RIBOSE IN THE TRITERPENE GLYCOSIDES OF Clematis vitalba. I

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Clematis vitalba (traveler's joy) has not been studied chemically hitherto. Investigations of other species of the genus Clematis, extracts of which possess anesthetic, bactericidal, and intact actions, have shown that the presence of an essential oil is characteristic of them [1]; from this oil anemonin and protoanemonin, and also saponin-like substances not so far investigated, have been extracted [2, 3]. Hederagenin and oleanolic acid have been found in the roots of Cl. paniculata [4], and four glycosides of the oligoside type have been obtained from Manchurian ground clematis and their complete structure has been established [5, 6].

A preliminary investigation of a methanolic extract from the roots and rhizomes of Clematis vitalba led to the discovery in the plant of free triterpenoids – hederagenin, oleanolic acid – and about 5% of saponins.

By chromatography on silica gel in several systems of solvents at various pH values we have established the presence of eight glycosides, which we have called in order of increasing polarity vitalbosides A, B, C, D, E, F, G, and H.

On acid hydrolysis, vitalboside A splits into glucose and oleanolic acid. On plates, the saponin has the same mobility and specific rotation as synthetic oleanolic acid 3-O-β-D-glucoside.

Under similar conditions, vitalbosides B and C decomposed into hederagenin and glucose. Saponins D and E additionally liberated arabinose and traces of rhamnose, and F-H arabinose, ribose, and rhamnose.

This is the first case of the isolation of saponins containing ribose as a carbohydrate component. Judging from the specific rotation, it belongs to the L series. This monosaccharide has not hitherto been known in nature. The identity of the ribose with an authentic sample was shown by paper chromatography, color reactions, and its mobility on electrophoresis in borate buffer.

EXPERIMENTAL

Chromatography was carried out with type S paper of the Volodarskii Leningrad Mill, type KSK silica gel, and neutral alumina (activity grade II) using the following solvent systems: 1) butan-1-ol–ethanol–water (10 : 2 : 5), 2) chloroform–methanol–water (65 : 35 : 10), 3) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3), 4) chloroform–methanol (9 : 1), and 5) butan-1-ol–acetic acid–water (4 : 1 : 5). The sugars were detected with aniline phthalate and p-anisidine, and the glycosides and aglycones with antimony trichloride in chloroform and conc. sulfuric acid.

Identification of Oleanolic Acid and Hederagenin. The comminuted roots of Clematis vitalba (4.5 kg) were extracted with ether. After concentration of the extract, hederagenin and oleanolic acid were identified by chromatography in a thin layer of silica gel in system 4 in the presence of markers. The methyl esters of the substances, obtained by treating the free aglycones with an ethereal solution of diazomethane, were also identical with authentic samples.

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TABLE 1

Glycoside	Aglycone	Carbohydrate composition of the glycosides
Vitalboside		
A	Oleanolic acid	
B	Hederagenin	Glucose
C		Glucose, arabinose, rhamnose
D		(traces)
E		Glucose, arabinose
F		
G		Glucose, arabinose, ribose, rhamnose
H		

Isolation of the Saponins. After defatting, the roots of Clematis vitalba were exhaustively extracted with 70% aqueous methanol. The extracts were evaporated to a volume of 1.5 liter. On standing in the cold, a precipitate deposited, and this was filtered off. The precipitate on the filter was found to contain free aglycones: hederagenin and oleanolic acid. The filtrate was washed with ether, and the glycosides were extracted with butan-1-ol (10 × 200 ml). The first butanolic extract, after the solvent had been distilled off, gave 10 g of product (I) consisting mainly of a mixture of vitalbosides A, B, and C. The subsequent butanolic fractions were combined and concentrated. This gave 80 g of product (II), containing the more polar glycosides. The aqueous fraction also gave a positive reaction for triterpene glycosides. After its evaporation, 130 g of product (III) was obtained.

Isolation of Vitalbosides A, B, and C. The combined saponins (2 g) were transferred to a column of silica gel (3 × 30 cm) and eluted in system 2. This gave 0.5 g of vitalboside A with mp 255°C, $[\alpha]_D^{20}$ +54° (c 2.23; methanol). Literature data for oleanolic acid β-D-glucopyranoside – mp 247-249°C: $[\alpha]_D^{20}$ +56° [7]. Further chromatography yielded 0.4 g of vitalboside B and 0.5 g of vitalboside C.

Isolation of Vitalbosides D, E, F, G, and H. The combined glycosides (2 g) were transferred to a column of silica gel and eluted in system 1. This gave 0.4 g of vitalboside D, 0.3 g of vitalboside E, 0.25 g of vitalboside F, 0.2 g of vitalboside G, and 0.1 g of vitalboside H.

Acid Hydrolysis of the Vitalbosides. For each glycoside, 50 mg was charged into a tube with 2% sulfuric acid and the mixture was heated at 100°C for 5 h. The precipitate of the aglycone was filtered off and identified by thin-layer chromatography on silica gel in system 4. The sugars in the filtrate were determined, after neutralization with barium carbonate, by paper chromatography in system 3 (Table 1).

Isolation of Ribose. The aqueous fraction (10 g) was hydrolyzed in 500 ml of 2% sulfuric acid (100°C, 5 h). The hydrolysate was filtered and neutralized with barium carbonate. The ribose was isolated by preparative chromatography on paper of type 3MM. This gave 100 mg of a substance with $[\alpha]_D$ +30° (c 1; methanol), identical with an authentic sample of ribose in solvent systems 3 and 5, the spots being revealed with aniline phthalate and p-anisidine. Literature data for L-ribose – mp 87°C, $[\alpha]_D^{20}$ 23.9° (in water) [8]. An additional proof was provided electrophoretically on an apparatus of the type ÉMÍB-Kiev 1965 at a voltage of 50 V/cm in borate buffer at pH 9.2.

SUMMARY

1. It has been shown that the roots of Clematis vitalba contain free hederagenin and oleanolic acid and also eight glycosides of these aglycones.

2. It has been established that vitalbosides F, G, and H contain in their carbohydrate moiety an unusual monosaccharide for saponins – ribose.

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