resin and evaporation, GLC in the presence of markers permitted the identification of methyl 2,3,4-tri-O-methyl-L-Rha$_p$ and methyl 3,4,6-tri-O-methyl-D-Glc$_p$.

Acetylation. A mixture of 30 mg of (VI), 3 ml of pyridine, and 5 ml of acetic anhydride was left at room temperature for 36 h. The resulting peraceate of (VI) was methylated by Hakamori's method and the product was hydrolyzed in methanol and deacetylated with 5% NaOH in MeOH, and the proudcts were analyzed by GLC. Methyl 4-mono-O-methyl-D-Glc$_p$ was identified.

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TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS

XXXII. CYCLOCARPOSIDE FROM Astragalus coluteocarpus

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A new triterpene glycoside of the cycloartane series, which has been called cyclocarposide, has been isolated from the epigeal part of the plant Astragalus coluteocarpus Boiss. (Leguminosae). The structure of cyclocarposide has been established on the basis of chemical transformations and spectral characteristics as 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 6-O-α-L-rhamnopyranoside 3-O-α-D-xylopyranoside.

Continuing investigations of cycloartane methylsteroids and their glycosides from plants of the genus Astragalus (Leguminosae), we have begun a study of Astragalus coluteocarpus Boiss. [1]. In a methanolic extract of epigeal parts of this plant, in various solvent systems on TLC, five products of triterpenoid nature were the most outstanding, and these have been designated in order of increasing polarity as substances 1-5. Substance 4 was isolated by column chromatography of the purified total material obtained from a methanolic extract of the epigeal part of A. coluteocarpus. This substance, of glycosidic nature, proved to be new and we have called it cyclocarposide (I). The present paper is devoted to a proof of the structure of this glycoside.

The PMR spectrum of glycoside (I), containing two one-proton doublets of an AB system at 0.10 and 0.30 ppm and the signals of seven methyl groups in the strong field permitted this compound to be assigned to the triterpenoids of the cycloartane series [2, 3]. An absorption band at 3055 cm^{-1} in the IR spectrum of cyclocarposide due to the stretching vibrations of the methylene group of the cyclopropane ring is in harmony with this conclusion.

The Smith degradation [4] of cyclocarposide gave the genin (II), which was identified as cyclosieversigenin [3].

It was shown by the GLC method that cyclocarposide contains D-xylose and L-rhamnose residues in a ratio of 1:1. This was also shown by the ^{13}C and ^{1}H NMR spectra of glycoside (I) in which the signals of two anomeric carbon atoms at 107.49 and 103.90 ppm (Table 1) and of two anomeric protons at 4.60 and 5.15 ppm, respectively, were readily traced.

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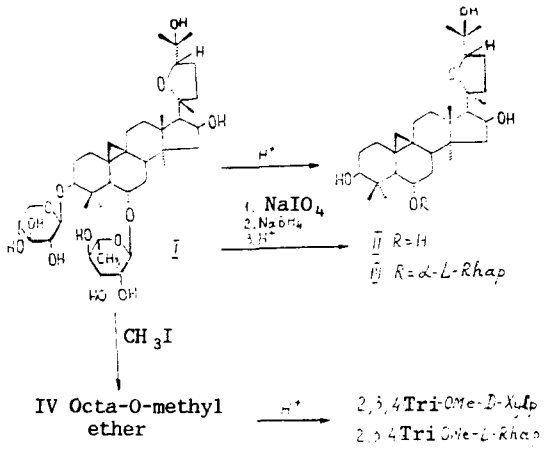

The partial hydrolysis of cyclocarposide gave, in addition to cyclosieversigenin, the progenin (III). GLC showed that the progenin (III) contained a L-rhamnose residue.

Cyclocarposide was methylated by Hakomori's method [6]. In the products of the methanolysis of the octa-O-methyl ether (IV) (M^+ 880) 2,3,4-tri-O-methyl-D-xylopyranose and 2,3,4-tri-O-methyl-L-rhamnopyranose were identified with the aid of GLC. As was to be expected, in the mass spectrum of the octa-O-methyl ether (IV) were observed the peaks of ions with m/z 175, 143, and 115, and m/z 189, 157, and 125, characterizing terminal pentose and terminal 6-deoxyhexose residues [7, 8]. It must be mentioned that ions with m/z 157 and 125 also arose from the side chain of the genin moiety of the permethylated (IV). Consequently, glycoside (I) consisted of a bisdesmoside. This conclusion was also confirmed by the ^{13}C NMR spectrum of cyclocarposide, in which the signals of the two carbinol carbon atoms of the genin moiety had undergone a glycosylation effect and were observed at 88.77 ppm (C-3) and 79.03 ppm (C-6). In the ^{13}C NMR spectrum of progenin (III), the signals of the C-3 and C-6 atoms were observed at 77.61 and 79.70 ppm. This means that the L-rhamnose was present at C-6 and the D-xylose residue at C-3.

The chemical shifts of the anomeric carbon atoms [9] and the difference in molecular rotations between compounds (I) and (III) and between (III) and (II) [10] showed the β- and α-configurations of the glycosidic bonds of the D-xylose and L-rhamnose residues, respectively. The anomeric proton of the D-xylopyranoside residue in the PMR spectra of compounds (I) and (IV) resonated in the form of a doublet with $^3J = 7$ Hz, which was an additional confirmation of the conclusion concerning the β-configuration of the glycosidic center of the D-xylose residue.

Thus, cyclocarposide has the structure of 20R,24S-epoxycycloartane-3β,6α,16β,25-tetraol 6-O-α-L-rhamnopyranoside 3-O-α-D-xylopyranoside.

EXPERIMENTAL

General Observations. The following solvent systems were used: 1) chloroform–methanol–water (140:14:1); 2) chloroform–methanol–water (70:12:1); 3) chloroform–methanol–water (70:23:4); 4) chloroform–methanol (15:1); and 5) benzene–ethyl acetate (2:1).

The ^{13}C and ^1H NMR spectra were recorded in deuteropyridine on a Tesla BS-567A instrument (δ, ppm, for ^{13}C, O–TMS, and for ^1H–HMDS).

For other observations, see [11].

Isolation and Separation of the Triterpenoids of Astragalus coluteocarpus. The air-dry epigeal parts of A. coluteocarpus (3.5 kg) collected in the territory of the Shugnanskii region of the Grono-Badakhshanskaya Autonomous Oblast, Tadzhik SSR (Bidzhundara Gorge) in the flowering phase was extracted with 80% aqueous methanol (40 liters). The extract was evaporated to a viscous syrupy consistency and was dissolved in 1 liter of water. The aqueous solution was treated with chloroform and with n-butanol (5 × 300 ml). The chloroform extract did not contain the desired substances. The butanol extract was evaporated to dryness (50 g) and chromatographed on a column of silica gel with elution successively with chloroform and with systems 1, 2, and 3. Fractions containing the minor substances 1-3 and the individual subsances 4 (the main component of the total material quantitatively) were obtained.

Cyclocarposide (I). The fraction containing substance 4 was recrystallized from methanol to give 8.75 g (0.25% on the air-dry raw material) of cyclocarposide, $C_{41}H_{68}O_{13}$, mp 284-285°C, $[\alpha]_D^{24}$ −28 ± 2° (c, 1.0; pyridine). By the GLC method, in a similar manner to that described in [5], it was found that glycoside (I) contained D-xylose and L-rhamnose residues in a ratio of 1.00:0.82. $[M]_{DI}$ −215°; $[M]_{DIII}$ 0°. $\Delta[M]_D$(I-III) −215°; $[M]_D$ of the methyl glycosides of D-xylopyranose: α, +253°; β, −108° [10]. ν_{max}^{KBr}, cm^{-1}: 3550-3260 (OH), 3055 (CH_2 of a cyclopropane ring). PMR spectrum, ppm: 0.10 and 0.30 (2 H-19, d, ^2J = 4 Hz); 0.83 (CH_3, s); 0.98 (CH_3, s); 1.15 (2 × CH_3, s); 1.25 (CH_3, s), 1.35 (CH_3, s), 1.43 (2 × CH_3, including the CH_3 of a L-rhamnose residue); 4.60 (anomeric proton of a D-xylose residue, d, ^3J = 7 Hz; 4.90 (H-16, m); 5.15 (anomeric proton of a L-rhamnose residue, br.s).

Smith Degradation of Cyclocarposide. A solution of 400 mg of sodium periodate in 5 ml of water was added to 200 mg of glycoside (I) in 40 ml of methanol, and the mixture was stirred at room temperature for 3 h and was then poured into 100 ml of water. To decompose the excess of oxidant, 0.5 ml of ethylene glycol was added. The solution was treated with chloroform. The residue after evaporation of the chloroform extract was dissolved in 25 ml of methanol. This solution was treated with 200 mg of sodium tetrahydroborate and the mixture was left at room temperature for 2 h. It was then acidified by the addition of 0.5 ml of concentrated sulfuric acid in 10 ml of methanol and was left for another 12 h at the same temperature. The reaction mixture was diluted with water and the products were extracted with chloroform. After the usual working up and evaparation of the chloroform extract, the residue was chromatographed on a column with elution by system 4. This gave 63 mg of genin (II) with mp 239-241°C (from methanol), $[\alpha]_D^{24}$ + 52 ± 2° (c 1.0; methanol), $[\alpha]_D^{24}$ + 22± 2° (c 1.0; pyridine), which was also identified as cyclosieversigenin by the characteristics of its PMR and mass spectra.

Cyclosieversigenin (II) and Cyclosieversigenin 6-O-α-L-rhamnopyranoside (III) from (I). Glycoside (I) (500 mg) was hydrolyzed in 30 ml of 0.5% methanolic sulfuric acid for 48 h. After the usual working up of the reaction mixture, the products were chromatographed on a column with elution by system 4. This gave 60 mg of cyclosieversigenin and 57 mg of glycoside (III), $C_{36}H_{60}O_9$, mp 283-286°C (from methanol), $[\alpha]_D^{24}$ 0 ± 3° (c 1.0; pyridine). It was shown with the aid of GLC [5] that glycoside (III) contained a L-rhamnose residue. $[M]_{DII}$ + 107.8°; $\Delta[M]_D$(III-II) −107.8°; $[M]_D$ of methyl glycosides of L-rhamnopyranose; α, −109°; β, +169° [10]. PMR spectrum, ppm: 0.16 and 0.38 (2 H-19, d, ^2J = 4 Hz), 0.85 (CH_3, s), 1.06 (CH_3, s), 1.16 (CH_3, s), 1.19 (CH_3, s), 1.30 (CH_3, s), 1.35 (CH_3, s), 1.45 (CH_3, s), 1.49 (CH_3 of L-rhamnose, ^3J = 6 Hz), 4.90 (H-16, m), 5.25 (anomeric proton of a L-rhamnose residue, br.s.).

TABLE 1. Chemical Shifts of the Carbon Atoms Bearing Oxygen Functions of Compounds (I-III) (δ, ppm, C_5D_5N, 0 − TMS)

C atom	Compound I	Compound II	Compound III
Genin moiety			
C-3	**87,77**	**78,21**	**77,61**
C-6	**79,03**	**68,27**	**79,70**
C-16	73,65	73,35	73,80
C-20	87,10	87,17	87,17
C-24	81,57	81,57	81,72
C-25	71,11*	71,19	71,25
C atom	**Compound I**	**Compound I**	**Compound III**
Residue	β-*D*-Xylp	α-*L*-Rhap	α-*L*-Rhap
C-1	107,49	103,90	104,13
C-2	75,37	72,83	72,85
C-3	78,43	72,55	72,35
C-4	71,11*	73,28	73,30
C-5	66,93	70,07	70,07
C-6		18,08	18,15

*The signals indicated by asterisks are superposed upon one another.

Octa-O-methyl Ether (IV) from (I). With constant stirring, 500 mg of sodium hydride was added in small portions to 500 mg of glycoside (I) in 50 ml of dry dimethyl sulfoxide, and the mixture was stirred for 1 h, after which 5 ml of methyl iodide was added dropwise and stirring was continued for 4 h. Then the reaction mixture was poured into 200 ml of a 2% aqueous solution of sodium hyposulfite, and the resulting mixture was diluted with water and extracted with chloroform. The residue after the usual working up of the chloroform extract and evaporation was chromatographed on a column with elution by system 5. This gave 135 mg of product (IV), $C_{49}H_{84}O_{13}$, mp 182-183°C (from methanol), $[\alpha]_D^{24}$ −5 ± 2° (c 1.0; methanol). The IR spectrum of this compound lacked the absorption due to hydroxy groups. In the products of the methanolysis of the permethylate (IV), 2,3,4-tri-O-methyl-D-xylopyranose (T_{rel} 0.39, 0.46) and 2,3,4-tri-O-methyl-L-rhamnopyranose (T_{rel} 0.50, 0.55) were identified with the aid of GLC [5].

Mass spectrum m/z (%): M^+ 880(2.3), 865(3.3), 848(1.0), 833(1.2), 816(0.5), 807(2.0), 759(3.0), 747(0.8), 743(0.7), 729(1.2), 715(0.8), 704(30.0), 689(8.0), 673(68.3), 657(10.0), 643(16.7), 641(16.7), 601(6.7), 555(5.0), 517(18.3), 498(68.3), 485(36.7), 482(25.0), 467 (60.0), 451(20.0), 435(38.3), 189(100), 175(100), 157(98.3), 143(91.7), 125(85.0), 115(56.7).

PMR spectrum, ppm: 0.13 and 0.39 (2 H-19, d, ^2J = 4 Hz), 0.88 (CH_3, s), 1.09 (2 × CH_3, s), 1.16 (CH_3, s), 1.25 (CH_3, s), 1.32 (2 × CH_3, s), ~1.35 (CH_3 of L-rhamnose, d), 3.00; 3.17; 3.26; 3.35; 3.39; 3.47; 3.51; 3.59 (8 × OCH_3, s), 4.47 (anomeric proton of a D-xylose residue, d, ^3J = 7 Hz); 5.10 (anomeric proton of a L-rhamnose residue, br.s.).

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