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# TRITERPENE GLYCOSIDES OF *Acanthophllum gypsophiloides*

## VI. STRUCTURE OF ACANTHOPHYLLOSIDE D

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In the present paper we give information on the structure of acanthophylloside D (III), which has been isolated from the roots of *Acanthophyllum gypsophiloides* Rgl. The structures of acanthophyllosides D and C, isolated from this plant previously, have been described in earlier papers [1-3]. Compound (III) was obtained in the individual form after repeated chromatography of the total glycosides.

Unlike acanthophyllosides B and C, acanthopylloside D is not a gypsogenin glycoside. The aglycone precipitating as the result of the hydrolysis of compound (III) is more polar than gypsogenin. The presence of absorption bands in the 2720 and 1720 $cm^{-1}$ regions in the IR spectrum and the peak of an aldehyde proton at 9.47 ppm in the PMR spectrum show that the genin contains an aldehyde group. According to mass spectrometry, the molecular weight of the sapogenin is 486. Furthermore, the substance isolated has a second hydroxy group as compared with gypsogenin. The peak with m/e $264^+$ detected in the mass spectrum shows that the hydroxy group is present in ring D or E, and the peaks with m/e 424, 246, and 202 show that it is located at C-16 of the aglycone. An analysis of literature information on the properties of quillaic acid [4-6] and a comparison of them with the results that we have obtained give grounds for considering that the genin of acanthophylloside D is in fact this acid (I). Although there is information in the literature on the presence of quillaic acid in some plants of the family Caryophyllaceae [4, 7, 8], no glycosides of this sapogenin have previously been known.

Investigations of a hydrolyzate by PC, TLC, and GLC showed that the carbohydrate chains of acanthophylloside D include D-glucuronic acid, D-galactose, D-quinovose, D-fucose, L-rhamnose, D-xylose, and L-arabinose in a ratio of 1:2:1:1:1:3:1. After the periodate oxidation of glycoside (III), the D-glucuronic acid, D-fucose, and D-xylose remained unchanged, as in the case of acanthophylloside B [1], which suggested the identity of the structure of the carbohydrate chains of the two compounds compared.

Alkaline cleavage of glycoside (III) gave a tetraoside (II) the carbohydrate chain of which consisted of D-glucuronic acid, D-galactose, and L-arabinose. In the oligosaccharides split off from the carboxy group of the aglycone we detected D-quinovose, L-rhamnose, and D-xylose. The absence of D-fucose from the hydrolysis products showed that it is directly attached to the carboxy group of the genin (I) and was destroyed during the reaction.

The methylation of acanthophylloside D and the hydrolysis of the permethylate obtained led to the same set of methylated monosaccharides as the analogous operation with acanthophylloside B [2, 3].

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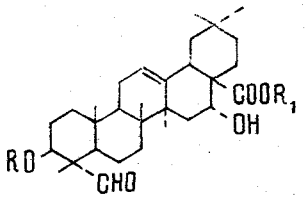
The Smith cleavage of glycoside (III) led to a bioside the carbohydrate part of which consisted of D-glucuronic acid and D-fucose, while the aglycone was a reduction product of quillaic acid. Because of its small amount, the latter was not isolated and characterized. In addition to the bioside, an oligosaccharide residue chromatographically identical with the polyol (IX) obtained as the result of the analogous reaction with acanthophylloside B and C [2] was detected in the reaction mixture. After a second Smith degradation of this oligosaccharide followed by hydrolysis with sulfuric acid of the product formed, D-xylose was detected in the reaction mixture. The product of secondary cleavage was methylated, the resulting permethylate was hydrolyzed, and 2,3,4-tri-O-methyl-D-xylose was identified in the hydrolyzate by the TLC method.

All the facts given above show that the structures of the carbohydrate chains in acanthophyllosides D and B are completely identical. The glycosides differ from one another only by their aglycones. The structure of acanthophylloside D corresponds to formula (III).




I. $R = R_1 = H$

II. $R = \beta\text{-D-Gal-}(1\rightarrow4)\text{-}\alpha\text{-}L\text{-Ara-}(1\rightarrow4)\text{-}\beta\text{-D-GlcUA-}$

$R^1 = H$ $\beta\text{-}D\text{-Gal-}(1\rightarrow2)$

III. $R = \beta\text{-}D\text{-Gal-}(1'\rightarrow4)\text{-}\alpha\text{-}L\text{-Ara-}(1\rightarrow4)\text{-}\beta\text{-}D\text{-GlcUA-}$

$\beta\text{-}D\text{-Gal-}(1\rightarrow2)$

$R_1 = \beta\text{-}D\text{-Xyl-}(1\rightarrow3)\text{-}D\text{-Xyl-}(1\rightarrow3)\text{-}D\text{-Xyl-}(1\rightarrow4)\text{-}L\text{-Rha-}(1\rightarrow4)\text{-}\beta\text{-}D\text{-Fuc-}$

$\beta\text{-}D\text{-Chi-}(1\rightarrow2)$

Until recently, only gypsogenin glycoside had been isolated from plants of the family Caryophyllaceae. In addition, V. E. Bukharov et al. have detected gypsogenic acid glycosides in *Saponaria officinalis* [9] and *Dianthus deltoides* [10], and medicagenic acid glycosides in *Herniaria glabra* [11]. We have found a gypsogenic acid glycoside in *Acanthophyllum paniculatum* [12]. The isolation of acanthophylloside D — a quillaic acid glycoside — is additional evidence of the fact that together with gypsogenin glycosides the plants of this family may contain glycosides of other aglycones of related structure.

## EXPERIMENTAL

For chromatography we used type "M" (slow) paper, KSK silica gel, and the following solvent systems: 1) chloroform—methanol—water (65:35:8); 2) butan-1-ol—acetic acid—water (4:1:5); 3) butan-1-ol—pyridine—water (6:4:3); 4) butan-1-ol—methanol—water (5:3:1); 5) chloroform—ethanol (25:1); 6) chloroform—ethanol (10:1); 7) benzene—acetone (2:1), and 8) toluene—ethanol (10:1).

The free monosaccharides were chromatographed on plates of silica gel impregnated with a 0.3 M solution of $NaH_2PO_4$. The glycosides were detected with a 2% solution of tungstophosphoric acid and the sugars with o-toluidine salicylate.

The gas—liquid chromatography of the silylated methyl glycosides was performed on a Tsvet-4 chromatograph with a flame-ionization detector using a column 2 m × 4 mm containing 5% of the silicone phase G-30 M on Diaforit (0.2-0.315 mm), temperature 170°C, carrier gas helium at a rate of flow of 50 ml/min.

The IR spectra were recorded on a UR-20 instrument, the mass spectra on a MKh-1303, and the PMR spectra on a JNM-4H/100/100 MHz spectrometer.

Isolation of Acanthophylloside D (III). The total glycosides (2 g) were fractionated on a column of silica gel in a ratio of 1:100, the substances being eluted with solvent system 1. After three separations of the fractions enriched with individual compounds we ob-

tained 0.5 g of glycoside B, $C_{86}H_{136}O_{49}$, mp 235-237°C, $[\alpha]_D^{20}$ −13.0 ±3° (c 1.5; water). The yield on the weight of the air-dry roots was 2.5%.

Acid Hydrolysis. Glycoside (III) (0.15 g) was hydrolyzed with 5% $H_2SO_4$ solution at 95°C for 8 h. The quillaic acid (I), $C_{30}H_{46}O_5$, that precipitated had, after recrystallization from aqueous methanol, mp 270-275°C, $[\alpha]_D^{20}$ +62.6 ± 2° (c 0.8114; $CH_3OH$); $M^+$ 486. $\nu_{max}^{KBr}$, $cm^{-1}$: 3520-3385 (OH), 2720, 1720 (CHO), 1700 (COOH). PMR spectrum (O — HMDS, δ, ppm, deuteropyridine): 0.78 (3 H, s), 0.9 (CH, s), 0.93 (3 H, s) 1.05 (3 H, s), 1.22 (3 H, s) 1.7 (3 H, s) — the signals of the protons of methyl groups; and 9.47 (1 H, s) — the signal of the proton of a CHO group. Mass spectrum: $M^+$ 486, m/e 468, 440, 424 ($M-H_2O-CO_2$), 409, 406, 394, 364, 346 (100%), 231, 219, 202.

Literature figures: mp 258-265°C, $[\alpha]_D^{20}$ +65° (methanol) [4]; mp 292-294°, $[\alpha]_D$ +56.1° (pyridine) [5].

The hydrolyzate was neutralized with $BaCO_3$ and evaporated. D-Glucuronic acid, D-galactose, D-quinovose, D-fucose, L-rhamnose, D-xylose, and L-arabinose were detected in the residue by PC (system 2 and 3), TLC (system 4), and GLC in a ratio of 1:2:1:1:1:3:1.

Periodate Oxidation. Glycoside (III) (0.9 g) was hydrolyzed in 100 ml of a 0.8% solution of $NaIO_4$ at 5°C for 24 h. A blank experiment was performed in parallel. The consumption of periodate was determined by titration with a 0.1 N solution of sodium thiosulfate, and the formic acid produced was titrated with a 0.01 N solution of NaOH. For each mole of glycoside, 10.2 moles of periodate were consumed and 3.63 moles of formic acid were formed.

After the addition of 0.3 ml of ethylene glycol (to decompose the excess of periodate), a separate part of the reaction mixture was hydrolyzed with 5% sulfuric acid. The $BaCO_3$-neutralized hydrolyzate was found by TLC in system 4 and PC in systems 2 and 3 to contain D-glucuronic acid, D-fucose, and D-xylose.

Alkaline Hydrolysis. Acanthophylloside D (0.1 g) was hydrolyzed in 20 ml of 10% aqueous caustic potash at 95°C for 7 h. The reaction mixture was neutralized with KU-2 cation-exchange resin and extracted with n-butanol, and the butanolic extracts were washed with water and evaporated to dryness. The dry residue was purified on a column in system 1. This gave 0.03 g of a tetraoside of quillaic acid (II) in the amorphous form.

The aqueous residue evaporated to dryness was purified on a column of silica gel in the same system. After hydrolysis with 5% sulfuric acid D-quinovose, L-rhamnose, and D-xylose were identified in it by TLC in system 4 and by GLC.

The tetraoside (II) was hydrolyzed under the same conditions, and D-glucuronic acid, D-galactose, and L-arabinose were detected in the hydrolyzate by the methods given above.

Methylation of Acanthophylloside D. Glycoside (III) (0.05 g) was methylated by Hakomori's method. The process was monitored by TLC in systems 7 and 8. The reaction mixture was poured into an aqueous solution of sodium thiosulfate and extracted with chloroform, and the chloroform extracts were washed with water, dried over sodium sulfate, and evaporated to dryness. The dry residue was purified on alumina (2-cm layer) and eluted with benzene (20 ml).

In a hydrolyzate of the glycoside permethylate the following compounds were identified by TLC in systems 7 and 8 and by GLC in the presence of authentic samples: 3-O-methyl-D-glucuronic acid, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-quinovose, 3-O-methyl-D-glucose, 2,3-di-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-xylose, 2,4-di-O-methyl-D-xylose, and 2,3-di-O-methyl-L-arabinose.

Smith Degradation. The oxidation of 0.1 g of glycoside (III) was carried out in 20 ml of a 4% aqueous solution of periodic acid at 5°C for 24 h. The process was monitored by TLC in system 1. The solution was neutralized with $BaCO_3$ and the precipitate that deposited was filtered off. The filtrate was treated with 0.1 g of $NaBH_4$ and was left at room temperature for 18 h.

The mixture was neutralized with acetic acid and was concentrated to 10 ml after which concentrated hydrochloric acid was added to pH ∿ 1 and the resulting mixture was left at 18-20°C for 6 h. Then it was neutralized with sodium bicarbonate and extracted with n-butanol, and the butanolic extracts were washed with water and evaporated to dryness. The dry residue was purified on a column of silica gel in system 1. This gave 20 mg of 16α-hydrohederagenin 28-β-D-fucopyranoside 3-O-[(α-D-glucopyranosid)uronic acid].

The aqueous residue after extraction with butanol was evaporated to dryness. Part of the residue was hydrolyzed with 5% sulfuric acid, and in the neutralized hydrolyzate D-xylose was identified by TLC in system 4.

Another part was resubjected to Smith degradation under the conditions described above. The product formed was methylated by Hakomori's method. The permethylate was subjected to methanolysis with 7% HCl, followed by hydrolysis after which 2,3,4-tri-O-methyl-D-xylose was detected by TLC in system 7.

Hydrolysis of the Diglycoside. The compound obtained in the preceding experiment (10 mg) was hydrolyzed with 5% sulfuric acid. The precipitate that deposited was separated off and washed with water. A substance with a $R_f$ value differing from that of quillaic acid (presumably 16α-hydroxyhederagenin) was detected by TLC in systems 5 and 6. D-Glucuronic acid and D-fucose were detected by the TLC method in the $BaCO_3$-neutralized hydrolyzate.

## SUMMARY

A new triterpene glycoside — acanthophylloside D — has been isolated from the roots of *Acanthophyllum gypsophiloides* Rgl. It is a decaoside of quillaic acid containing O-glycosidic and O-acylosidic carbohydrate chains identical in structure with the analogous fragments of acanthophylloside B.

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