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TRITERPENE GLYCOSIDES OF Silphium perfoliatum.

V. THE STRUCTURE OF SILPHIOSIDE A

É. S. Davidyants, Zh. M. Putieva, V. A. Bandyukova, and N. K. Abubakirov

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From the epigeal part of Silphium perfoliatum L. we have isolated glycoside F, identified as oleanolic acid 3-O-β-D-glucopyranosiduronic acid and a new triterpene glycoside – silphioside A – for which the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-(methyl β-D-glucopyranosiduronate) has been established.

Continuing a study of the triterpene glycosides of Silphium perfoliatum L. [1, 2], we have isolated compounds A and F from the epigeal part of this plant. This is the first time that glycoside A has been described, and we have called it silphioside A (I).

The acid hydrolysis of glycoside (I) gave a genin – oleanolic acid (II) – and in the carbohydrate fraction D-glucose and D-glucuronic acid were identified.

In the PMR spectrum of silphioside A the signals of two anomeric protons appeared in the form of doublets at 4.92 and 6.23 ppm, which showed the presence of two sugar residues in the compound under investigation. The downfield shift of one of the signals (6.23 ppm) showed the addition of one of the monoses to the carboxy group of the aglycone, and the spin-spin coupling constants for both signals of 7.5 Hz showed the β configuration of the glycosidic bonds.

The results obtained showed that the qualitative and quantitative compositions of the sugars of glycoside (I) and of glycoside G (III) isolated previously from the same plant were identical (see following page).

A comparison of the PMR spectra of silphiopsides A (I) and G (III) likewise showed a similarity of their main spectral characteristics (Table 1). However, in the PMR spectrum of (I), unlike that of compound (III), there was a three-proton singlet at 3.72 ppm showing the presence of a methoxy group in the molecule of glycoside A.

To determine the site of attachment of the methoxy group, we used the method of selective homonuclear H_i–{H_j} double resonance in the usual and extended variants. An assignment of the signals of the carbohydrate protons of glycosides (I) and (III) was made with its aid.

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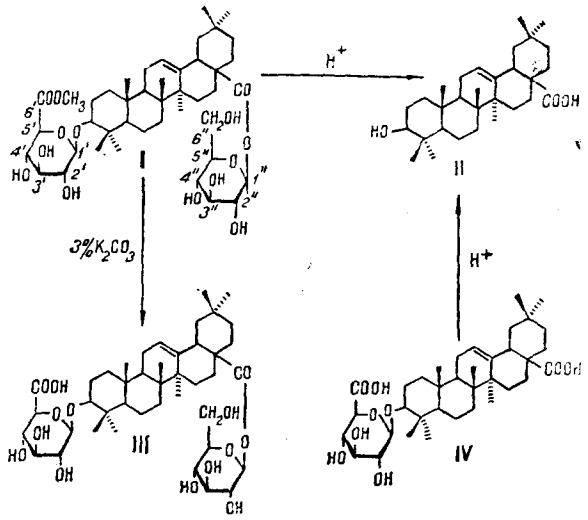

Below, we give the chemical shifts of the carbohydrate protons in the PMR spectra of silphioside A (I) and glycoside G (III) (C_5D_5N, δ, ppm, O – TMS; the spin-spin coupling constants in Hertz are given in parentheses; d – doublet; t – triplet; q – quartet; m – multiplet):

Position of the proton	Compound I	Compound III
H-1′	4,92, d, (7,5)	4,96, d, (7,5)
H-2′	4,00, t, (7,5)	4,05, t, (7,5)
H-3′	4,17, t, (8,5)	4,20, t, (3,5)
H-4′	4,38, t, (8,5)	4,51, t, (8,5)
H-5′	4,47, d, (8,5)	4,57, d, (8,5)
H-1″	6,23, d, (7,5)	6,23, d, (7,5)
H-2″	4,11, t, (7,5 and 8,5)	4,11, t, (7,5)
H-3″	4,18, t, (8,5)	4,19, t, (8,0)
H-4″	4,23, q, (7,0 and 8,0)	4,24, q, (8,0 and 9,0)
H-5″	3,95, m,	3,94, m,
H_{A}-6″	4,39, q, (3,0 and 2,0)	4,39, q, (3,0 and 12,0)
H_{B}-6″	4,29 m, (4,5 and 10,5)	4,30, q, (4,5 and 10,5)

The agreement of the values of the chemical shifts of the D-glucose protons in compounds (I) and (III) excludes the possibility that the methoxy group is located in this part of the molecule. The signals of the H-2, H-3, and H-4 protons of the D-glucuronic acid residue are also characterized by close values of the chemical shifts, and a slight displacement (by not more than 0.1 ppm) of some of these signals cannot show the attachment of a methoxy group to one of the hydroxyls under consideration of the D-glucuronic acid residue.

The facts given, and also the value of the chemical shift of the OCH_3 group (3.72 ppm) permit the conclusion that this group is attached to the carboxy group of the D-glucuronic acid. A confirmation of this is the formation of the glycoside G (III) in mild alkaline hydrolysis of silphioside A (I).

Thus, silphioside A has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-(methyl β-D-glucopyranosiduronate).

Glycoside F (IV) was a more polar compound than silphioside A.

The acid hydrolysis of glycoside (IV) showed that its aglycone was also oleanolic acid, and its carbohydrate moiety consisted of D-glucuronic acid.

The IR spectrum of compound (IV) lacked the absorption characteristic for an ester group. Consequently, the sugar substituent can be present only at C-3 of the aglycone.

On the basis of the results of acid hydrolysis, elementary analysis, and IR spectroscopy, and also a TLC comparison with an authentic sample, glycoside F was identified as oleanolic acid 3-O-β-D-glucopyranosiduronic acid [3, 4]. The IR spectra of the two compounds under comparison coincided.

EXPERIMENTAL

For general observations, see [1]. The following solvent systems were used: 1) chloroform–methanol–water (65:35:8); 2) chloroform–methanol–water (85:25:3); 3) chloroform–methanol (25:1); 4) chloroform–methanol (10:1); 5) chloroform–methanol (8:1); and 6) cutan-1-ol-methanol–water (5:3:1).

PMR spectra were recorded on a Bruker WM-250 instrument.

Isolation of the Glycosides. The total saponins (for their isolation, see [1]) were chromatographed on a column of silica gel (system 2). The separation of the glycosides was monitored by TLC in system 1. The fractions containing compounds A and F were rechromatographed in systems 5 and 2. In this way, 45 mg (0.0025%) of silphioside A and 53 mg (0.003%) of glycoside F were isolated.

Silphioside A. $C_{43}H_{68}O_{14}$, mp 197-199°C, $[\alpha]D^{25}$ +4.5° (c 1.0; methanol). PMR (C_5D_5N, ppm): 0.85, 0.88, 0.89, 0.96, 1.07, 1.25 (21 H, s, 7 CH_3); 3.72 (3 H, s, OCH_3) (the signals of carbohydrate protons; see above).

Glycoside F. $C_{43}H_{56}O_9$, mp 213-215°C (chloroform–methanol–water); $[\alpha]D^{25}$ +17.0 ± 2° (c 0.5; methanol); ν_{max}^{KBr} (cm^{-1}): 3320-3530 (OH); 1700 (C=O of carboxy groups).

Acid Hydrolysis of Silphioside A (II from I). A solution of 20 mg of glycoside (I) in 2 ml of methanol was treated with 2 ml of 10% sulfuric acid, and the reaction mixture was heated in the water bath for 5 h.

It was then diluted with water (5 ml) and the methanol was distilled off. The precipitate that deposited was filtered off, washed with water to neutrality, and chromatographed on a column of silica gel with elution by system 4. This gave 7 mg of oleanolic acid, mp 305-307°C (ethanol), $[\alpha]D^{24}$ 78.2 ± 2° (c 0.68; methanol). Thin-layer chromatography in system 3 with an authentic sample showed their identity.

The aqueous solution was heated at 90°C for 4 h to destroy the methyl glycosides. After neutralization and concentration, D-glucose, and D-glucuronic acid were detected in the hydrolysate by TLC (system 6).

Alkaline Hydrolysis of Silphioside A [(III) from (I)]. A solution of 17 mg of glycoside (I) in 5 ml of 3% aqueous methanolic K_2CO_3 was left at room temperature for 2 h. Then the mixture was diluted with water and the methanol was evaporated off. The precipitate that deposited was filtered off, washed with water to neutrality, and dried. After recrystallization from aqueous methanol, 12 mg of glycoside (III) with mp 198-200°C was obtained. Compound (III) was shown to be identical with glycoside G from its melting point and its R_f value in TLC (system 2).

Acid Hydrolysis of Glycoside F [(II) from (IV)]. Glycoside F (20 mg) was hydrolyzed by the method described above. This gave 8 mg of oleanolic acid with mp 303-305°C (ethanol). In the neutralized hydrolysate D-glucuronic acid was identified with the aid of TLC (system 6).

SUMMARY

Glycoside F, which has been identified as oleanolic acid 3-O-β-glucopyranosiduronic acid, and a new triterpene glycoside – silphioside A – for which the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-(methyl β-D-glucopyranosiduronate) has been established have been isolated from the epigeal part of Silphium perfoliatum L.

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