TRITERPENE GLYCOSIDES OF *Silphium perfoliatum*.

III. STRUCTURE OF SILPHIOSIDE E

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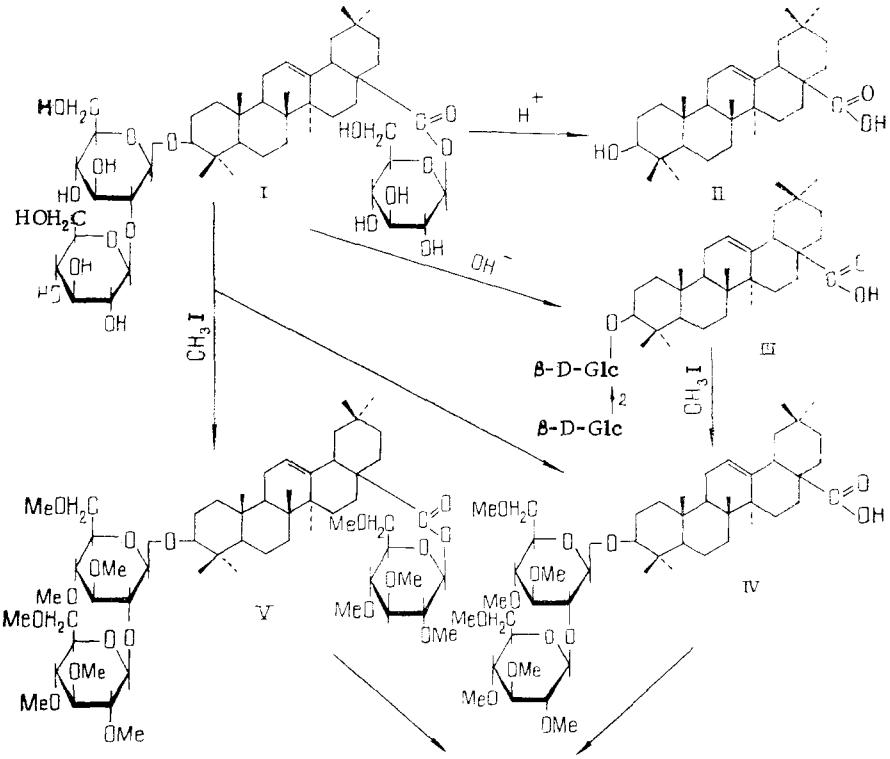
A new triterpene glycoside — silphioside E — has been isolated from the epigeal part of *Silphium perfoliatum* L. and its structure has been established on the basis of chemical transformations and spectral characteristics as oleanolic acid 28-O-β-D-glycopyranoside 3-O-[O-β-D-glycopyranosyl-(1 → 2)-β-D-glucopyranoside].

Continuing a study of the triterpene glycoside of the epigeal part of *Silphium perfoliatum* L. (family Asteraceae) we have isolated from the combined extractive substances [1] a compound which we have called silphioside E (I). In the quantitative respect, together with silphioside B, this is one of the main glycosides of the plant.

The acid hydrolysis of glycoside (I) gave a genin (II), which was identified as oleanolic acid. It was established by GLC and TLC that the carbohydrate moiety of glycoside E consisted of D-glucose. The IR spectrum of the compound under investigation had absorption bands at 1750 and 1240 cm^{-1} that are characteristic for an ester group.



3,4,6-Tri-OMe-D-Glc

2,3,4,6-Tetra-OMe-D-Glc

The alkaline hydrolysis of substance (I) gave a progenin (III) with a free carboxy group at C-28.

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Progenin (III) was subjected to Hakomori methylation. The octa-O-methyl ether (VI) (with M^+ 892) was isolated from the products of the methylation reaction. After methanolysis, the carbohydrate moiety of compound (IV) yielded 3,4,6-tri-O-methylglycopyranose and 2,3,4,6-tetra-O-methylglucopyanose. Consequently, progenin (III) was a bioside in which the carbohydrate chain consisted of two D-glucose residues linked in the 1 → 2 positions.

The Hakomori methylation of silphioside E led to the hendeca-O-methyl ether (V) (M^+ 1096). Together with the main product, the octa-O-methyl ester (IV) described above was also obtained.

It was shown with the aid of GLC that the carbohydrate moiety of the ether (V), like that of the ether (IV), included 3,4,6-tri-O-methylglycopyranose and 2,3,4,6-tetra-O-methylglycopyranose residues. However, in view of the mass of the molecular ion of compound (V), we can go to the conclusion that in glycoside (I) not one but two D-glucose residues were terminal.

Silphioside E (I) is a trioside in which one molecule of D-glucose is directly attached to the carboxy group at C-28 of oleanolic acid and the other two molecules, in the form of the disaccharide sophorose, are attached to the hydroxy group of the genin at C-3.

The configuration of the glycoside centers of the D-glucopyranose residues were determined with the aid of the PMR spectrum. The spectrum of silphioside E showed the signals of the anomeric protons in the form of three doublets at 4.65, 5.10, and 6.04 ppm with spin-spin coupling constants of 7 Hz. This shows the Cl conformation of the pyranose rings and, consequently, the β configuration of the glycosidic centers of the D-glucose residues. A calculation of molecular rotation differences confirmed this conclusion.

Thus, silphioside E has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glycopyranoside].

EXPERIMENTAL

General Observations. Type L 5/40 silica gel and Silufol were used for thin-layer chromatography (TLC). Silica gel 40-100 μ and 100-160 μ was used for column chromatography, with the following solvent systems: 1) chloroform—methanol—water (65:35:8), 2) chloroform—methanol—water (85:35:3), 3) chloroform—methanol (25:1), 4) chloroform—methanol (6:1), 5) benzene—acetone (15:1), 6) benzene—acetone (10:1), and 7) butan-1-ol—methanol—water (5:1:3).

The genine and the glycosides and their derivatives were revealed on TLC with a 20% methanolic solution of tungstophosphoric acid, and the monosaccharides with o-toluidine salicylate on plates impregnated with a 0.3 M aqueous solution of NaH_2PO_4 followed by heating at 100-120°C for 5-10 min.

The gas—liquid chromatography (GLC) of the silylated methylglycosides was performed on a Chrom-5 chromatograph with a 3.7 m × 3 mm column containing 5% of the silicone phase SE-30 on Chromaton NAW at 190°C with helium as the carrier gas at a rate of flow of 50 ml/min. IR spectra were taken on a UR-20 instrument in KBr. Mass spectra were obtained on a MKh-1310 mass spectrometer at an ionizing voltage of 50 V and a temperature of 130-170°C. PMR spectra were obtained on a JNM-4H-100 × 100 MHz instrument (δ, ppm, 0 — JMDS).

Isolation of Biphioside E. For the isolation of the combined saponins, see [1]. The fractions enriched with silphioside E were rechromatographed on columns of silica gel in system 2. The process was monitored by TLC in system 1. The eluents containing the individual glycosides were evaporated, and the dry residue was dissolved in aqueous butan-1-ol. On standing, acicular crystals of silphioside E deposited. The yield of glycoside on the weight of the air-dry raw material was 0.16%.

Silphioside E, $C_{48}H_{78}O_{18}$, mp 218-221°C (from aqueous methanol), $[\alpha]_D^{25}$ +20.0 ± 3° (c 0.88; methanol). ν_{max}^{KBr} cm^{-1}: 3240-3560 (OH), 1750, 1240 (ester group). PMR (C_5D_5N): 0.71-1.11 (7 × CH_3, s); 4.65, 5.10 (1 H each, d, J = 7 Hz, anomeric protons); 5.23 (1 H, br.s., >C=C—H),); 6.04 (1 H, d, J = 7 Hz, anomeric proton).

Oleanolic Acid (II) from Silphioside E (I). A solution of 200 mg of silphioside E in 10 ml of methanol containing 5% of sulfuric acid was heated at 100°C for 5 h. Then the reaction mixture was diluted with water and the methanol was eliminated by evaporation. The precipitate that had deposited (85 g) was filtered off, washed with water, and dried. After repeated recrystallization from ethanol, 30 mg of the genin (II) was obtained with mp 306-307°C, $[\alpha]_D^{24}$ +78.6 ± 2° (c 0.9; methanol). The genin was shown to be identical with an

authentic sample of oleanolic acid by a mixed melting point and from its R_f value on TLC in system 3.

The aqueous solution was evaporated to half its initial volume and was again heated on the water bath for 4 h to decompose methyl glycosides. Then it was neutralized with $BaCO_3$, the resulting precipitate of $BaSO_4$ was separated off, and the filtrate was concentrated to the minimum volume. D-Glucose was found in the resulting syrupy mass by TLC (system 7) and GLC.

Oleanolic Acid Sophoroside (III) from (I). A solution of 500 mg of silphioside E and 10 ml of 5% KOH was heated on the water bath for 1 h. Then the reaction mixture was neutralized with dilute sulfuric acid solution and was extracted repeatedly with butan-1-ol. The butanolic extract was washed with water and evaporated. After chromatographic purification in system 4, the dry residue yielded 300 mg of progenin (III).

Oleanolic acid 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside], mp 255-257°C, $[\alpha]_D^{25}$ +40.6 ± 3° (c 0.94; methanol), ν_{max}^{KBr} cm^{-1}: 3250-3550 (OH), 1700 (C=O of the carboxy group of a triterpene acid). PMR (C_5D_5N): 0.71-1.15 (7 × CH_3, s); 4.70, 5.14 (1 H each, d, J = 7 Hz, anomeric protons); 5.30 (1 H, br.s, >C=C–H).

The Octa-O-methyl Ether (IX) from (III). With constant stirring, 150 mg of sodium hydride was added in small portions to a solution of 150 mg of the progenin (III) in 25 ml of dry dimethyl sulfoxide. After 1 h, 1.5 ml of methyl iodide was added dropwise to the reaction mixture and it was stirred at room temperature for another 4 h. Then it was poured into 200 ml of 2% sodium hyposulfite solution and was exhaustively extracted with chloroform. The chloroform extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated. The reaction products were separated by column chromatography on a column, with elution by system 5. This gave, in anhydrous form, 80 mg of the octa-O-methyl ether (IV), $C_{50}H_{84}O_{13}$, $[\alpha]_D^{25}$ + 30.4 ± 3 (c 0.67; chloroform), M^+ 1096. PMR (CCl_3): 0.68-1.00 (7 × CH_3, s); 3.19-3.51 (3 H × 11, s, OCH_3); 4.15, 4.56 (1 H each, d, J = Hz, anomeric protons); 5.18-5.30 (2 H, m, anomeric protons and >C=C–H).

Hendeca-O-methyl Ether of Silphioside E (V) from (I). Silphioside E (400 mg) was methylated as described. After the appropriate working up of the reaction mixture and chromatography on a column in system 5, 75 mg of the octa-O-methyl ether (IV) was obtained in the amorphous form with $[\alpha]_D^{25}$ +30.3 ± 3° (0.66; chloroform). The permethylate was found to be identical with the compound obtained in the preceding experiment from its R_f value on TLC in system 6.

The continued elution of the column with the same solvent mixture yielded 120 mg of the amorphous hedeca-O-methyl ether $C_{59}H_{100}O_{18}$, $[\alpha]_D^{25}$ +22.5 ± 3° (c 0.56; chloroform), M^+ 1096. PMR (CCl_3): 0.68-1.00 (7 × CH_3, s); 3.19-3.51 (3 H × 11, s, OCH_3); 4.15, 4.56 (1 H each, d, J = 7 Hz, anomeric protons); 5.18-5.30 (2 H, m, anomeric proton and >C=C–H).

The methyl ethers (IV) and (V) were subjected to methanolysis with 5% methanolic HCl at 100°C for 4 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3, and the precipitate was filtered off. The solvent was distilled off, and 3,4,6-tri-O-methyl-D-glucopyranose and 2,3,4,6-tetra-O-methyl-D-glucopyranose were detected in the residue by the GLC method.

SUMMARY

A new triterpene glycoside has been isolated from the epigeal part of *Silphium perfoliatum* L. — oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranoside].

LITERATURE CITED

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