TRITERPENE GLYCOSIDES OF Acanthophyllum gypsophiloides

III. STRUCTURE OF THE O-GLYCOSIDIC CARBOHYDRATE CHAIN OF ACANTHOPHYLLOSIDES B AND C

Zh. M. Putieva, L. G. Mzhel'skaya,
T. T. Gorovits, E. S. Kondratenko,
and N. K. Abubakirov

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The isolation from the roots of Acanthophyllum gypsophiloides Rgl., family Caryophyllaceae of acanthophylloside B – a glycoside of gypsogenin having the same qualitative set of monosaccharides as gypsoside [3] but differing from it in the structure of the carbohydrate chains – has been reported previously [1, 2].

On a more careful study of acanthophylloside B with the aid of TLC on silica gel, we found that it separates into two spots with very similar distances of migration. This would appear to confirm the hypothesis of the presence in glycosides containing as their bases a gypsogenin glucuronoside two forms – semiacylal and open – like gypsoside [4]. In actual fact, when the glycoside from A. gypsophiloides was subjected to alkaline treatment a transition from a more polar form to a less polar form was observed, as in the case of gypsoside. However, the reverse transition did not take place on acidification.

Repeated chromatography on a column permitted the isolation of two forms of the acanthophylloside, which proved to be individual compounds distinguished by their qualitative sugar composition. For the less polar of them we retained the name acanthophylloside B (I) and we called the more polar one acanthophylloside C (II).

According to the results of acid hydrolysis and GLC of the silyl derivatives of the methyl glycosides [5], acanthophylloside B contains D-galactose, D-xylose, L-arabinose, D-fucose, L-rhamnose, and D-glucuronic acid in a ratio of 2:3:1:1:2:1. Acanthophylloside C contains the same sugars in the same ratio and, in addition, one molecule of D-glucose. Consequently, acanthophylloside B with the composition $C_{86}H_{136}O_{48}$ is a decaoside, and acanthophylloside C with the composition $C_{92}H_{146}O_{53}$ is an undecaoside of gypsogenin.

On a GC chromatogram of the trimethylsilyl derivatives of the sugars of both glycosides, in addition to the peaks of the monosaccharides mentioned another peak of unknown origin can be well seen (Fig. 1), appearing between the peaks of the xylose and the galactose. However, when both the free and the methylated sugars were analyzed by other methods, we were unable to detect any other compounds whatever. It must be noted that the same peak appears on GC chromatograms of other complex glycosides of gypsogenin present in our collection: gypsoside [3], saponaside D [6], and trichoside D [7]. The reason for the appearance of the peak has not yet been elucidated.

Another feature of acanthophyllosides B and C is the impossibility of obtaining the full acetates. The products obtained even after repeated treatment of the glycosides with acetic anhydride in pyridine continue to show absorption in the region of hydroxy groups in the IR spectrum. This can be explained by the presence of sterically hindred hydroxy groups due to the branching of the carbohydrate chains.

In actual fact, when acanthophyllosides B and C were subjected to periodate oxidation by a known method [8], the D-glucuronic acid, the D-fucose, and the D-xylose were preserved, which shows the presence either of 1–3 bonds or of branchings. The absence of furanose forms of the monosaccharides was confirmed by the hydrolysis of the glycosides with dilute oxalic acid. This led to no change in acantho-

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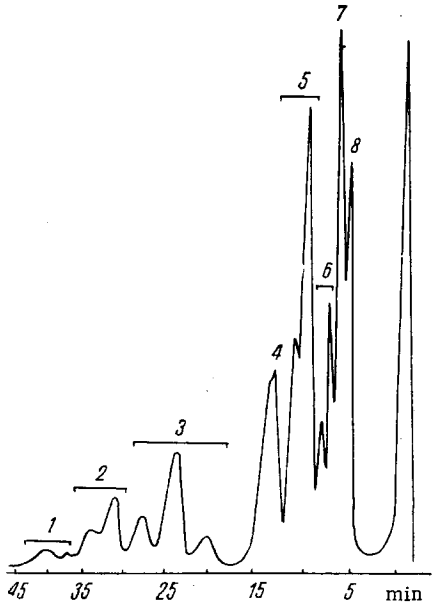


Fig. 1. GC chromatogram of the silylated methylglycosides of acanthophylloside C: 1) D-glucuronic acid; 2) D-glucose; 3) D-galactose; 4) unknown substance; 5) D-xylose; 6) D-fucose; 7) L-rhmanose; 8) L-arabinose.

phyllosides B and C. As mentioned above, acanthophylloside C is converted into acanthophylloside B under the action of alkaline agents. It follows from this that the D-glucose split off in this process is attached to the carboxy group of the D-glucuronic acid, as in the case of polemonioside C [9]. D-Glucose was not detected in the alkaline hydrolyzate: Like all sugars attached to a carboxy group, it is decomposed on saponification with alkali. On more severe alkaline treatment, the acyloside bond at the carboxyl of the aglycone was saponified, and the two glycosides formed the same progenin – a tetraoside of gypsogenin $C_{53}H_{82}O_{24}$ (III). This compound includes two molecules of D-galactose and one molecule each of L-arabinose and D-glucuronic acid (GLC). The exhaustive methylation of the tetraoside of gypsogenin (III) followed by the hydrolysis of its permethylate led to 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-arabinose, and 3-O-methyl-D-glucuronic acid (in the Scheme, the permethylates of the glycosides are omitted, and only the methylated sugars formed from the hydrolysis of the permethylates are shown). We arrive at a similar result by excluding from the products of the methylation of acanthophyllosides B and C the O-methyl derivatives of D-fucose, L-rhamnose, and D-xylose. These sugars are absent from the O-glycosidic moiety and are present only in the O-acylosidic moiety of the glycoside molecules.

It only remains to be added that a permethylate of acanthophylloside C gave 2,3,4,6-tetra-O-methyl-D-glucose, as well, which confirms the terminal position of the glucose residue. If we take into account the fact that the acid hydrolysis of both acanthophyllosides forms not only gypsogenin but also its β-D-glucuronoside, then both molecules of galactose in the tetraoside of gypsogenin are terminal. One of them is attached directly to the glucuronic acid and the second through arabinose. These results are confirmed by periodate oxidation of the gypsogenin tetraoside (III), in which two molecules of formic are formed and only the D-glucuronic acid is unaffected.

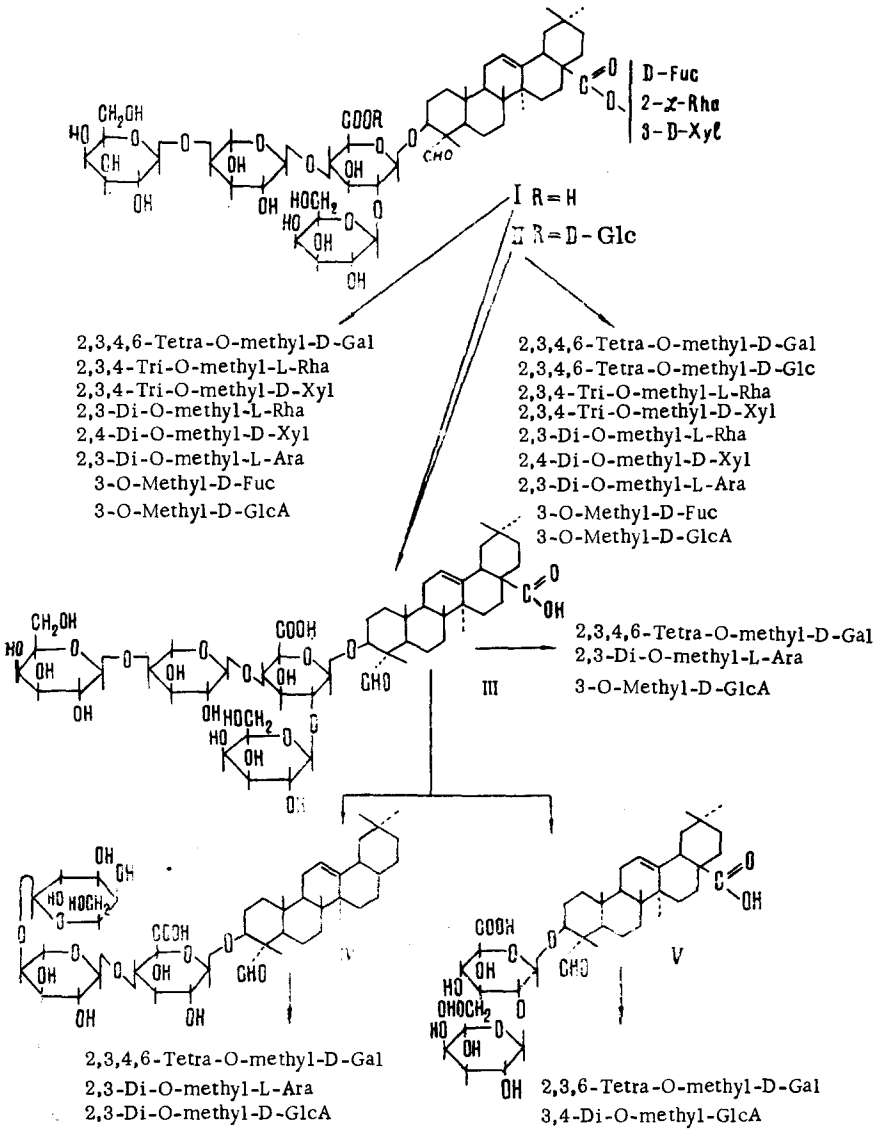
For a detailed elucidation of the structure of the O-glucosidic chain of acanthophyllosides B and C we performed a partial hydrolysis of the gypsogenin tetraoside (III) with 0.25% sulfuric acid. Among the other hydrolysis products we detected two glycosides. One of them – a gypsogenin bioside (V) – contained only D-galactose and D-glucuronic acid, and the second – a gypsogenin trioside (IV) – also included L-arabinose.

Hydrolysis of the permethylate of (V) led to 2,3,4,6-tetra-O-methyl-galactose and 3,4-di-O-methyl-D-glucuronic acid, and hydrolysis of the permethylate of the trioside (IV) led to 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-arabinose, and 2,3-di-O-methyl-D-glucuronic acid. Consequently, in the O-glycosidic chain of the gypsogenin tetraoside one molecule of D-galactopyranose is attached to the hydroxyl at C_2 of the D-glucuronic acid. A second molecule of D-galactopyranose is attached to the fourth hydroxyl of the L-arabinose, and the arabinose itself to the hydroxyl at C_4 of the D-glucuronic acid. This clearly shows the partial structure of acanthophyllosides B (I) and C (II).

The configuration of the glycosidic bonds has been taken in accordance with Klyne's rule [11] (see Scheme on next page).

EXPERIMENTAL METHOD

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-o-ol–ethanol–25% NH_4OH (7 : 2 : 5); 3) butan-1-ol–acetic acid–water (4 : 1 : 5); 4) water-saturated methyl ethyl ketone; 5) butan-1-ol–ethanol–water (5 : 1 : 4); 6) benzene–acetone (2 : 1); 7) chloroform–methanol (6 : 1); 8) butan-1-ol–methanol–water (5 : 3 : 1); 9) chloroform–methanol (25 : 1); 10) chloroform–methanol (10 : 1); 11) toluene–methanol (9 : 1); 12) butan-1-ol–pyridine–water (6 : 4 : 3).



The glycosides were revealed with an ethanolic solution of phosphotungstic acid, and the sugars with o-toluidine salicylate.

The identification of the methylated monosaccharides was performed by chromatographic comparison (PC, TLC, GLC) with authentic samples using color reactions with diphenylamine–aniline [2] and with tetraphenyltetrazolium chloride [13], and also periodate oxidation both of the partially methylated sugars and of their methyl glycosides.

The GLC of the silylated methyl glycosides was performed on a UKh-1 chromatograph using a copper column (1 m × 4 mm), containing 5% of the silicone phase G-30-M on Diaforit (0.2-0.315 mm), column temperature 176°C, carrier gas hydrogen at the rate of 55 ml/min. The GLC of the acetates of the aldononitriles of the monosaccharides [14] and of the methyl glycosides of the methylated sugars was performed on a "Tsvet-2" chromatograph using spiral stainless-steel columns (100 × 0.3 cm) containing 10% of poly-(butane-1,4-diyl succinate) on Chromosorb W (45-60 mesh), temperature of the evaporator 280°C, carrier gases hydrogen and nitrogen at the rate of 60 ml/min. The work on the GLC of the acetates of the aldononitriles of the monosaccharides [14] and of the methyl glycosides of the methylated sugars was performed at the Tashkent Institute of Industrial Biology and Organic Chemistry of the Far Eastern Institute of Cytology of the Siberian Branch of the Academy of Sciences of the USSR.

Extraction and Isolation of the Glycosides. The comminuted roots were repeatedly extracted with hot methanol. The precipitate that deposited when the methanolic extract cooled was separated off and chromatographed on a column of silica gel in system 2. The process was monitored by TLC in the same system. The fractions enriched in acanthophyllosides B and C were reseparated on a column and eluted with

system 1. This gave acanthophylloside B (I) with mp 240-242°C, $[\alpha]_D^{20}$ +40.3 ± 2° (c 1.2; water) and acanthophylloside C (II) with mp 241-246°C, $[\alpha]_D^{30}$ −10.9 ± 2° (c 0.92; water). The combined amount of glycosides B and C amounted to 10% of the dry weight of the roots.

Acid Hydrolysis of Acanthophyllosides B (I) and C (II). The hydrolysis of 20 mg of acanthophylloside B (I) was performed in 8 ml of 5% H_2SO_4 at 100°C for 6 h. The precipitate that deposited was separated off, washed with water, dried, and identified by means of TLC in systems 9 and 10 as gypsogenin and gypsogenin glucuronoside [10].

The hydrolyzate was neutralized with $BaCO_3$, and D-glucuronic acid, D-galactose, L-arabinose, D-xylose, D-fucose, and L-rhamnose were detected by paper chromatography (PC) in systems 3 and 12 and on plates of silica gel impregnated with a 0.3 M solution of NaH_2PO_4 in system 8.

Under the same conditions, acanthoside C (II) gave D-glucuronic acid, D-galactose, D-glucose, L-arabinose, D-xylose, D-fucose, and L-rhamnose.

Hydrolysis of Acanthophyllosides B and C with 0.25 N $H_2C_2O_4$. A mixture of 0.02 of glycoside C and a 0.25 N solution of oxalic acid was heated at 80°C for 5 h. The hydrolyzate was neutralized with AV-16 anion-exchange resin and evaporated to dryness. The initial acanthophylloside C was isolated. On being heated with oxalic acid under the same conditions, acanthophylloside B likewise remained unchanged.

Saponification of Acanthophyllosides B (I) and C (II). A mixture of 10 mg of acanthophylloside C (II) and 3 ml of 10% NH_4OH was heated in the boiling-water bath for 4 h. TLC in system 1 showed the presence of a mixture of acanthophyllosides B and C.

The saponification of 2 g of acanthophylloside C was performed in 300 ml of 10% aqueous KOH at 95°C for 7 h. The reaction mixture was neutralized with KU-2 cation-exchange resin and repeatedly 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 0.35 g of gypsogenin tetraoside (III), $C_{53}H_{82}O_{24}$, mp 284-290°C (decomp.), $[\alpha]_D^{20}$ +35.6 ± 2° (c 1.01; water). In an acid hydrolyzate (5% H_2SO_4) of the tetraoside (III) D-glucuronic acid, L-arabinose, and D-galactose were identified by TLC in system 8.

When acanthophylloside B (I) was saponified, again the gypsogenin tetraoside (III), identical with that obtained from (II), was obtained.

Periodate Oxidation of (I) and (II). The oxidation of 0.1 g of glycoside C was performed in 100 ml of a 0.8% solution of $NaIO_4$ at 5°C for 24 h. The reaction was monitored by TLC in system 1. To decompose the excess of periodate, 0.3 ml of ethylene glycol was added to the solution. The reaction mixture was evaporated in vacuum to a volume of 15 ml, and then $BaCO_3$ was added and was filtered off. The filtrate was acidified with sulfuric acid to a concentration of 5% and was hydrolyzed at 100°C for 5 h.

The hydrolyzate was neutralized with $BaCO_3$, and D-xylose, D-fucose, and D-glucuronic acid were detected by GLC in system 8 and PC in systems 3 and 12.

The oxidation of compound (II) under the same conditions gave similar results.

Methylation of Acanthophyllosides B (I) and C (II). Separately, 100 mg each of (I) and (II) was dissolved in 10 ml of dimethyl sulfoxide, 100 mg of NaH was added, and the mixture was stirred for an hour. Then 2.5 ml of CH_3I was added and stirring was continued for another 4 h. The reaction was monitored by TLC in system 11. The reaction mixture in each case was poured into a concentrated solution of sodium thiosulfate and extracted with chloroform. The chloroform extracts were washed with water, dried with Na_2SO_4, and evaporated to dryness. The dry residues were dissolved in the minimum amount of benzene and filtered through a layer of Al_2O_3 (3 cm), the alumina being washed additionally with benzene (30 ml).

2,3,4-Tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-rhamnose, 2,3-di-O-methyl-L-arabinose, 2,4-di-O-methyl-D-xylose, 3-O-methyl-D-fucose, and 3-O-methyl-D-glucuronic acid were identified in the hydrolyzate of the permethylate of glycoside B by paper chromatography in systems 4 and 5 and by TLC in systems 6 and 7.

On hydrolysis, the permethylate of glycoside C gave the same set of methylated sugars and, in addition, 2,3,4,6-tetra-O-methyl-D-glucose.

Methylation of the Gypsogenin Tetraoside (III). The tetraoside (50 mg) was methylated and worked up as described above for (I) and (II). The tetraoside permethylate (15 mg) was hydrolyzed with a 5% solution of H_2SO_4 in methanol for 4 h, and then the mixture was diluted with water, the methanol was distilled

off, and hydrolysis was continued for 3 h. The hydrolyzate was neutralized with $BaCO_3$, concentrated, and chromatographed with markers by PC in systems 4 and 5 and TLC in systems 6 and 7. 2,3,4,6-Tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-arabinose, and 3-O-methyl-D-glucuronic acid were detected.

Periodate Cleavage of the Tetraoside (III). The tetraoside (0.0835 g) was oxidized with a 1% solution of sodium metaperiodate. In part of the reaction mixture the consumption of sodium periodate was determined by titration with a 0.1 N solution of $Na_2S_2O_3$, and the amount of formic acid liberated on oxidation by titration of an aliquot with a 0.01 N solution of NaOH. The oxidation of one molecule of tetraoside consumed 4.76 mole of $NaIO_4$ and liberated 2.01 mole of HCOOH.

After the hydrolysis of the reaction mixture with 5% H_2SO_4, D-glucuronic acid was identified by TLC in system 8.

Partial Hydrolysis of the Gypsogenin Tetraoside (III). A mixture of 0.5 g of compound (III) and 150 ml of a 0.25% aqueous methanolic solution (2 : 1) of H_2SO_4 was heated at 80°C for 4 h. The precipitate that deposited was separated off and chromatographed on a column of silica gel in system 1. A gypsogenin trioside (IV, 60 mg), a bioside (V, 40 mg), and gypsogenin glucuronoside (100 mg) were isolated.

Acid Hydrolysis of the Gypsogenin Trioside and Bioside. The trioside (IV) (0.01 g) was hydrolyzed with a 5% aqueous methanolic solution (2 : 1) of H_2SO_4 at 80°C for 5 h.

The hydrolyzate was neutralized with $BaCO_3$, and D-galactose, L-arabinose, and D-glucuronic acid were found in it by TLC in system 8.

Under the same conditions, the bioside (V) gave D-galactose and D-glucuronic acid.

Methylation of the Gypsogenin Trioside and Bioside. The trioside (IV) and bioside (V) (0.03 g each) were methylated and worked up as described above for (I) and (II). In a hydrolyzate of the permethylate of the trioside by TLC in systems 6 and 7 we identified 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-L-arabinose, and 2,3-di-O-methyl-D-glucuronic acid. On hydrolysis of the permethylate of the bioside we found 2,3,4,6-tetra-O-methyl-D-galactose and 3,4-di-O-methyl-D-glucuronic acid.

SUMMARY

From an extract of the roots of Acanthophyllum gypsophiloides we have isolated new triterpene glycosides – acanthophylloside B (a gypsogenin decaoside) and acanthophylloside C (a gypsogenin undecaoside). Acanthophylloside C differs from acanthophylloside B by the presence of glucose, bound to the carboxy group of D-glucuronic acid.

On saponification with alkali, acanthophyllosides B (I) and C (II) split off the acyloside carbohydrate chain and form a progenin – a gypsogenin tetraoside (III). The carbohydrate chain of (III) is attached at position 3 and has the structure [O-D-galactopyranosyl-(1 → 4)-O-L-arabopyranosyl-(1 → 4)]-[O-D-galactopyranosyl-(1 → 2)]-D-glucuronopyranose.

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