TRITERPENE GLYCOSIDES OF Gleditschia triacanthos

II. THE STRUCTURE OF TRIACANTHOSIDE C

T. A. Badalbaeva, E. S. Kondratenko,
L. G. Mzhel'skaya, and N. K. Abubakirov

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In the pericarps of the decorative tree Gleditschia triacanthos L. (family Leguminosae) we have found triterpene glycosides – triacanthosides A, B, C, and D [1]. In a more careful study of them it was found that glycosides A, B, and D are not individual substances but consist of mixtures of several compounds. It has been established by thin-layer chromatography that triacanthoside A consists of three glycosides – A, A_1, and A_2 – and triacanthoside B of two – B and B_1. Because of their very small amounts, the glycosides mentioned (apart from A_1) have not yet been isolated in the individual state. It has been found merely that their aglycones are oleanolic and echinocystic acids and the sugar components include glucose, xylose, arabinose, and rhamnose.

Triacanthoside D has been separated into four compounds – triacanthosides D, E, F, and G. Triacanthosides D, F, and G are glycosides of echinocystic acid and E of oleanolic acid. The sugar moiety of all four substances consists of the same sugars – glycose, xylose, arabinose, and rhamnose. Thus, it has been established that the pericarps of the common honeylocust contain not less than ten triterpene glycosides. Triacanthosides C and G are present in predominating amount, and the others in very small amount.

As already shown [1], triacanthoside C is a glycoside of oleanolic acid. According to gas-liquid chromatography of the silyl derivatives, the sugar moiety of the glycoside comprises D-glucose, D-xylose, L-arabinose, and L-rhamnose in a ratio of 2 : 1 : 2 : 1 and, consequently, triacanthoside C is a hexaoside, $C_{63}H_{102}O_{29}$.

The alkaline saponification of triacanthoside C gave a trioside of oleanolic acid, $C_{46}H_{74}O_{16}$, containing D-glucose, D-xylose, and L-arabinose (1 : 1 : 1). An acid hydrolyzate of the oligosaccharide split off on alkaline saponification was found to contain D-glucose and L-arabinose. The absence of L-rhamnose shows that this is directly connected to the carboxyl of the aglycone and is destroyed on alkaline hydrolysis.

When triacanthoside C was subjected to periodate oxidation, D-glucose and L-arabinose were unaffected, and when the trioside obtained by alkaline saponification was subjected to oxidation under the same conditions the residual sugar was D-glucose. Consequently, the sugars mentioned form centers of branching of the carbohydrate chain or have substituents at the hydroxyl in position 3, and the residual glucose is part of the composition of the trioside, while the arabinose is present in the acyloside chain.

Basic information on the structure of the carbohydrate chains of triacanthoside C was obtained by a study of the products of exhaustive methylation. In a hydrolyzate of completely methylated triacanthoside C we found 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. A permethylate of the trioside obtained by alkaline hydrolysis gave 2,3,4-tri-O-methyl-D-xylose, 2,3-di-O-methyl-L-arabinose, and 2,4,6-tri-O-methyl-D-glucose.

It follows from this that in the acyloside chain L-rhamnose is directly attached to the carboxyl, L-arabinose is attached directly to this at the fourth hydroxyl, and then there is a terminal D-glucose residue. This is attached to the arabinose at the C_3 hydroxyl. So far as concerns the second carbohydrate chain,

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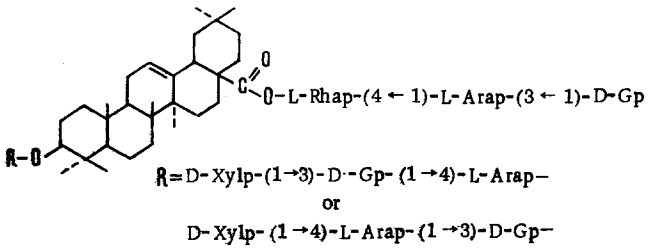
here, undoubtedly, the terminal sugar is D-xylose. The sequence of attachment of the other two sugars (glucose and arabinose) requires additional investigations, although it may be considered as already established that the linkage is effected at the third hydroxyl of the glucose and at the fourth hydroxyl of the arabinose.

On the basis of all this information, the following probable structure may be put forward for triacanthoside C.



EXPERIMENTAL

Chromatography was performed on type M (slow) paper of the Goznak Leningrad Mill and type KSK silica gel with the following systems of solvents: 1) butan-1-ol–ethanol–25% ammonia (10 : 2 : 5); 2) the same components (5 : 2 : 5); 3) chloroform–methanol–water (65 : 35 : 10); 4) butan-1-ol–acetic acid–water (4 : 1 : 5); 5) butan-1-ol–pyridine–water (6 : 4 : 3); 6) chloroform–ethanol (25 : 2); 7) benzene–acetone (2 : 1); and 8) methyl ethyl ketone saturated with water.

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

The 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 a rate of 55 ml/min.

Extraction and Isolation of the Combined Glycosides and Oligosaccharides. The comminuted pericarps were defatted with chloroform and were then exhaustively extracted with hot methanol. The combined extracts were dried at 40°C in vacuum. The residue from the methanolic extract was dissolved in water and extracted with butanol. The butanolic extracts were combined and evaporated to dryness in vacuum.

Purification and Fractionation of the Glycosides. The substances extracted by butanol (20 g) were chromatographed on a column 1.5 m high containing type KSK silica gel (2 kg) in system 2. The fractions were monitored by thin-layer chromatography in systems 1 and 2. The fractions containing triterpene glycosides but free from oligosaccharides were combined, evaporated to dryness in vacuum, and rechromatographed on a column of silica gel in system 3 with monitoring by the same method. Fractions containing the glycosides A, A_1, A_2, B, and B_1; B, B_1, C, and D; C, D, E, and F; E, F, and G; and F and G were isolated.

The dry residue from the fraction containing the glycosides A, A_1, A_2, B, and B_1 was hydrolyzed by being heated with 5% sulfuric acid (6 h). The precipitate that deposited was identified by thin-layer chromatography in system 6 as consisting of oleanolic and echinocystic acids. The hydrolyzates was shown by paper chromatography in systems 4 and 5 to contain D-glucose, D-xylose, L-arabinose, and L-rhamnose.

Isolation of Triacanthoside C. The combined fractions with substances B, B_1, C, and D and with C, D, E, and F were chromatographed on a column of silica gel in system 3 with monitoring in the same system. The eluates enriched in triacanthoside C were evaporated and were chromatographed several more times on a column in the same system. The fractions containing triacanthoside C alone were evaporated to dryness in vacuum, and the residue was dissolved in hot water-saturated butanol. Acicular crystals of triacanthoside C, $C_{63}H_{102}O_{29}$, were deposited with mp 230-234°C (decomp.), $[\alpha]_D^{20}$ −14° (c 0.8; 70% methanol).

Acid Hydrolysis of Triacanthoside C. The glycoside (50 mg) was hydrolyzed in 5 ml of 5% sulfuric acid with heating (5 h). The resulting precipitate was found by thin-layer chromatography in system 6 to contain oleanolic acid and the hydrolyzate by paper chromatography in systems 4 and 5 to contain D-glucose, D-xylose, L-arabinose, and L-rhamnose.

Alkaline Hydrolysis of Triacanthoside C. A mixture of 100 mg of the glycoside and 10 ml of 10% caustic potash in 70% ethanol was heated in the boiling water for 10 h, and then the reaction mixture was neutralized with dilute sulfuric acid. The precipitate that deposited (a trioside of oleanolic acid, $C_{46}H_{74}O_{16}$) had mp 200-208°C (from aqueous methanol), $[\alpha]_D^{20}$ −5.5° (c 1.03; methanol).

The neutralized aqueous solution after the separation of the trioside was evaporated, chromatographed in system 1 on a column of silica gel, and then hydrolyzed with 5% sulfuric acid. After neutralization with barium carbonate the hydrolyzate was found by paper chromatography in systems 4 and 5 to contain D-glucose and L-arabinose.

Acid Hydrolysis of the Trioside. The trioside of oleanolic acid (20 mg) was hydrolyzed by heating with 2 ml of 5% sulfuric acid for 5 h. After neutralization with barium carbonate, the hydrolyzate was found by paper chromatography in systems 4 and 5 to contain D-glucose, D-xylose, and L-arabinose (ratio 1 : 1 : 1, GLC).

Periodate Oxidation of Triacanthoside C and the Trioside. To a solution of 50 mg of the glycoside in 15 ml of water was added 0.15 g of sodium metaperiodate, and the mixture was left in the dark at room temperature for two days. Then the excess of periodate was destroyed with ethylene glycol, and the mixture was evaporated and extracted with methanol. The material from the methanolic extract was hydrolyzed with 5% sulfuric acid. The hydrolyzate was found by paper chromatography in systems 4 and 5 to contain the residual sugars D-glucose and L-arabinose. A hydrolyzate of the trioside oxidized under the same conditions was found to contain D-glucose.

Methylation of Triacanthoside C and the Trioside. The triacanthoside (200 mg) was methylated by Hakomori's method [2]. The permethylate was heated in a 5% methanolic solution of hydrochloric acid (100°C, 5 h). The mixture was diluted with water, the aglycone was separated off, and the filtrate was heated for another 2 h. The hydrolyzate was neutralized with AV-16 anion-exchange resin, and then by thin-layer chromatography in system 7 and paper chromatography in system 8 with markers the following methylated sugars were identified: 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 oleanolic acid trioside, 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.

SUMMARY

It has been shown that the pericarps of Gleditschia triacanthos L. contain not less than ten triterpenoid glycosides. The partial structure of triacanthoside C – a hexaoside of oleanolic acid – has been established.

LITERATURE CITED

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