TRITERPENE GLYCOSIDES OF Silphium perfoliatum.

IV. STRUCTURE OF SILIPHIOSIDE C

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The epigeal part of Silphium perfoliatum has a yielded a new triterpene glycoside, silphioside C – $C_{50}H_{80}O_{19}$, mp. 207-210°C (from aqueous methanol), $[\alpha]_D^{25}$ +19.3 ± 2°C (c 0.88; methanol). On the basis of acid hydrolysis, mild alkaline saponification, and the results of GLC and of IR, mass, and ^1H and ^{13}C spectroscopy the structure of silphioside C had been established as 28-β-D-glucopyranosyl 3-O-[O-β-D-glucopyranosyl-(1→2)-(6'-O-acetyl-β-D-glucopyranosyl)]oleanolate.

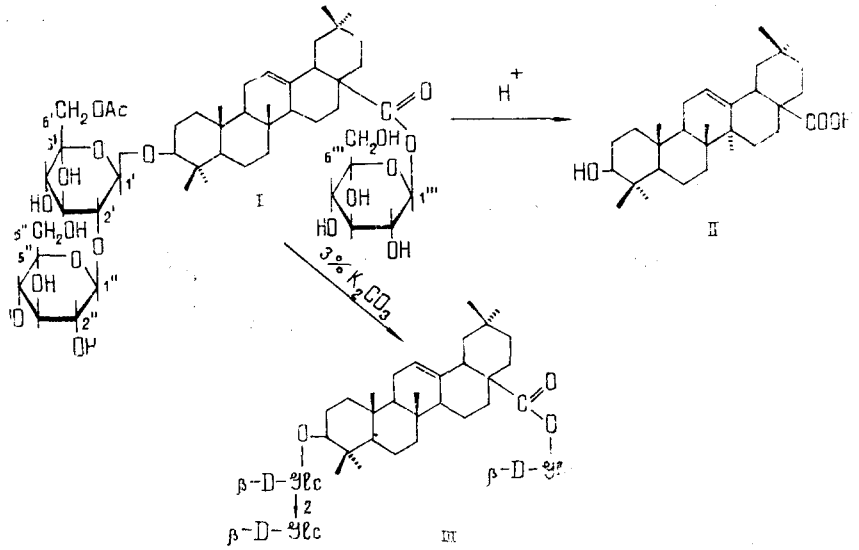
Continuing a study of the triterpene glycosides of the epigeal part of Silphium perfoliatum L. [1], we have isolated from this plant a compound that we have called silphioside C (I).

The acid hydrolysis of glycoside (I) gave oleanolic acid (II), while D-glucose was identified in the hydrolysis by TLC and GLC.

The PMR spectrum of compound (I) had the signals of anomeric protons in the form of three distinct doublets at 4.83, 5.34, and 6.29 ppm, indicating the presence of three sugar residues in the glycoside. The spin-spin coupling constants (SSCCs), which were 7.5 Hz, showed the β configurations of the glycosidic bonds, and the weak-field shift of one of the signals (6.29 ppm) indicated the attachment of one of the glucose residues directly to the carboxy group of the aglycone.

Absorption bands in the 1730 and 1260 cm^{-1} regions in the IR spectrum of glucoside (I), and also a three-proton singlet at 2.05 ppm in the PMR spectrum gave grounds for assuming the presence of an acetyl group in it.

The ^{13}C NMR spectrum of silphioside C (I) had signals at 170.70 and 20.75 ppm corresponding to the resonances of the CO and CH_3 of a O-acetyl group [2].



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The alkaline hydrolysis of silphioside C (I) under mild conditions led to the formation of silphioside E (III) [1].

To determine the position of attachment of the acetyl group in glycoside (I) we used the methods of ^1H and ^{13}C-spectroscopy.

In the ^1H NMR spectrum of compound (I), from the system of signals of carbohydrate protons (3.79-4.56 ppm for (III)) the signals of the AB part of a ABX system stood out in the relatively weak field with SSCCs J_{AB} = 10 Hz, and J_{AX} = 4 Hz (signal at 4.75 ppm), and J_{AB} = 10 Hz and J_{BX} = 1.5 Hz (signal at 4.87 ppm). The general form, chemical shifts, and SSCCs indicated that these signals belonged to the two protons at C-6 of one of the glucopyranose residues: these protons were located geminally to an acetyl group.

A signal with a double intensity at 62.90 ppm in the ^{13}C NMR spectrum of glycoside (III) belonged to the C-6 atoms of two glucopyranose residues (Table 1). In the analogous spectrum of silphioside C (I), the intensity of a signal with the same chemical shift was only half of this and at the same time a new signal appeared in the form of a triplet of unit intensity in a weaker field (64.50 ppm). Furthermore, in place of the signal at 78.40 ppm (for C-5 in the spectrum of (III)) a signal with a chemical shift of 74.60 ppm was observed in the spectrum of glycoside (I). This nature of the change in the spectrum of the monoacetylated glycoside as compared with the unacetylated compound unambigously showed the presence of the OAc at C-6 of one of the three glycopyranose residues. The shift of one of the C-6 signals downfield is explained by the α-effect of the substitution of a OH group by –OAc, and the upfield shift of one of the signals from the general region of resonance of the C-5 atoms of β-D-glucopyranose is the characteristic β-effect arising with this type of substitution [2].

The small shift of the signal of C-3 in the aglycone moiety (from 89.20 in (III) to 89.50 ppm in (I)) permitted the assumption that the acetyl substituent was present at C-6 of one of the glucose residues of sophorose.

It was revealed by homonuclear double resonance that the anomeric protons, which resonated in the 4.83 and 5.34 ppm regions in the ^1H NMR spectrum of compound (I), interacted with the H-2' and H-2" protons, giving triplet signals at 4.17 and 4.08 ppm, respectively.

Selective heteronuclear resonance showed that the H-2' and H-2" protons were attached to carbon atoms resonating at 83.20 and 76.90 ppm, respectively. It is obvious that the signal at 83.20 ppm relates to C-2', since it is precisely this carbon atom that experiences the glycosylation effect. Consequently, the signals of the anomeric protons at 4.83 and 15.34 ppm relates to H-1' and H-1".

To determine the chemical shift of the signal of the proton at C-5' of the acetylated D-glucopyranose residue we used the method of homonuclear double resonance. When the B component of the ABX system referred to above (4.87 ppm) was irradiated we found that the proton under investigation resonated at 3.92 ppm

The next stage in the determination of the position of the acetyl group in glycoside (I) was the use of the nuclear Overhauser effect. When the H-1' and H-1" protons were irradiated alternatively, it was found that presaturation of the H-1' proton led to an increase in the intensity of the signal with a chemical shift of 3.92 ppm (2% in the difference spectrum). This signal represented the resonance of the H-5' proton.

At the same time, presaturation of the H-1" proton caused an increase in the intensity of the signals at 4.17 ppm (2%) and 4.05 ppm (1%) relating to H-2' and H-5", respectively.

The increase in the intensity of the H-2' signal indicated the spatial propinquity of the H-1" and H-2' protons and confirmed the presence of an 1→2-bond in the disaccharide.

The intensification of the signal with a chemical shift of 3.92 ppm (H-5') served as a proof of the localization of the acetyl group at C-6'.

Thus, silphioside C is 28- D-glucopyranosyl 3-O-[O-β-D-glucopyranosyl-(1→2)-(6'-O-acetyl β-D-glucopyranosyl)]oleanolate.

EXPERIMENTAL

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

TABLE 1. ^{13}C Chemical Shifts of the Carbohydrate Component of Silphiosides C (I) and E (III) (δ, ppm, relative to TMS)

C-atom	Silphioside C (I)	Silphioside E (III)
1′	104,90	104,90
2′	83,20	83,35
3′	78,10	78,00
4′	71,55	71,75
5′	74,60	78,40
6′	64,80	62,90
1″	105,90	105,85
2″	76,90	76,90
3″	78,00	78,00
4″	71,90	71,90
5″	78,10	78,10
6″	62,90	62,90
1‴	95,75	95,70
2‴	74,15	74,10
3‴	78,85	78,85
4‴	71,40	71,40
5‴	79,10	79,50
6‴	62,50	62,50
C=O	170,70	
CH_3	20,75	

^1H and ^{13}C NMR spectra were recorded on a Bruker WM-250 instrument at working frequencies of 250 and 60.9 MHz, respectively, in C_5D_5N (0 – TMS).

Isolation of Silphioside C. The fractions obtained when the column was eluted with system 1 [1] were rechromatographed in system 3. The separation was monitored by TLC, using system 2. The individual silphioside C was obtained with a yield of 0.147% calculated on the weight of the air-dry raw material.

Silphioside C (I) $C_{50}H_{80}O_{19}$, mp 207-210°C (from aqueous methanol), $[\alpha]_D^{25} + 19{,}3 \mp 2°$ (c 0,88; methanol). ν_{max}^{KBr}, cm^{-1}: 3570-3240 (OH); 1730, 1260 (ester group). PMR (C_5D_5N): 0.90-1.27 (21 H, s, 7 × CH_3); 2.05 (3 H, s, $OCOCH_3$); 3.18-3.29 (2 H, doublet-like signals); 3.92 (1 H, m, H at C-5'); 4.05 (1 H, t, H at C-5"); 4.08 (1 H, t, H at C-2"); 4.1 (1 H, t, H at C-2'); 4.75, 4.87 (H^A, H^B, J_{AB} = 10 Hz, J_{AX} = 4 Hz, J_{BX} = 1.5 Hz, 2 H at C-6'; 4.83 (1 H, d, J = 7.5 Hz, anomeric proton at C-1"); 5.41 (1 H, pseudotriplet, >C=C–H) ; 6.29 (1 H, d, J = 7.5 Hz, anomeric proton at C-1‴).

Acid Hydrolysis of Silphioside C. A solution of 100 mg of silphioside C in 10 ml of 5% methanolic sulfuric acid was heated at 75°C for 5 h. Then the reaction mixture was diluted with water and the methanol was evaporated off. The precipitate that deposited was filtered off, washed with water, and dried. After repeated recrystallization from ethanol, 15 mg of oleanolic acid was obtained with mp 305-307°C, $[\alpha]_D^{25} + 78{,}6 \pm 2°$; methanol), M^+ 456.

The genin was identified from its PMR and mass spectra and also by TLC in the presence of an authentic sample of oleanolic acid (system 4).

The aqueous solution was evaporated to half its initial volume and was again heated in the water bath for 4 h to break down the methyl glycosides. After neutralization with $BaCO_3$ and concentration, D-glucose was detected in the residue by TLC (system 5) and GLC.

Silphioside E (III) from Silphioside C (I). A solution of 320 mg of silphioside C in 30 ml of aqueous methanol containing 3% of K_2CO_3 was left at room temperature for 2 h. Then the reaction mixture was diluted with water and methanol was evaporated off.

The aqueous solution was extracted with n-butanol. The butanolic extracts were washed with water to neutrality and concentrated. The concentrated aqueous butanolic solution deposited crystals of glycoside (III) with mp 216-220°C, $[\alpha]_D^{25} + 20{,}6 \pm 2°$ (c 0,94 ; methanol). PMR (C_5D_5N): 0.86-1.29 (21 H, s, 7 × CH_3); 3.19 (1 H, dd, J = 13 and 4 Hz); 3.29 (1 H, dd,

J = 11 and 4 Hz); 4.89 (1 H, d, J = 7.5 Hz, anomeric proton at C-1'); 5.35 (1 H, d, J = 7.5 Hz, anomeric proton at C-1''); 5.41 (1 Hz, pseudotriplet, > C=C—H);); 6.30 (1 H, d, J = 7.5 Hz, anomeric proton at C-1'''). On TLC in system 2 silphioside E was identified.

SUMMARY

From the epigeal part of Silphium perfoliatum L a new triterpene glycoside C, has been isolated which is 28-β-D-glucopyranosyl 3-O-[O-β-glucopyranosyl-(1→2) -(6'-O-acetyl-β-D-glucopyranosyl)]oleanolate.

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PHYTOECDYSTEROIDS OF Silene nutans.

III. NUSILSTERONE

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A new ecdysteroid — nusilsterone — has been insolated from the whole plant Silene nutans L. It has been shown that it is 1β,2β,3β,14α,20R,22R,24ξ,25-octahydroxy-5-β-cholest-7-en-6-one.

We have previously [1, 2] reported the isolation from Silene nutans L. (family Caryophyllaceae) by high-pressure liquid chromatograpy of ecdysterone, polypodin B and 22-deoxyecdysterone. In the present paper we have given experimental details of the finding in this plant of another pytoecdysteroid which we have called nusilsterone (I).

The UV spectrum of the new ecdysteroid had a storng maximum at 244 nm (log ε 4.09), which is characteristic for 6-keto-7-ene steroids, and in the IR spectrum, in addition to the absorption of hydroxy groups (3350-3450 cm^{-1}) there was a band at 1660 cm^{-1} corresponding to a keto group conjugated with a double bond.

The mass spectrum of nusilsterone (I) lacked the molecular ion, but in the region of high mass numbers the leaks of ions with m/z 494 ($M - H_2O^+$), 476, 458, and 440 ($C_{27}H_{36}O_5$) were observed. In the region of the PMR spectrum where olefinic protons usually resonate the new compound had a single broadened one-proton singlet at 6.12 ppm which is characteristic for the protons at C-7 ecdysteroids. It followed from the facts given that the substance that we had isolated belonged to the series of ecdysteroids containing eight OH groups in the molecule, and its molecular weight was 512.

The key fragments formed on the cleavage of the C-20—C-22 contained 379, 361, 343, and 325 m.u., and in this compound an ion with m/z 316 ($C_{19}H_{24}O_4$) characterized the breakdown at the C-17—C-20 bond. These facts showed on the one hand, the presence of an OH group at C-20 and, on the other hand, the presence of four hydroxyls in the steroid nucleus.

The PMR spectrum of nusilsterone (I) had a broadened singlet at 4.10 ppm recalling in its nature the signal of the three protons of integristerone A (II) [3] located geminally to secondary hydroxy groups at C-1, C-2, and C-3. By analogy with integristerone A, we were justified in assuming that in the new ecdysteroid, as well, secondary hydroxy groups were present at C-1, C-2, and C-3 of the steroid nucleus and had the β orientation. One of the two-proton multiplets at 3.69-3.74 ppm had to be assigned to a proton at C-22, and the other to one at C-24.

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