TRITERPENE GLYCOSIDES OF *Caltha polypetala*

GLYCOSIDES A, B, D, E, and F

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Five individual triterpene glycosides — A, B, D, E, and F — which are hederagenin mono- and biosides, have been isolated from the roots of *Caltha polypetala* (Great Marsh Marigold), family *Ranunculaceae*. Glycosides E and F proved to be new compounds for which, on the basis of the results of acid and alkaline hydrolyses, methylation, methanolysis, and physicochemical methods of investigation, the following structures are proposed: E — hederagenin 3-O-D-glucopyranoside, 28-O-L-rhamnopyranoside; and F — heteragenin 28-O-[O-L-rhamnopyranosyl-(1 → 6)-D-glucopyranoside].

Plants of the genus *Caltha* (marsh marigold), family *Ranunculaceae*, are common in various regions of the world, and six species grow in the USSR, two of them in Georgia [1, 2]. In repeated studies of representatives of the marsh marigold genus, considerable amounts of triterpene saponins have been detected in them [3-9].

We have detected nine triterpene saponins and a free genin in the epigeal organs of *Caltha Polypetala* Hochst. — great marsh marigold — growing in Georgia and have called the saponins in order of increasing polarity glycosides A, B, C, D, E, F, G, H, and I. The qualitative compositions of the triterpene saponins of the epigeal and hypogeal parts of the plant were identical. The yield of saponins from the hypogeal part of the marsh marigold amounted to 8.5%, and from the epigeal part 5%. The complete acid hydrolysis of the purified total saponins isolated from a methanolic extract of the roots of the plant showed that the glycosides were hederagenin derivatives and contained L-rhamnose, L-arabinose, and D-glucose residues in the carbohydrate moieties.

From the enriched combined saponins by chromatography on a column of silica gel we succeeded in isolating eight individual compounds, one of which proved to be hederagenin [10] while the others were glycosides A, mp 238-239°C; B, mp 234-235°C; D, mp 224-225°C; E, mp 119-200°C; F, mp 139-140°C; G, mp 172-173°C; and I, mp 217-218°C.

In the present paper we gave information on the investigation of the weakly polar glycosides A, B, D, E, and F. The following polyol acetates were identified by GLC in the carbohydrate moieties of the glycosides under investigation as the result of acid hydrolysis followed by a reduction and acetylation: for glycoside A, L-rhamnitol acetate; B, L-arabitol acetate; D,L-arabitol and D-sorbitol acetates in a ratio of 1:1, E and F, L-rhamnitol and D-sorbitol acetates in a ratio of 1:1. The presence of a single monosaccharide residue in each of glycosides A and B, and also the nature of the attachment of the monosaccharides in the carbohydrate chains of glycosides D, E, and F were established by the Hakomori methylation [11] of the compounds under investigation. As a result of the methanolysis of the methylated glycosides the following were identified: for compound A, methyl 2,3,4-tri-O-methyl-α,β-L-rhamnopyranoside; for B, methyl 2,3,4-tri-O-methyl-α,β-L-arabinopyranoside; for D, methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucopyranoside and methyl 3,4-di-O-methyl-α,β-L-arabinopyranoside; for E, methyl 2,3,4-tri-O-methyl-α,β-L-rhamnopyranoside and methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucopyranoside; and for F, methyl 2,3,4-tri-O-methyl-α,β-D-glucopyranoside and methyl 2,3,4-tri-O-methyl-α,β-L-rhamnopyranoside. As a result of the hydrolysis of the methylated glycosides, the methyl ester of 23-O-methylhederagenin was identified for compounds A, B, and D; 23-O-methylhederagenin for E; and 3,23-di-O-methylhederagenin for F.

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In the carbohydrate moieties of the glycosides under investigation, as a result of reduction followed by the acetylation of the hydrolysates, the acetates of partially methylated polyols were identified: for A, 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol; for B, 1,5-diacetyl-2,3,4-tri-O-methyl-L-arabitol; for D, 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-sorbitol and 1,2,5-tri-O-acetyl-3,4-di-O-methyl-L-arabitol; for E, 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-sorbitol; and for F, 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol and 1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl-D-sorbitol. The IR spectra of compounds A, B, and D each had the absorption band in the 1700 cm^{-1} region that is characteristic for an unsubstituted carboxy group, and the spectra of E and F each had a band in the 1740 cm^{-1} region characterizing the presence of an ester group at C-28 of the genin.

On the basis of the results obtained, it was possible to conclude that glycosides A and B were hederagenin monosides with L-rhamnose and L-arabinose residues, respectively, at C-3 of the genin, glycoside A proving to be identical with fatsin, isolated previously from *Fatsia japonica* [12], and glycoside B being identical with pastuchoside C, isolated from *Hedera pastuchovii* [13]. Glycosides D, E, and F were hederagenin biosides, glycoside D being identical with calthoside D, isolated previously from *Caltha sylvestris* [3].

In order to determine the localization of the carbohydrate chains in glycosides E and F, they were subjected to alkaline hydrolysis. As a result of the alkaline hydrolysis of glycoside E, a progenin and L-rhamnose were detected. The acid hydrolysis of the progenin permitted the identification of hederagenin and D-glucose. The results obtained were in complete agreement with the results of methylation indicating the presence in glycoside E of two terminal monosaccharide residues of which L-rhamnose was obviously present at C-28 and D-glucose at C-3 of the genin. As a result of the alkaline hydrolysis of glycoside F, we detected hederagenin and an oligosaccharide fraction the acid hydrolysis of which permitted the identification of L-rhamnose and D-glucose. It follows from the facts given that in glycoside F the disaccharide chain was attached at C-28 of the genin and the terminal monosaccharide residue was L-rhamnose linked to D-glucose by a (1 → 6)-bond.

Thus, on the basis of the combination of available information it is possible to propose the following structures for glycosides E and F: E — hederagenin 3-O-D-glycopyranoside, 28-O-L-rhamnopyranoside; F — hederagenin 28-O-[O-L-rhamnopyranosyl-(1 → 6)-D-glucopyranoside].

EXPERIMENTAL

The roots of *Caltha polypetala* gathered in 1977 in the village of Zedazeni, Georgian SSR, were investigated. The plant material after collection was dried and comminuted.

For the chromatography of the compounds investigated we used type "M" ["slow"] paper from the Leningrad mill, for column chromatography silica gel L 40/100, and for thin-layer chromatography KSK silica gel, with the following solvent systems: 1) chloroform—methanol (20:1); 2) butanol—methanol—water (5:3:1); 3) pyridine—ethyl acetate—water (2:8:1); and 4) chloroform—methanol—water (26:14:3). As the revealing agent for the saponins we used a 25% solution of tungstophosphoric acid in ethanol, and for monosaccharides o-toluidine salicylate.

GLC analysis was performed on a Chrom-5 instrument using a column containing 5% of XE-60 on Chromaton N-AW HMDS, FID, with helium as the carrier gas at a rate of flow of 40 ml/min. IR spectra were taken on a UR-20 instrument (in paraffin oil).

Isolation of the Glycosides. The air-dried comminuted roots of great marsh marigold (500 g) were extracted with methanol at the boil. The combined methanolic extract was concentrated and dried. This gave 102 g of combined triterpene saponins in the form of a brown amorphous powder, 50 g of which was dissolved in system 4 and chromatographed on a column of silica gel in the same system. The saponin fractions freed from impurities were combined, giving 25 g of purified saponins. Of this enriched combination of weakly polar saponins, 3 g was chromatographed on a column of silica gel in system 1. As the result of repeated chromatography, the individual weakly polar glycosides A, B, D, E, and F were isolated.

Determination of the Monosaccharide Compositions of the Glycosides. In each ase, 20 mg of glycosides A, B, D, E, and F were hydrolyzed with 2 N HCl at 100°C for 3 h. The resulting aglycon was separated off, recrystallized from acetone, and identified as hederagenin. The hydrolysate was evaporated to dryness to eliminate the last traces of HCl. By PC in system 3 in comparison with markers, L-rhamnose was identified for glycoside A, L-arabinose for B, L-arabinose and D-glucose for D, and L-rhamnose and D-glucose for E and F. The monosaccharides

were dissolved in 2 ml of 50% aqueous methanol and were reduced with sodium tetrahydroborate at room temperature for 12 h, after which the mixtures were neutralized with KU-2 cation-exchange resin, filtered, and evaporated to dryness. The carefully dried substance was acetylated in a mixture of acetic anhydride and pyridine (2 ml in each case) at room temperature for 12 h, and then the reaction mixture was evaporated to dryness and extracted with chloroform. The following polyol acetates were identified by GLC in comparison with markers: rhamnitol acetate for glycoside A, L-arabitol acetate for B, L-arabitol and D-sorbitol acetates in a ratio of 1:1 for D, and L-rhamnitol and D-sorbitol acetates in a ratio of 1:1 for E and F.

Methylation of the Glycosides. Glycosides A, B, D, E, and F — 50 mg in each case — were methylated by Hakomori's method. The methylated saponins were subjected to methanolysis (5% HCl/MeOH, 100°C, 4 h). The methanolysates were neutralized with aqueous ammonia, evaporated to dryness, and extracted with chloroform. The following were identified by GLC in comparison with markers: for glycoside A, methyl 2,3,4-tri-O-methyl-α,β-L-rhamnopyranoside; for B, methyl 2,3,4-tri-O-methyl-α,β-L-arabinopyranoside; D, methyl 2,3,4,6-tetra-O-methyl-α,β-D-glucopyranoside and methyl 3,4-di-O-methyl-α,β-L-arabinoside; E, methyl 2,3,4-tri-O-methyl-α,β-L-rhamnopyranoside and methyl 2,3,4-tetra-O-methyl-α,β-D-glucopyranoside; F, methyl 2,3,4-tri-O-methyl-α,β-L-rhamnopyranoside and methyl 2,3,4-tri-O-methyl-α,β-D-glucopyranoside.

As a result of the hydrolysis of the permethylates of the glycosides in system 1 in comparison with markers, the methyl ester of 23-O-methyl hederagenin was identified for compounds A, B, and D, 23-O-methyl hederagenin for E, and 3,23-di-O-methyl hederagenin for F. In the carbohydrate moieties of the glycosides under investigation, after their reduction of the hydrolysates to polyols followed by acetylation as described above, the following were identified by GLC in comparison with authentic samples: for A, 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol; B, 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-arabitol; D, 1,5-di-O-acetyl-2,3,4-tetra-O-methyl-D-sorbitol and 1,2,5-tri-O-acetyl-3,4-di-O-methyl-L-arabitol; E, 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-sorbitol; and F, 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol and 1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl-D-sorbitol.

Alkaline Hydrolysis. Glycosides E and F, 100 mg each, were subjected to alkaline hydrolysis (5% KOH, 100°C, 1.5 h). The hydrolysates were neutralized with KU-2 cation-exchange resin and filtered. The progenins were extracted with butanol, and the aqueous and butanolic fractions were evaporated. For glycoside E, L-rhamnose was identified in the aqueous fraction by TLC in system 2. The butanolic fraction, containing a progenin, was subjected to acid hydrolysis (2 N HCl, 100°C, 3 h); hederagenin was separated off, and D-glucose was identified in the filtrate.

For glycoside F, alkaline hydrolysis led to the detection of hederagenin and an oligosaccharide fraction which was subjected to acid hydrolysis as described above. L-Rhamnose and D-glucose were identified in the hydrolysate by TLC in system 2. The hydrolysate was reduced with sodium tetrahydroborate and was acetylated in a similar manner to that described above. The acetates of L-rhamnitol and of D-sorbitol were identified in a ratio of 1:1 by comparison with authentic samples by the GLC method.

SUMMARY

The roots of the *Caltha polypetala* Hochst. (family *Ranunculaceae*) have yielded a combination of triterpene glycosides containing nine glycosides (A, B, C, D, E, F, G, H, and I) — all derivatives of hederagenin. Eight glycosides have been isolated, and structures have been proposed for five of them — the weakly polar glycosides A, B, D, E, and F. Glycosides E and F proved to be new compounds, and for these the following structures are proposed: E — hederagenin 3-O-D-glucopyranoside, 28-O-L-rhamnopyranoside, and F — hederagenin 28-O-[O-L-rhamnopyranosyl-(1 → 6)-D-glucopyranoside].

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INFLUENCE OF THE CHEMICAL MODIFICATION OF THE FUNCTIONAL GROUPS OF THE β-1,3-GLUCANASE L IV ON ITS CAPACITY FOR HYDROLYSIS AND TRANSGLYCOSYLATION REACTIONS

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The influence of the chemical modification of the functional groups of the β-1,3-glucanase L IV on its capacity for performing hydrolysis and transglycosylation reactions has been investigated. On the modification of the lysine, tryptophan, histidine, and dicarboxylic acid residues and on the oxidation of the carbohydrate component in the L IV molecule the ratio of the hydrolase and transglycosylating activities does not change. It is likely that the hydrolysis and transglycosylation reactions take place at the same active site with the participation of the same catalytic groups.

It is known that endoglycanases and glycosidases catalyze hydrolysis and transglycosylation reactions simultaneously. A capacity for transglycosylation has been detected in endo-β-1,3-glucanases from marine molluscs [1, 2]. Investigations of the laws of the combined occurrence of hydrolysis and transglycosylation reactions under the action of β-1,3-glucanases L IV from *Spisula sachalinensis* and L_{Cr} from *Chlamys albidus* have shown that the pH optima of the two reactions coincide [2]. The hypothesis has been expressed that identical catalytic groupings of the active sites of the enzyme studied take part in the processes of hydrolysis and of transglycosylation. To check this hypothesis we have used the method of chemically modifying functional groups, which has been used previously with success for studying the influence of modification mainly on the hydrolase activity of the β-1,3-glucanase L IV [3-7]. In an investigation of the chemical modification of lysine residues [8], the kinetics of the action on laminarin of β-1,3-glucanase L IV modified with pentane-2,4-dione was analyzed in detail in comparison with the native enzyme. It was established that modification of the lysine residues led to no change in the hydrolase and transglycosylating activities.

All the chemical modifications were carried out by the procedures described previously for β-1,3-glucanase L IV, the degree of modification and the change in activity being monitored. Transglycosylating activity was determined from the accumulation of p-nitrophenol [1] and hydrolase activity from the increase in reducing power by Nelson's method [9]. The results are given below: (Top, following page.)

The oxidation by N-bromosuccinimide of more than one tryptophan residue in the β-1,3-glucanase L IV molecule and the esterification of the carboxy groups led to the complete loss of both the hydrolase and the transglycosylating capacity of the enzyme.

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