STRUCTURE OF THE TRITERPENE GLYCOSIDES FROM THE ROOTS OF Chenopodium anthelminticum

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UDC 547.913+547.918

In popular medicine aqueous alcoholic extracts of the roots of Chenopodium anthelminticum (drug wormseed goosefoot), which contains saponins, are used as a sedative and analgesic agent [1].

We have investigated the roots of drug wormseed goosefoot collected in October, 1968 in the Poltava oblast of the Ukrainian SSR. After the purification of the extract by thin-layer chromatography, we established the presence in it of two compounds which we have called chenopodium saponins A and B.

In both cases, the hydrolytic cleavage of the glycosides yielded the aglycone – echinocystic acid [2]. In substance A, glucose, arabinose, and xylose were found, while saponin B also contains rhamnose. The selective alkaline saponification of glycoside B led to its conversion into saponin A.

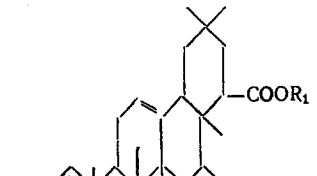
The type of bond between the monosaccharides was determined by methylation [3]. After the cleavage of the permethylated saponin A, 2,3,4-tri-O-methyl-D-xylose, 2,3,6-tri-O-methyl-D-glucose, and 3,4-di-O-methyl-L-arabinose were identified, while for the permethylated glycoside B 2,3-di-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-glucose were found in addition. The aluminum hydride cleavage of the methylated chenopodium saponin B gave a reduced glycoside having the same set of sugars as in the case of compound A, and also an oligosaccharide decomposing into 2,3-di-O-methyl-L-rhamnitol and 2,3,4,6-tetra-O-methyl-D-glucose.

The attachment of one carbohydrate chain to the hydroxyl at C_3 of the aglycone was assumed by analogy with other triterpene glycosides [4].

The sequence of sugars in the carbohydrate chain at the C_3 of the aglycone was established by the isolation, on partial acid hydrolysis, of echinocystic acid arabinoside and a disaccharide consisting of arabinose and xylose.

On the basis of these results, the most probable structure of chenopodium saponins A and B can be represented by the following formulas, I and II:

I R = DXylp 1→6DGl$_p$ →2LAr$_p$ →
R_1=H
II R = DXyl$_p$ 1→6DGl$_p$ →2LAr$_p$ →
R_1=DGl$_p$ 1→4LRha$_p$ →



Institute of Chemistry, Academy of Sciences of the Moldavian SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 27-30, January, 1971. Original article submitted October 23, 1970.

EXPERIMENTAL

The work was carried out with type KSK silica gel and alumina (activity grade II) and with chromatographic paper of the Volodarskii Leningrad Mill with the use of the following solvent systems: 1) butan-1-ol–acetic acid–water (4:1:5); 2) butan-1-ol–benzene–pyridine–water (5:1:3:3); 3) toluene–ethanol (10:1); 4) benzene–acetone (2:1); 5) chloroform–methanol–water (55:35:100); and 6) chloroform–ethyl acetate (2:1).

The comminuted air-dry roots (1 kg) were extracted with 70% aqueous methanol. Yield 50 g. The evaporated extract was dissolved in 500 ml of water and extracted successively to decoloration with chloroform, diethyl ether, and petroleum ether. The contaminating substances were removed, and the aqueous solution was extracted with butanol. The organic extracts were concentrated to dryness giving 15 g of residue.

Separation of the Chenopodium Saponins. The combined substances (3 g) were dissolved in 30 ml of mixture 5 and the solution was transferred to a column of silica gel (100 × 7 cm). Elution was performed with the same mixture, 10-ml fractions being collected. The process was monitored in thin layers of silica gel in systems 1 and 5. Fractions 3 to 5 contained saponin A with mp 257-259°C, $[\alpha]_D^{20}$ +53° (c 0.77; methanol) and fractions 13 to 15 saponin B with mp 230-232°C, $[\alpha]_D^{20}$ +15° (c 1.3; H_2O).

Hydrolysis of Chenopodium Saponins A and B. Glycoside A (2 g) was heated with 40 ml of 7% HCl in methanol (1:1) at 100°C for 6 h. The hydrolysate was diluted with water, and the precipitate that deposited was filtered off and dried. Glucose, arabinose, and xylose were identified in the filtrate by paper chromatography in system 2. The genin obtained, after recrystallization from absolute methanol, had mp 304-306°C, $[\alpha]_D^{20}$ +35.5° (c 2.5; chloroform) and, from its R_f value (system 6), was identical with echinocystic acid. The analogous operations were performed for the second saponin. The hydrolysate of the chenopodium saponin B was found to contain glucose, arabinose, xylose, and rhamnose, and the aglycone was echinocystic acid.

Alkaline Hydrolysis of the Chenopodium Saponins. A mixture of 500 mg of the saponin B and 50 ml of 5% aqueous ethanolic KOH was heated in the water bath for 4 h. The reaction mixture was neutralized with acetic acid and extracted with butanol. Evaporation of tbe organic extracts gave 300 mg of a glycoside having the same R_f value as chenopodium saponin A. The progenin obtained (20 mg) was heated with 7 ml of 2% H_2SO_4 at 110°C for 4 h. After cleavage, the hydrolysate was found to contain glucose, arabinose, and xylose

The analogous reactions were performed on 100 mg of chenopodium saponin A. Thin-layer chromatography in silica gel in systems 1 and 5 showed that the glycoside had undergone no change.

Stepwise Hydrolysis of Chenopodium Saponin B (II). The chenopodium saponin II (200 mg) was heated with 30 ml of 10% oxalic acid at 80°C for 3 h. An analysis of the reaction mixture by thin-layer chromatography in system 1 showed the presence of three glycosides of different polarities (III, IV, V). The separation of these substances on a column of silica gel in the same system gave 20 mg of (III), 35 mg of (IV), and 40 mg of (V). Ten milligrams of each substance was heated with 5 ml of 2% sulfuric acid at 110°C for 4 h. By paper chromatography in system 2, glucose, arabinose, and xylose were identified in the case of the progenin (V), glucose and arabinose for the glycoside (IV), and arabinose for the least polar substance.

Methylation of Saponins A and B. Glycoside B (100 mg) was methylated by Hakomori's method [3]. The course of the reaction was monitored by chromatography in a thin layer of alumina in system 4 and by IR spectroscopy. After the end of the reaction, the mixture was extracted with chloroform. The evaporated extracts were dissolved in 4 ml of mixture 3 and deposited on a column of alumina. Elution was performed with the same system, 3-ml fractions being collected. The separation was monitored in a thin layer of alumina in system 2. Yield 80 mg.

Saponin A (150 mg) was methylated by the same method. The yield of permethylate was 120 mg.

Methanolysis of the Methylated Chenopodium Saponins. The product obtained after the methylation of saponin B was dissolved in 5 ml of absolute methanol and 3 ml of conc. hydrochloric acid, and the solution was heated in a sealed tube at 100°C for 5 h.

The hydrolysate was shown by gas-liquid, paper, and thin-layer chromatography in the presence of authentic reference materials to contain 2,3,4-tri-O-methyl-D-xylose, 2,3,6-tri-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-glucose, 3,4-di-O-methyl-L-arabinose, and 2,3-di-O-methyl-L-rhamnose.

In the case of chenopodium saponin A, in a similar manner, 3,4-di-O-methyl-L-arabinose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4-tri-O-methyl-D-xylose were obtained.

To 0.5 g of completely methylated chenopodium saponin B in 50 ml of absolute tetrahydrofuran was added 0.25 g of lithium aluminum hydride. The mixture was heated with stirring for 10 h. After the solution had been worked up as described previously [5], 3,4-di-O-methylarabinose, 2,3,4-tri-O-methylglucose, and 2,3,4-tri-O-methylxylose were found in the reduced glycoside by thin-layer chromatography, and 2,3-di-O-methylrhamnitol and 2,3,4,6-tetra-O-methylglucose in the oligosaccharide.

Periodate Oxidation of the Chenopodium Saponins. To 30 mg of $NaIO_4$ in 25 ml of water were added 10 mg of sodium bicarbonate and then 15 mg of saponin, and the mixture was left at room temperature for 60 h. After this, 0.05 mg of ethylene glycol was added to the mixture and it was evaporated and the residue was subjected to acid hydrolysis. Paper chromatography established the complete absence of monosaccharides.

SUMMARY

1. It has been shown that the roots of Chenopodium anthelminticum contain two triterpene glycosides.

2. Chenopodium saponin A is a trioside and chenopodium saponin B a pentaoside of echinocystic acid.

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