TRITERPENE GLYCOSIDES OF Gleditschia triacanthos

III. THE STRUCTURE OF TRIACANTHOSIDE G

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It is known [1, 2] that the pericarps of Gleditschia triacanthos L. (family Leguminosae) contain not less than ten triterpene glycosides, and the structure of one of them – triacanthoside C – has been partially determined.

In the present paper we give information on the determination of the structure of the following glycoside – triacanthoside G, $C_{63}H_{102}O_{30}$.

As found on acid hydrolysis, triacanthoside G consists of an aglycone (echinocystic acid) and the sugars D-glucose, D-xylose, L-arabinose, and L-rhamnose in a ratio of 2 : 1 : 2 : 1 (GLC).

The alkaline saponification of triacanthoside G gave a trioside of echinocystic acid, $C_{46}H_{74}O_{17}$, containing D-glucose, D-xylose, and L-arabinose (1 : 1 : 1). Hydrolysis of the oligosaccharide obtained on saponification yielded D-glucose and L-arabinose. The absence of L-rhamnose shows that it was attached directly to the carboxyl of the aglycone and underwent degradation under the conditions of alkaline hydrolysis. Thus, triacanthoside G consists of a hexaoside of echinocystic acid and contains not less than two carbohydrate chains, one of which is attached to the carboxyl of the aglycone and consists of D-glucose, L-arabinose, and L-rhamnose.

On hydrolysis of the product of the exhaustive methylation of triacanthoside G, 16-O-methylechinocystic acid, 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-xylose, 2,4,6-tri-O-methyl-D-glucose, 2,3-di-O-methyl-L-arabinose, 2,3-di-O-methyl-L-rhamnose, and 2,4-di-O-methyl-L-arabinose were identified. It is clear from this that the second carbohydrate chain of triacanthoside G is attached to the C_3 hydroxyl of echinocystic acid and its terminal monosaccharide is D-xylose.

In addition, it is obvious that the terminal sugar of the acyloside chain is D-glucose. The absence of monomethylated derivatives of the pentoses and of dimethyl derivatives of glucose shows that both carbohydrate chains are straight with no branching.

On hydrolysis, the permethylate of the trioside of echinocystic acid isolated from the alkaline hydrolysis of triacanthoside G gave 2,3,4-tri-O-methyl-D-xylose, 2,4,6-tri-O-methyl-D-glucose, and 2,3-di-O-methyl-L-arabinose. By comparing the composition of the methylated monosaccharides of the trioside and of triacanthoside G, also taking into account the direct attachment of the L-rhamnose to the carboxyl group of the aglycone, the structure of the acyloside chain may be represented as follows: –COO–L-Rhap-(4 ← 1)-L-Arap-(3 ← 1)-D–Gp. The structure of the carbohydrate chain attached to the hydroxyl at C_3 of echinocystic acid cannot be decided definitely from the information obtained so far. It is clear merely that the terminal sugar is D-xylose and this is attached to glucose by its third hydroxyl or to arabinose by its fourth hydroxyl.

The sequence of bonds in the two chains is also confirmed by the fact that on periodate oxidation of triacanthoside G the residual sugars were D-glucose and L-arabinose, while on the oxidation of the trioside of echinocystic acid only D-glucose was found.

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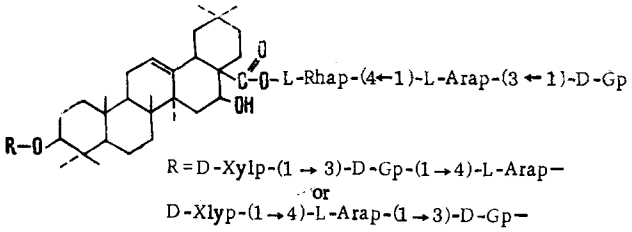
On this basis, the following probable structure may be put forward for triacanthoside G:



It is interesting to note that the structures of the carbohydrate chains in triacanthosides C and G are similar. The acyloside chains in them are completely identical. The glycoside chains are also probably identical, but in any case they have the same monosaccharide components and the same positions of the linkages between them. In this case, for glycosides with different aglycones the hypothesis put forward previously [3] that the formation of acyloside chains may take place directly in large blocks, i.e., with oligosaccharides of a definite structure, is confirmed.

EXPERIMENTAL

Chromatography was performed on type M (slow) paper of the Goznak Leningrad Mill and with type KSK silica gel using the following solvent systems: 1) chloroform–methanol–water (65 : 35 : 10); 2) butan-1-ol–acetic acid–water (4 : 1 : 5); 3) butan-1-ol–pyridine–water (6 : 4 : 3); 4) chloroform–ethanol (25 : 2); 5) benzene–acetone (2 : 1); and 6) methyl ethyl ketone saturated with water.

Gas-liquid chromatography 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) at a column temperature of 176°C with hydrogen as the carrier gas at the rate of 55 ml/min.

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

Isolation of Triacanthoside G. The fractions with glycosides E, F, and G and with F and G obtained as described in the preceding paper [2] were chromatographed on a column of silica gel in system 1, the fractions being monitored on plates in the same system. The eluates enriched in triacanthoside G were combined, evaporated, and rechromatographed on a column of silica gel in system 1. After recrystallization from aqueous butanol, the triacanthoside G, $C_{63}H_{102}O_{30}$, had mp 170-178°C (decomp.), $[\alpha]_D^{20}$ −13.6° (c 1.44; 70% methanol).

Acid Hydrolysis of Triacanthoside G. A mixture of 50 mg of the glycoside and 5 ml of 5% sulfuric acid was heated for 5 h. The precipitate that deposited was identified by thin-layer chromatography in system 4 as echinocystic acid. After neutralization with barium carbonate, the aqueous solution was found by paper chromatography in systems 2 and 3 to contain D-glucose, D-xylose, L-arabinose, and L-rhamnose. The ratio of the sugars was 2 : 1 : 2 : 1 (GLC).

Alkaline Saponification of Triacanthoside G. The glycoside (100 mg) in 10 ml of 10% caustic potash was dissolved in 70% ethanol and the solution was heated on the water bath for 10 h and was then neutralized with dilute sulfuric acid. After recrystallization from aqueous methanol, the precipitate of an echinocystic acid trioside, $C_{46}H_{74}O_{17}$, had mp 213-215°C, $[\alpha]_D^{20}$ −16.5° (c 1.8; methanol).

The aqueous solution from which the trioside had been separated was evaporated and the residue was hydrolyzed with 5% sulfuric acid. The hydrolyzate was shown by paper chromatography in systems 2 and 3 to contain D-glucose and L-arabinose.

Acid Hydrolysis of the Trioside. A mixture of 20 mg of the echinocystic acid trioside and 2 ml of 5% sulfuric acid was heated for 5 h. After neutralization with barium carbonate, the hydrolyzate was found by paper chromatography in systems 2 and 3 to contain D-glucose, D-xylose, and L-arabinose in a ratio of 1 : 1 : 1 (GLC).

Methylation of Triacanthoside G and of the Trioside. Triacanthoside G (200 mg) was methylated by Hakomori's method [4]. The permethylate was heated in a 5% methanolic solution of hydrochloric acid at 100°C for 5 h. Then the mixture was diluted with water, the aglycone was separated off, and the solution

was heated for another 2 h. The hydrolyzate was neutralized with AV-16 ion-exchange resin and was then found by thin-layer chromatography in system 5 with markers and by paper chromatography in system 6 to contain 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-L-arabinose, 2,3-di-O-methyl-L-rhamnose, 2,4-di-O-methyl-L-arabinose, and 2,4,6-tri-O-methyl-D-glucose.

The precipitate of the aglycone that deposited was found by thin-layer chromatography in system 4 with a marker to be 16-O-methylechinocystic acid.

The trioside of echinocystic acid, after methylation and hydrolysis under the same conditions, gave 2,3,4-tri-O-methyl-D-xylose, 2,4,6-tri-O-methyl-D-glucose, and 2,3-di-O-methyl-L-arabinose.

Periodate Oxidation of Triacanthoside G and the Trioside. A solution of 50 mg of triacanthoside G in 15 ml of a 1% solution of sodium metaperiodate was left in the dark at room temperature for two days. After the end of the reaction, the excess of periodate was destroyed with ethylene glycol, the solution was evaporated, and the residue was extracted with methanol. The evaporated methanolic extract was hydrolyzed with 5% sulfuric acid. The barium-carbonate-neutralized hydrolyzate was analyzed by paper chromatography in systems 2 and 3. The residual sugars D-glucose and L-arabinose were detected.

Only D-glucose was found in a hydrolyzate of the trioside of echinocystic acid oxidized under the same conditions.

SUMMARY

The partial structure of triacanthoside G, a hexaoside of echinocystic acid isolated from the pericarps of Gleditschia triacanthos L., has been determined. The acyloside chain has the structure of D-glucopyranosyl-(1→3)-L-arabopyranosyl-(1 →4)-L-rhamnopyranoside. So far as concerns the chain attached to the hydroxyl at C_3 of the aglycone, its qualitative and quantitative composition and the possible sequence of bonds have been determined.

LITERATURE CITED

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