 (CH_3, s), 1.18 (3 × CH_3, s), 1.28 (CH_3, s), 1.44 (CH_3, s), 1.85 (CH_3, s), 4.84 (2 H, anomeric proton of a D-glucopyranose residue, d, ^3J = 8 Hz and H-16; the signal of the latter was completely overlapped by the signal of the anomeric proton).

Acid Hydrolysis of Cycloaraloside A (I). Glycoside (I) (50 mg) was heated in 10 ml of methanol containing 0.5% sulfuric acid at 50°C for 5 h. Then the reaction mixture was diluted with water and the methanol was distilled off. The precipitate that deposited was filtered off, washed with water, and chromatographed on a column with elution by system 3. This gave 17 mg of a genin with mp 239-241°C (from methanol), $[\alpha]_D^{20}$ + 51 ± 2° (c 1.1; methanol), identified as cyclosieversigenin (II) [2, 3].

The filtrate was concentrated to a volume of 5 ml and was boiled for 2 h. After cooling and neutralization of the reaction mixture with ARA-8p anion-exchange resin the solution was evaporated to dryness. D-Glucose was found in the residue by PC in system 5. GLC, performed with the use of D-xylose as standard, showed that glycoside (I) contained only one D-glucose residue.

The Hepta-O-Methyl Ether (III) from (I). With stirring, 200 mg of sodium hydride was added in small portions to 195 mg of cycloaraloside A in 25 ml of absolute dimethyl sulfoxide. Then 5 ml of methyl iodide was added to the reaction mixture dropwise and stirring was continued for another 5 h. The reaction mixture was poured into 100 ml of 2% aqueous sodium hyposulfite solution, and the products were extracted with chloroform. The residue after the usual working up of the chloroform extract and evaporation of the solvent was chromatographed on a column with elution by system 6. This led to the isolation of 30 mg of the amorphous product (III), $C_{43}H_{74}O_{10}$, $[\alpha]_D^{22}$ + 26.8 ± 2° (c 0.67; methanol). In the IR spectrum of the permethylate (III) there were no absorption bands of hydroxy groups. Mass spectrum m/z (%): M^+ 750 (0.5), 735 (0.5), 720 (1.1), 718 (0.7), 703 (0.5), 677 (0.9), 645 (1.6), 613 (0.9), 545 (0.8), 530 (19.3), 514 (19.3), 499 (22.8), 482 (12.3), 467 (15.8), 457 (6.3) 451 (9.8), 441 (5.4), 435 (8.4), 427 (5.3), 425 (4.0), 419 (6.1), 417 (3.5), 409 (5.8), 395 (3.3), 393 (2.5), 377 (3.5), 341 (3.9), 219 (12.3), 218 (15.8), 187 (100), 157 (96.5), 155 (24.6), 125 (43.9), 111 (61.4). PMR spectrum ($CDCl_3$): 0.15 and 0.40 (2H-19, d, ^2J = 4 Hz), 0.87 (2 × CH_3, s), 1.02 (CH_3, s), 1.10 (2 × CH_3, s), 1.15 (CH_3, s), 1.20 (CH_3, s), 3.03, (CH_3O, s), 3.14 (CH_3O, s), 3.18 (CH_3O, s), 3.30 (CH_3O, s), 3.43 (CH_3O, s), 3.53 (2 × CH_3O, s), 4.18 (1H, anomeric proton of a D-glucopyranose residue, d, ^3J = 7.5 Hz).

In the products of the methanolysis of the hepta-O-methyl ether (III), 2,3,4,6-tetra-O-methyl-D-glucopyranose was detected by the GLC method.

The Hexaacetate (IV) and Heptaacetate (V) of Cycloaraloside A from (I). Glycoside (I) (102 mg) was acetylated with 3 ml of acetic anhydride in 4 ml of dry pyridine at room temperature for 30 days. After the solvents had been evaporated off, the reaction products were chromatographed on a column with elution by system 7. This led to the isolation of 30 mg of the amorphous heptaacetate (V), $C_{50}H_{74}O_{17}$, $[\alpha]_D^{25}$ + 48 ± 2° (c 0.75; methanol). ν_{max}^{KBr}, cm^{-1}: 1770-1745, 1270-1230 (ester groups). Mass spectrum, m/z (%): $(M - 15)^+$ 931(0.2), 886(2.6), 844(0.7), 826(1.5), 766(2.6), 538(27.8), 478(11.1), 331(24.4), 185(66.7), 169(77.8), 125(100). PMR spectrum (C_5D_5N): 0.12 and 0.37 (2H-19, d, ^2J = 4 Hz), 0.79 (CH_3, s), 0.90 (CH_3, s), 1.02 (CH_3, s), 1.16 (CH_3, s), 1.25 (CH_3, s), 1.47 (2 × CH_3, s), 1.87 (CH_3COO, s), 1.90 (2 × CH_3COO, s), 1.96 (CH_3COO, s), 1.99 (3 × CH_3COO, s), 3.28 (H-3, dd, $^3J_1$ = 10 Hz, $^3J_2$ = 4Hz), 4.89 (1H, anomeric proton of D-glucopyranose residue, d, ^3J = 9 Hz).

When elution of the column was continued with the same system, 57 mg was obtained of the hexaacetate (IV), $C_{48}H_{72}O_{16}$, mp 114-115°C (from system 7), $[\alpha]_D^{25}$ + 50 ± 2° (c 0.6; methanol). ν_{max}^{KBr}, cm^{-1}: 3490 (OH), 3040 (CH_2 of a cyclopropane ring), 1770-1740, 1270-1220 (ester groups). Mass spectrum, m/z (%): $(M-15)^+$ 889 (1.0), 886 (0.4), 844 (6.3), 784 (3.5), 572 (0.8), 556 (3.8), 513 (2.6), 496 (66.7), 453 (6.9), 437 (6.9), 377 (5.6), 331 (58.3), 169 (58.3), 143 (100), 125 (17.4). PMR spectrum (C_5D_5N): 0.10 and 0.37 (2H-19, d, ^2J = 4 Hz), 0.76 (CH_3, s), 0.92 (CH_3, s), 1.02 (CH_3, s), 1.14 (CH_3, s), 1.22 (2 × CH_3, s), 1.27 (CH_3, s), 1.87 (CH_3COO, s), 1.89 (CH_3COO, s), 1.95 (CH_3COO, s), 1.96 (2 × CH_3COO, s), 2.01 (CH_3COO, s), 3.25 (H-3, dd, ^3J = 10 Hz, $^3J_2$ = 4 Hz), 4.85 (1H, anomeric proton of D-glucopyranose residue, d, ^3J = 9 Hz).

SUMMARY

In addition to β-sitosterol, cyclosieversigenin, and β-sitosterol β-D-glucopyranoside, the roots of the plant Astragalus amarus Pall. (Leguminosae) have yielded a new triterpene glycoside of the cycloartane series or – cycloaraloside A, which has the structure of 20R, 24S-epoxycycloartane-3β,6α,16β,25-tetraol 3-O-β-D-glucopyranoside.

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