THE STRUCTURE OF CALENDULOSIDE A

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We have previously [1] isolated a mixture of eight glycosides of oleanolic acid from the roots of Calendula officinalis L. and have proposed a partial structure for the least polar glycoside – calenduloside A (II). In the present paper we give the results of the isolation and the determination of the complete structure of (II) and also of a minor component of the mixture of glycosides – a monoside of oleanolic acid (I), which we obtained also by the incomplete acid hydrolysis of (II).

The monoside (I), with the composition $C_{36}H_{58}O_8$, which is present in the roots of C. officinalis in the form of traces was detected in small amounts when the combined glycosides were chromatographed on a column of silica gel and was also isolated from the products of the incomplete hydrolysis of this mixture (in the latter case, the yield was 7.5%). The identity of the samples was confirmed by the absence of a depression of the melting point of a mixture and by their IR spectra.

Calenduloside A (II), $C_{42}H_{68}O_{13} \cdot H_2O$, was obtained by chromatographing the combined glycosides on a column of silica gel; yield 3%. The two glycosides give a tetraacetate $C_{44}H_{66}O_{12}$ and a heptaacetate $C_{56}H_{82}O_{20}$, respectively, and the permethylates, $C_{41}H_{68}O_8$ and $C_{50}H_{84}O_{13}$.

According to the UV spectrum, glycosides (I) and (II) contain a trisubstituted olefinic group ($\lambda_{max}^{70\%C_2H_5OH}$ 204-206 nm, ε 4514), while the IR spectra show the presence of a free carboxy group (1700 cm^{-1}) and a hydroxy group (3440 cm^{-1}).

The presence of a carboxy group is also confirmed by the solubility of (I) and (II) in alkalis (they are practically insoluble in water), by potentiometric titration, and by the formation of methyl oleanolate on hydrolysis of the permethylates.

When (I) and (II) were subjected to complete hydrolytic decomposition, in addition to oleanolic acid the hydrolysate was found to contain D-glucose in the case of (I), and D-glucose and D-galactose in the case of (II).

The partial hydrolysis of (II) by dilute mineral acids and 10% oxalic acid led to a mixture of D-galactose, D-glucose, the monoside (I), and oleanolic acid. No disaccharide (lactose) was found in the products. On the acetolysis of (II) and subsequent diacetylation [2], lactose was identified by PC and TLC.

TABLE 1

Substance	Mol. wt.	$[\alpha]_D^{20}$, methanol	$[M]_D^{20}$	ΔC	Form of bond
		degrees			
Methyl α-D-glucopyranoside	194,2	+149	+289,4		
Methyl β-D-glucopyranoside		−25	− 48,6		
Methyl α-D-galactopyranoside		+161	+312,7		
Methyl β-D-galactopyranoside		+5	+ 9,7		
Calenduloside A	799,0	+41,4	+330,8		
The monoside	618,86	+55,7	+329,3	+1,5	β
Oleanolic acid	456,7	+80,0	+365,3	−36	β

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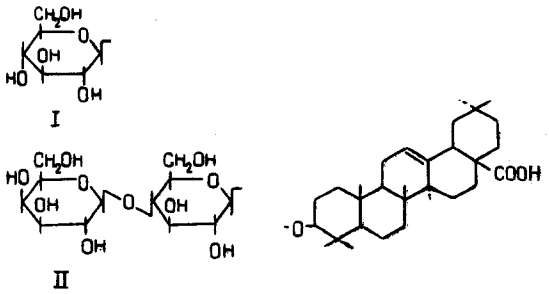
The difficulty of the hydrolysis with dilute mineral acids [3] shows that the sugars have the pyranose form, which is also confirmed by the formation of 2,3,4,6-tetra-O-methyl-D-glucopyranose in the hydrolysis of the permethylate of (I) and of 2,3,6-tri-O-methyl-D-glucopyranose and 2,3,4,6-tetra-O-methyl-D-galactopyranose in the hydrolysis of the permethylate of (II).

Methylation was effected with methyl iodide in the presence of sodium hydride in dimethyl sulfoxide [4, 5].

The configurations of the glycosidic centers were established from the difference in the molecular rotations between the bioside (II) and the monoside (I), and between the monoside (I) and oleanolic acid in accordance with Klyne's rule [6] (Table 1), by comparison with the molecular rotations of methyl α- and β-D-glucosides and α- and β-D-galactosides given in the literature [7].

On the basis of the information given, the complete structures of the monoside (I) and of calenduloside A (II) can be represented in the following way:



The 3-O-β-D-glucopyranoside of oleanolic acid in the form of the hydrate $C_{36}H_{58}O_8 \cdot 2H_2O$ has been obtained synthetically [8, 9] and has recently been found in Beta vulgaris [10].

EXPERIMENTAL

Chromatography was carried out on type KSK silica gel and type M ("slow") paper of the Leningrad Volodarskii Mill with the following systems of solvents: 1) chloroform–methanol–water (61:32:7); 2) chloroform–methanol (9:1); 3) butan-1-ol–pyridine–water (6:4:3); 4) benzene–butan-1-ol–pyridine–water (1:5:3:3); 5) ethyl acetate–n-propanol–water (2:7:1); 6) chloroform–ethanol (25:1); 7) chloroform–ethyl acetate (10:1); 8) butan-1-ol–ethanol–water (5:1:4); 9) benzene–acetone–water (5:5:1); 10) chloroform–acetone (10:1); 11) chloroform–methanol–water (10:2:3); 12) chloroform–acetone (4:1); and, 13) chloroform–methanol (7:3).

The sugars were revealed by aniline phthalate and a mixture of aniline, diphenylamine, and phosphoric acid, and the glycosides and their derivatives with 20% sulfuric acid.

The IR spectra of the substances were taken on a UR-10 spectrophotometer (paraffin oil) and the UV spectra on a "Hitachi" recording spectrophotometer. The potentiometric titration was performed on a LP-58 pH-meter.

The gas-liquid chromatography was carried out on a "Pye" chromatograph (column filled with Chromosorb W impregnated with 10% of neopentyl glycol succinate with nitrogen as the carrier gas at 150°C) and a "Chrom 2" instrument [Chromosorb W impregnated with 5% of poly(neopentyl glycol succinate) with argon as the carrier gas at 169°C].

The NMR spectra were recorded on a Varian HA-100 spectrometer (with deuteropyridine as the solvent and tetramethylsilane as internal standard).

The melting points were determined on a Kofler block. The analytical figures for all the compounds corresponded to those calculated.

Isolation of the Glycosides. The comminuted and chloroform-defatted roots of C. officinalis (4 kg) were exhaustively extracted with methanol. The concentrated methanolic extracts deposited a precipitate which was filtered off and washed with acetone. Yield 100 g. According to TLC on silica gel in system 1, the mixture obtained consisted of eight glycosides.

This mixture (27 g) was transferred to a column (7.5 × 80 cm) containing 800 g of silica gel and was chromatographed in system 1, fractions amounting to 50-60 ml being collected and examined by TLC on silica gel in the same system. Fractions with similar compositions were combined. This gave 0.05 g of a mixture containing a monoside and less polar impurities. After rechromatography on a column of silica gel successively with chloroform and system 2, 0.02 g of a monoside was obtained which was chromatographically homogeneous on a silica gel plate in system 1 (R_f 0.72), mp 240-242°C (methanol), $[\alpha]_D^{20}$ +55.7° (c 0.5; methanol).

Found: mol. wt. 618; 622 (potentiometrically) [11];646 (spectrophotometrically) [12]. $C_{36}H_{58}O_8$. Calculated: mol. wt. 618.9.

Literature data for the hydrate of the 3-O-β-D-glucopyranoside of oleanolic acid – 247-249°C [8], 242-244°C [9, 10], $[\alpha]_D^{20}$ +56 ± 2°C (c 4, pyridine) [8].

The products of the incomplete hydrolysis of the combined glycosides (5.6 g) were chromatographed on a column of silica gel in systems 2, 3, and 1, successively. The fractions were analyzed by TLC in system 1, and 0.75 g of a monoside with mp 240-242°C (methanol) was obtained.

When 27 g of the combined glycosides was subsequently chromatographed on a column as described above, after the elution of the monoside 0.85 g of calenduloside A was obtained; it was homogeneous in system 1 (R_f 0.66) and had mp 260-262°C (chloroform–ethanol), $[\alpha]_D^{20}$ + 41.4° (c 0.5, methanol).

Found: mol. wt. 799.795 (potentiometrically). $C_{42}H_{68}O_{13} \cdot H_2O$. Calculated: mol. wt. 799.0.

Acid Hydrolysis. The hydrolysis of 30.6 mg of the monoside was performed with 8 ml of Kiliani's mixture [conc. HCl–CH_3COOH–H_2O (10:35:55)]. Oleanolic acid was identified by TLC on silica gel in systems 1 and 2, and D-glucose by PC in system 3.

Calenduloside A (20 mg) was hydrolyzed with 5 ml of Kiliani's mixture. The resulting precipitate of oleanolic acid was recrystallized from methanol, mp 306-308°C. The filtrate was evaporated to dryness, and D-glucose and D-galactose were identified by PC in systems 3 and 4.

A mixture of 85.8 mg of calenduloside A in 20 ml of methanol and 20 ml of 5% HCl was heated with stirring for 9 h. The resulting precipitate was filtered off and chromatographed on a column of silica gel and then in systems 2 and 13. This gave oleanolic acid and a monoside with mp 240-242°C, identical with the samples obtained previously with respect to chromatographic behavior and IR spectrum.

Acetolysis. A solution of 0.1 g of calenduloside A in 5 ml of acetic anhydride and 5 ml of 2% H_2SO_4 in acetic anhydride was left at room temperature for 48 h. Then 25 ml of water was added and the mixture was neutralized with Na_2CO_3 and extracted with chloroform. After the solvent had been distilled off, the product was dissolved in absolute methanol and treated with gaseous ammonia for 30 min. Lactose was identified by PC (system 3) and TLC (system 5).

Acetylation. The monoside (54 mg) was acetylated with 3 ml of a mixture of pyridine and acetic anhydride (2:1). The yield of chromatographically homogeneous (system 7) acetate, $C_{44}H_{66}O_{12}$, was 56.8 mg, mp 219-221°C (ethanol) $[\alpha]_D^{20}$ + 70° (c 0.5; methanol).

In the NMR spectrum there are four three-proton singlets in the region around 2 ppm (2.00, 2.02, 2.06, and 2.11 ppm) due to the protons of an acetoxy group, which confirms the presence of four acetyl groups.

Similarly, 53.8 mg of calenduloside A gave 55.8 mg of an acetate with the composition $C_{56}H_{82}O_{20}$, mp 165-167°C, $[\alpha]_D^{20}$ +55.7° (c 0.5; methanol).

In the NMR spectrum, in the region of the signals of methyl groups of acetoxy groupings there is a series of overlapping peaks with a total intensity of 21 proton units [1.93 (3H), 2.00 (6H), 2.02 (3H), 2.17 (9H)], which shows the presence of seven acetyl groups.

Methylation. A solution of 0.48 g of the monoside in 10 ml of dimethyl sulfoxide was treated with 0.45 g of sodium hydride and 3.5 ml of methyl iodide. The reaction mixture was stirred at room temperature for 14 h, and was then partially evaporated in vacuum, mixed with 150 ml of chloroform, and extracted with water (8 × 150 ml). In the penultimate extraction an aqueous solution of $Na_2S_2O_3$ was added for decoloration. The chloroform solution was distilled, giving 0.5 g of resinous residue. By chromatography on

a column of silica gel and elution with system 7, 0.22 g of the completely methylated monoside, with the composition $C_{41}H_{68}O_8$, mp 98-100°C, R_f 0.63 (TLC in system 7), was obtained.

The IR spectrum lacked the absorption bands of OH groups.

Calenduloside A (0.2 g) was treated in 10 ml of dimethyl sulfoxide with 0.3 g of sodium hydride and 3 ml of dimethyl sulfoxide. The reaction was performed in a current of nitrogen. After stirring for 4 h, 3 ml of methyl iodide was added at such a rate that the temperature did not rise above 25°C.

The reaction mixture was poured into water and extracted with chloroform (5 × 100 ml). The chloroform extracts were washed with $Na_2S_2O_3$ solution and with water and were evaporated to dryness. This gave 0.15 g of chromatographically homogeneous (systems 7 and 10), completely methylated calenduloside A with the composition $C_{50}H_{84}O_{13}$, mp 109-110.5°C.

No absorption band of OH groups was present in the IR spectrum.

Acid Hydrolysis of the Permethylates. A mixture of 0.1 g of the permethylate of the monoside, 10 ml of methanol, and 0.5 ml of conc. hydrochloric acid was heated with stirring for 5 h. Then 7 ml of water was added and it was heated for another 3 h. The methanol was distilled off and the precipitate of methyl oleanolate that had deposited was filtered off, dried, and recrystallized from ethanol, mp 198-200°C. A mixture with an authentic sample gave no depression of the melting point.

The filtrate was neutralized with an anion-exchange resin (Dowex-3, OH^- form) and evaporated to dryness. This gave a methylated monosaccharide (0.02 g). By PC (system 8), TLC (system 9), and GLC with an authentic sample, it was identified as 2,3,4,6-tetra-O-methyl-D-glucose.

A mixture of 0.12 g of the permethylate of calenduloside A, 6 ml of methanol, and 0.4 ml of conc. hydrochloric acid was heated with stirring for 10 h, and then 2.5 ml of water was added and it was heated for another 5 h. The methanol was distilled off and the precipitate that deposited was filtered off and transferred to a column of silica gel. It was eluted with chloroform, giving chromatographically homogeneous methyl oleanolate, mp 198-200°C (ethanol). Yield 0.07 g.

The methylated sugars fraction was neutralized with an anion-exchange resin (Dowex-3, OH^- form) and evaporated to dryness. Yield 0.038 g. By TLC (systems 11 and 12) and GLC with authentic samples, 2,3,4,6-tetra-O-methyl-D-galactose and 2,3,6-tri-O-methyl-D-glucose were identified.

SUMMARY

1. The structure of a new triterpene glycoside from the roots of Calendula officinalis L. – calenduloside A – has been established as the 3-O-[O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside] of oleanolic acid.

2. The 3-O-β-D-glucopyranoside of oleanolic acid has been isolated from C. officinalis for the first time.

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