TRITERPENE GLYCOSIDES OF Gleditschia triacanthos

V. STRUCTURE OF TRIACANTHOSIDE C AND THE MINOR GLYCOSIDES

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We have previously reported the determination of the partial structure of triacanthoside C – a triterpene glycoside from the pericarps of Gleditschia triacanthos L. (common honeylocust) [1]. In the present paper we give information to prove the complete structure of this glycoside and the results of an investigation of the chemical structures of the minor glycosides from the green pods and leaves of this plant.

In triacanthoside C (I) the sequence of the sugars in the oligosaccharide bound by a glycosidic bond with the aglycone remained obscure. We have performed the stepwise hydrolysis of the oleanolic acid trioside (III) (the progenin of triacanthoside C) obtained by the alkaline saponification of (I). A bioside (V) and a monoside (VII) were isolated in preparative amounts. The acid cleavage of (V) yielded glucose and arabinose, and that of (VII) yielded glucose. In a hydrolyzate of permethylated (VII) 2,3,4,6-tetra-O-methyl-D-glucose was identified, and in a hydrolyzate of the permethylate of (V) 2,4,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-arabinose. Consequently, in compound (III) the glucose is attached to the hydroxyl of the oleanolic acid, and the arabinose is attached to the glucose at C_3. The trioside has structure (III) and triacanthoside C structure (I).

As can be seen from the formula of triacanthoside C, its two carbohydrate chains are identical in structure with the oligosaccharides from the hexaoside of echinocystic acid – triacanthoside G (II) [2]. In an extract of the ripe pods we also found a trioside of echinocystic acid (the progenin of triacanthoside G) – triacanthoside A_1 (IV). Such cases of the simultaneous presence in plants of glycosides with identical structures of the sugar moieties attached to the hydroxyls at C_3 but, respectively, acylated and nonacylated at the C_{28} carboxy group have been described in the literature repeatedly [3-5], and a report has appeared according to which the acylosides are usually formed by the direct addition of a large oligosaccharide molecule [3].

However, the progenin of triacanthoside C – the oleanolic acid trioside (III) – could not be detected in the ripe pods of the honeylocust. In view of the fact that in the ripe pods the processes of biosynthesis are already completed, in the main, simple glycosides must be sought in the still vegetating organs of the plant. For this purpose, we performed a preliminary chromatographic study of an extract of green pods and the leaves of the honeylocust. The results of the analysis of a butanol extract of the green pods showed that it contained, in addition to the glycosides A, A_1, A_2, B, B_1, C, D, E, F, and G [1], which have been found in the ripe pericarps and formed the bulk of this extract, oleanolic and echinocystic acids in the free state, and also compounds with a glycosidic nature less polar than the triacanthosides.

From the time of the first publications on complex triterpene glycosides (oligosides) [6, 8], a tradition has grown up of giving them trivial names with additional letter symbols. The least polar glycoside was given the first letter of the Latin alphabet A and the more polar ones, usually containing longer carbohydrate chains, the following letter symbols B, C, and so on. The inconvenience of this system was shown as soon as intermediate compounds remaining undetected in the first investigations were isolated. This problem was solved by the additional symbols A_1, A_2, A_3, etc. [9-10]. The position with the triterpene glycosides mentioned was complicated after glycosides of different sapogenins began to be found in one and the same plant. It also occurred that glycosides with different lengths of their carbohydrate chains proved to

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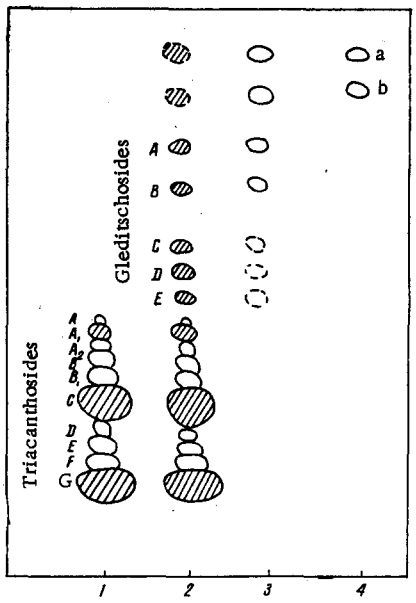


Fig. 1. Thin-layer chromatography of triterpene glycosides from various organs of the honeylocust [$CHCl_3$–CH_3OH–H_2O (65:35:10) system]: 1) combined triacanthosides from the pericarps of the ripe pods; 2) combined gleditschosides and triacanthosides from the green pods; 3) gleditschosides from the leaves; 4) oleanolic (a) and echinocystic (b) acids (compounds isolated in the individual state hatched).

be close to one another and, conversely, compounds with the same number of sugar residues were given letter symbols remote from one another because of the different polarities of the sapogenins.

Consequently, the question of the terminology of the triterpene glycosides has required special discussion. We have maintained the gradually established tradition by not giving special trivial names to simple monoglycosides, limiting ourselves to combining the name of the aglycone and that of the sugar residue (for example, echinocystic acid β-D-glucopyranoside). However, beginning with the diglycosides, because of the multiplicity of possible combinations connected with the differences in the positions of attachment of the sugar residues, the size of the oxide ring, and anomerism, the observance of this semirational nomenclature will apparently be difficult. In the present paper, for the minor glycosides of the honeylocust we have been forced to introduce new trivial names – gleditschosides A, B, C, D, and E – which have been given to the individual compounds in order of their increasing polarity. Only the gleditschosides and the aglycones have been detected in the leaves, while the triacanthosides are apparently absent from the green parts (Fig. 1).

By preparative chromatography (on columns and plates), from a butanolic extract of the green pods we have isolated, in addition to the triacanthosides, gleditschosides A, B, C, D, and E, and also oleanolic and echinocystic acids. (See scheme on following page.)

The results of a comparison of the physicochemical constants, chromatographic behavior, acid hydrolysis, and exhaustive methylation have shown that gleditschoside A is oleanolic acid β-D-glucopyranoside (VII), gleditschoside B is echinocystic acid β-D-glucopyranoside (VIII), gleditschoside C has structure (V), gleditschoside D corresponds to structure (III) and is identical with the progenin of triacanthoside C, and gleditschoside E corresponds to structure (VI). The configurations of the glycosidic centers were calculated by molecular-rotation differences.

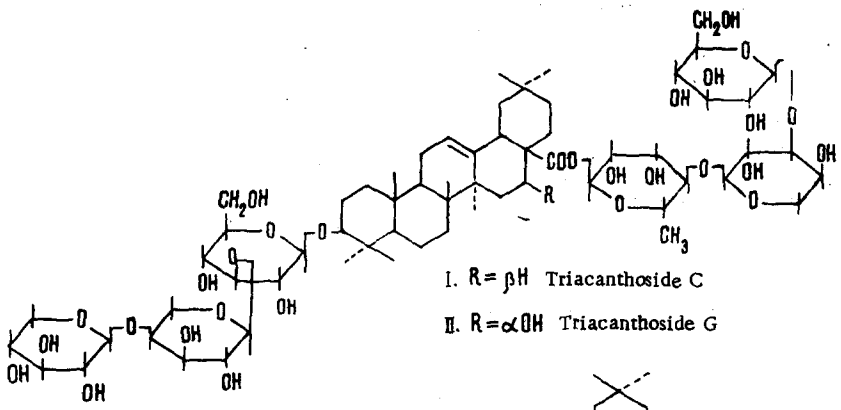
Thus, gleditschosides A, C, and D, as has been shown above, are identical with the intermediate glycosides obtained by the cleavage of triacanthoside C (I), and gleditschosides B and E are identical with the glycosides obtained by the stepwise hydrolysis of triacanthoside A_1 (IV) [2].

Rough estimates of the amounts of glycosides in the air-dried plant raw material are as follows (%): 1) in the ripe pods, total triacanthosides 1.5, triacanthoside A_1 0.1, triacanthoside C 0.6, and triacanthoside G 0.4; 2) in the green pods, combined triacanthosides 0.8, gleditschoside A 0.005, gleditschoside B 0.008, gleditschoside C 0.004, gleditschoside D 0.130, gleditschoside E 0.010, and oleanolic and echinocystic acids – traces.

The results of a comparison of the qualitative compositions of the glycosides in the ripe pericarps, green pods, and leaves permit some conclusions concerning the biogenesis of these substances in the honeylocust. It is obvious that the synthesis of the simple glycosides takes place in the leaves where the carbohydrate chain at the hydroxyl in position 3 grows gradually by the addition of one monosaccharide in each step. Then in the green pods the transition from the simple O-glycosides to the complex O-acylosides takes place by the addition to the carboxyl of the aglycone of the whole trisaccharide fragment [3]. The synthesis is completed in the ripe pericarps, and we find mainly complex O-acylglycosides.

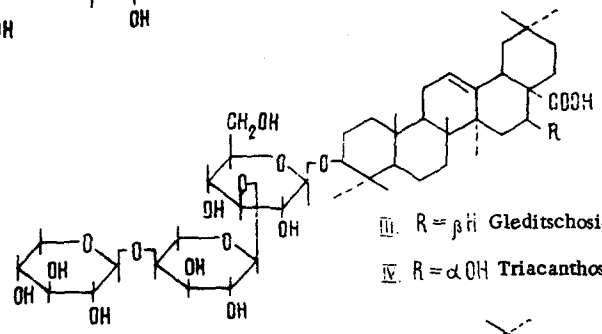
EXPERIMENTAL

Chromatography was performed with type KSK silica gel, "slow" paper, and the following solvent systems: 1) chloroform–methanol–water (68:35:10); 2) chloroform–methanol (25:2); 3) butan-1-ol–ace-



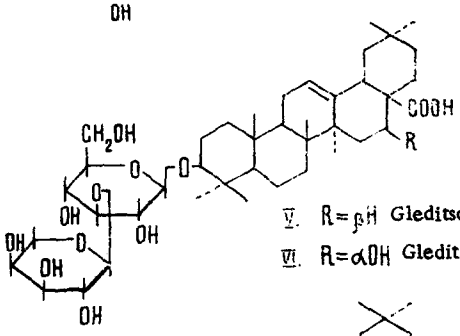
I. R = βH Triacanthoside C

II. R = αOH Triacanthoside G



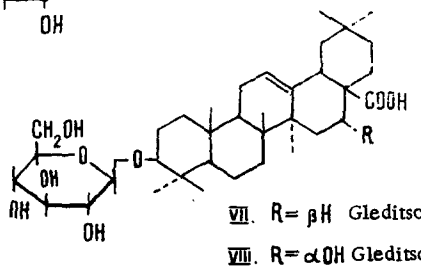
III. R = βH Gleditschoside D

IV. R = αOH Triacanthoside A



V. R = βH Gleditschoside C

VI. R = αOH Gleditschoside E



VII. R = βH Gleditschoside A

VIII. R = αOH Gleditschoside B

tone–water (4 : 5 : 1); 4) benzene–acetone (2 : 1); and 5) water-saturated methyl ethyl ketone. The free sugars were chromatographed in a thin layer of KSK silica gel impregnated with a 0.2 M solution of sodium dihydrogen phosphate [11].

Stepwise Hydrolysis of the Oleanolic Acid Trioside (III). The glycoside (III) (500 mg) obtained by the method described previously [1] was heated in 30 ml of a 0.25% solution of sulfuric acid in aqueous methanol (1 : 1) at 90°C for 50 min. The reaction mixture was diluted with 30 ml of water, the methanol was distilled off, and the acid hydrolyzate was extracted with butan-1-ol. The butanolic extracts were washed with water and evaporated to dryness. The hydrolysis products were separated preparatively by thin-layer chromatography (TLC) on plates (25 × 36 cm) in system 1. The following were isolated in the individual state: 23 mg of the monoside (VII) with mp 241-244°C, $[\alpha]_D^{20}$ + 53.2 ± 2° (c 1.08; methanol) [literature data [12] for oleanolic acid β-D-glucopyranoside: mp 247-249°C, $[\alpha]_D^{20}$ + 56° (pyridine)], and 55 mg of the bioside (V) with mp 209-214°C, $[\alpha]_D^{20}$ + 1.9 ± 2°, (c 2.2; methanol).

Hydrolysis of the Monoside (VII) and of the Bioside (V). The monoside (VII) (8 mg) and the bioside (V) (15 mg) were each, separately, heated with 3 ml of a 5% solution of sulfuric acid in aqueous methanol (1 : 1) at 90°C for 5 h. The solid matter was separated off and the reaction mixture was neutralized with barium carbonate. The hydrolyzate of the monoside (VII) was shown by TLC in system 3 to contain D-glucose, and in the hydrolyzate of the bioside (V) D-glucose and L-arabinose were identified similarly. The precipitate that had deposited was identified by TLC in system 2 as oleanolic acid in each case.

Methylation of the Monoside (VII) and of the Bioside (V). The monoside (VII) (10 mg) was methylated by Hakomori's method [13], and the methylation product was hydrolyzed with 5% H_2SO_4. The hydrolyzate was shown by paper chromatography (PC) in system 5 and by TLC in system 4 with markers to contain 2,3,-4,6-tetra-O-methyl-D-glucose.

The bioside (V) (30 mg) was methylated under the same conditions. After the hydrolysis of the permethylated glycosides, 2,3,4-tri-O-methyl-L-arabinose and 2,4,6-tri-O-methyl-D-glucose were identified in the reaction products by the method described above.

Extraction of the Green Pods and the Leaves. The air-dried green pods (1 kg) collected in June were first defatted with chloroform and were exhaustively extracted with boiling methanol. The residue obtained after the distillation of the solvent in vacuum (112 g) was dissolved in 1 liter of water and extracted with butan-1-ol. The yield of combined saponins was 12 g.

The air-dried leaves (200 g), previously treated with ether and chloroform, were extracted with methanol with heating. The concentrated methanolic extract was dissolved in water and extracted with butan-1-ol. The yield of combined products was 1.3 g.

Isolation of Gleditschosides A, B, C, D, and E and the Aglycones. A dry butanolic extract of the green pods (12 g) was transferred to a column of silica gel (700 g). Elution in system 1 gave a fraction containing a mixture of oleanolic and echinocystic acids and then fractions with gleditschosides ABC, BCD, and CDE and with triacanthosides $AA_1A_2BB_1$, BB_1CDE, and DEFG. The glycoside compositions of the fractions were checked by TLC in the same system. From the fraction of the free aglycones in system 2 (plates of the size shown) we performed the preparative isolation of oleanolic acid with mp 301-306°C, $[\alpha]_D^{20}+78.8°$ (c 0.60; chloroform) and echinocystic acid with mp 304-306°C, $[\alpha]_D^{20}+31°$ (c 0.75; chloroform).

To obtain the individual gleditschosides A, B, C, D, and E, the fractions containing mixtures of these substances were also separated preparatively on plates in system 1. In this way we obtained 16 mg of oleanolic acid β-D-glucopyranoside (VII), $C_{36}H_{58}O_8$, with mp 243-246°C, $[\alpha]_D^{20}+50\pm3°$ (c 0.61; methanol), 17.5 mg of echinocystic acid β-D-glucopyranoside (VIII), $C_{36}H_{58}O_9$, mp 265-268°C, $[\alpha]_D^{20}+16.5\pm2°$ (c 0.54; methanol), 20.3 mg of gleditschoside C (V), $C_{41}H_{66}O_{12}$, mp 210-218°C (decomp.), $[\alpha]_D^{20}+2.0\pm1°$ (c 0.8; methanol), 25 mg of gleditschoside E (VI), $C_{41}H_{66}O_{13}$, mp 220-229°C (decomp.), $[\alpha]_D^{20}-13.2\pm2°$ (c 1.1; methanol), and 21 mg of gleditschoside D (III), $C_{46}H_{74}O_{16}$, mp 212-224°C (decomp.), $[\alpha]_D^{20}-16.8\pm2°$ (c 0.75; methanol).

Acid Hydrolysis of Gleditschosides A (VII) and B (VIII) and the Products of Their Methylation. Glycoside (VII) (3 mg) was heated in 2 ml of a 5% solution of sulfuric acid in aqueous methanol (1 : 1) at 90°C for 5 h. In the hydrolyzate, D-glucose was found by TLC in system 3 and oleanolic acid in system 2. Glycoside (VIII) (5 mg) was hydrolyzed under similar conditions. The hydrolyzate was shown by TLC in system 3 to contain D-glucose and in system 2 echinocystic acid.

Gleditschosides (VII) and (VIII) (10 mg each) were methylated by Hakomori's method [13]. In the hydrolysis products of the permethylates of compounds (VII) and (VIII), each investigated separately by PC in system 5 and by TLC in system 4 with markers, 2,3,4,6-tetra-O-methyl-D-glucose was identified.

Acid Hydrolysis of Gleditschosides C (V) and E (VI) and of the Products of Their Exhaustive Methylation. Compounds (V) and (VI) (5 mg each) were hydrolyzed under the conditions given above. By the TLC method, D-glucose and L-arabinose were found in the hydrolyzates of (V) and (VI) (system 3); in addition to the sugars of glycoside (V), oleanolic acid was found among the hydrolysis products, and echinocystic acid was found from the glycoside (VI) (TLC, system 2). Gleditschosides (V) and (VI) (10 mg each) were methylated by Hakomori's method [13]. After the cleavage of the permethylated products, in each of the hydrolyzates from (V) and (VI) 2,4,6-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-arabinose were found by PC in system 5 and TLC in system 4 with markers.

Acid Hydrolysis of Gleditschoside D (III) and Its Permethylate. The native glycoside (III) (10 mg) was hydrolyzed as described above. The hydrolyzates were shown by TLC in system 3 to contain D-glucose, L-arabinose, and D-xylose and, in system 2, oleanolic acid.

Glycoside (III) (10 mg) was methylated under conditions similar to those for the other glycosides. In the products of the hydrolysis of the permethylate by PC in system 5 and TLC in system 4 with markers, 2,3,4-tri-O-methyl-D-xylose, 2,3-di-O-methyl-L-arabinose, and 2,4,6-tri-O-methyl-D-glucose were identified.

SUMMARY

The complete structure of the triterpene glycoside triacanthoside C (I) isolated from the pericarps of the honeylocust, Gleditschia triacanthos L. (family Leguminosae) has been established as a hexaoside of oleanolic acid.

From the green pods of the honeylocust, in addition to the ten triacanthosides described previously, oleanolic and echinocystic acids have been isolated, and also five other triterpene glycosides—gleditschosides A, B, C, D, and E. Gleditschoside A, $C_{36}H_{58}O_8$, is oleanolic acid β-D-glucopyranoside (VII), gleditschoside B, $C_{36}H_{58}O_9$ is echinocystic acid β-D-glucopyranoside (VIII), gleditschoside C, $C_{41}H_{66}O_{12}$, is oleanolic acid 3-O-[α-L-arabinopyranosyl-(1 → 3)-O-β-D-glucopyranoside] (V), gleditschoside E, $C_{41}H_{66}O_{13}$, is echinocystic acid 3-O-[α-L-arabinopyranosyl-(1 → 3)-O-β-D-glucopyranoside] (VI), and gleditschoside D, $C_{46}H_{74}O_{16}$, is oleanolic acid 3-O-[β-D-xylopyranosyl-(1 → 4)-O-α-L-arabopyranosyl-(1 → 3)-O-β-D-glucopyranoside]. Gleditschosides A, B, C, D, and E have also been detected in the leaves of the plant.

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