TRITERPENE GLYCOSIDES OF Caulophyllum robustum

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Caulophyllum robustum is a perennial relict plant of the family Berberidaceae whose area of growth is restricted to eastern Asia. Taxonomically the placing of the genus Caulophyllum in the family Berberidaceae is a matter of doubt, since, on the basis of recent systematic studies [1], the closely related genus Diphylleia, originally assigned to the Berberidaceae, has been transferred to the family Podophyllaceae.

Previously saponins – caulosaponin [2], whose aglycone is hederagenin, and caulophyllosaponin [3]– have been isolated by precipitation from the American species Caulophyllum thalictroides growing in central America. The papers describing these isolations give no information at all on the structure of the saponins. A knowledge of the methods used for the extraction of saponins indicates that caulosaponin and caulophyllosaponin are not individual compounds but mixtures of triterpene glycosides having closely related properties.

A chemical study of the roots of C. robustum was limited to obtaining alkaloids: methylcytisine and thaspine [4].

According to chromatography in a thin layer of silica gel, a methanolic extract of the rhizomes and roots of C. robustum* contains five glycosides revealed by the Carr-Price reagent [5] which we have called, in order of their increasing polarity, "caulosides" A, B, C, D, and E. To isolate the individual caulosides, we used partition chromatography on silica gel saturated with water [6] using a neutral system of solvents and gel filtration through Sephadex A-25.

The hydrolysis of each of the caulosides A, C, D, and E in the presence of 20% hydrochloric acid in methanol gave the same aglycone, identical with hederagenin in its physicochemical constants and NMR and mass spectra, and in the analogous characteristics of its methyl ester [7]. The hydrolysis of cauloside B under similar conditions led to an aglycone with mp 305-308°C whose IR spectrum had an absorption band corresponding to a lactone group (ν KBr 1755 cm^{-1}). When cauloside B was hydrolyzed under milder conditions, an aglycone with mp 277-280°C was obtained whose IR spectrum had a band characteristic for a free carboxy group (ν KBr 1702 cm^{-1}). The mass spectrum of this aglycone had a molecular peak with m/e 488, which corresponds to the molecular weight calculated for the empirical formula $C_{30}H_{48}O_5$. The nature of the fragmentation of the molecular ion shows that the aglycone of cauloside B is a pentacyclic triterpenoid, but it has not yet been identified. An analysis of the hydrolyzates of caulosides A, B, C, D, and E by paper chromatography has shown that caulosides D and E contain glucose, arabinose, and rhamnose; cauloside C, glucose and arabinose; and caulosides B and A, arabinose.

The IR spectra of caulosides A-E show that in caulosides A, B, and C the carboxy group of the aglycone is free (ν KBr 1710 cm^{-1}). Caulosides D and E have absorption bands in the IR spectra corresponding to ester groups (ν KBr 1745 cm^{-1}), which makes it likely that they contain an O-acyl glycoside bond. This conclusion is confirmed by the negative result of attempts to methylate caulosides D and E with diazomethane and alkaline hydrolysis. The hydrolyzates of caulosides D and E yielded (TLC) progenins identical, according to thin-layer chromatography on silica gel, with caulosides A and C.

*The samples of Caulophyllum robustum were collected and identified by P. G. Gorov (Institute of Biologically Active Substances).

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The glycosides found in C. robustum are similar in nature to the kalopanax saponins A and B isolated previously from Kalopanax septemlobum and to the leontosides A-E isolated from Leontice eversmanii [9, 10]. However, direct comparison by thin-layer chromatography of caulosides A, C, D, and E and a methanolic extract from Kalopanax septemlobum showed that not one of the caulosides is identical with kalopanax saponins A and B (the question of the identity of caulosides A-E and leontosides A-E still remains open).

EXPERIMENTAL

Chromatographic analysis was carried out with plates having a fixed layer of silica gel in the following solvent systems: 1) chloroform–methanol (2 : 1)/H_2O and 2) chloroform–methanol (9 : 1)/H_2O (the spots of the glycosides and aglycones on the plates were revealed with a saturated solution of $SbCl_3$ in chloroform or with conc. H_2SO_4); paper chromatography was carried out in system 3) butan-1-ol–benzene–pyridine–H_2O (5 : 1 : 3 : 3). The preparative isolation of caulosides A-E was effected on columns filled with silica gel (200-270 and 50-100 mesh) saturated with water. The substances were eluted in systems 4) toluene–butanol saturated with water, 5) benzene–ethyl acetate, 6) chloroform–methanol, and 7) chloroform–ethyl acetate. For acid hydrolysis caulosides A-E were heated for 5 h with the mixtures of 8) 20% HCl in methanol, and 9) 2 ml of conc. H_2SO_4 in 50 ml of 50% aqueous methanol. The standard treatment of the hydrolyzate consisted in separation of the aglycone that had deposited and neutralization of the aqueous fraction with Dowex 1 × 4 (HCO_3^- form). The analytical results for hederagenin and its methyl ester corresponded to the calculated figures.

Isolation of the Glycoside Fraction. The roots and rhizomes of Caulophyllum robustum, ground to a powder in the dry state (1 kg), were exhaustively extracted with methanol with heating. Evaporation of the methanolic extract gave 194 g of dry residue (19.5%).

A 98-g quantity of the dry methanolic extract was chromatographed on a column of silica gel in system 4 (2 : 1 → 0 : 1). This gave the following fractions: 1 (8.5 g), caulosides A and B; 2 (11.6 g), D and C; and 3 (20.8 g), D and E.

Isolation of Caulosides C, D, and E. Fractions 2 and 3 were repeatedly chromatographed on columns of silica gel in system 4 (3 : 1 → 0 : 1). After gel filtration through Sephadex A-25, cauloside E had mp 216-218°C (from butan-1-ol–methyl ethyl ketone). Found %: C 55.24, 55.20; H 8.06, 7.84.

The acetate of cauloside E, after chromatographic purification in system 5 (100 : 0 → 30 : 70), had mp 128-130°C (from ethanol), $[\alpha]_D^{21}$ +8.2° (c 0.098, chloroform). Found %: C 56.78, 56.71; H 6.70, 6.71. Mp of cauloside D, 202-205°C (from butan-1-ol–methyl ethyl ketone–methanol), $[\alpha]_D^{21}$ 0° (c 0.1%, methanol). Found %: C 58.16, 57.90; H 8.46, 8.49. For cauloside C, mp 248-251°C (butan-1-ol), $[\alpha]_D^{21}$ +58.12° [c 0.125% chloroform–methanol (1 : 1)]. Found %: C 62.91, 62.94; H 8.86, 8.22.

Isolation of Caulosides A and B. Fraction 1 was chromatographed repeatedly on columns of silica gel in system 6 (2 : 1 → 1 : 1). Cauloside A had mp 276-280°C (decomp.) (from aqueous methanol), $[\alpha]_D^{21}$ + 58.73° [c 0.126, chloroform–methanol (1 : 1)]. For cauloside B, mp 256-259°C (from butanol), $[\alpha]_D^{21}$ +41.5° [c 0.106%, chloroform–methanol (1 : 1)]. Found %: C 66.99, 67.07; H 9.27, 9.24.

Acid Hydrolysis of Caulosides A, C, D, and E. Two grams of cauloside E was hydrolyzed in 160 ml of mixture 8. The aglycone of cauloside A obtained by the standard working up of the hydrolyzate was found to be hederagenin with mp 326-327°C (from methanol). Mass spectrum: M^+ 472. Main peaks, m/e: 248 and 203. IR spectrum (KBr, cm^{-1}): 3480 (bound H of OH) and 1710 (COOH). The mp of the methyl ester of the aglycone, which was methylated with diazomethane, after chromatographic purification on a column of silica gel in system 5 (100 : 0 → 30 : 70), was 242-243°C (from methanol). Mass spectrum: M 486. Main peaks, m/e: 262 and 203. IR spectrum (KBr, cm^{-1}): 3630 (OH), 3500 (bound H of OH), and 1730 ($COOCH_3$). The aglycones of caulosides A, C, and D were identical with hederagenin in their R_f values on a thin-layer chromatogram in system 2 and by mixed mp's. The hydrolyzates were shown by paper chromatography in system 3 to contain glucose, arabinose, and rhamnose in caulosides D and E; glucose and arabinose in cauloside C; and arabinose in caulosides A and B.

Acid Hydrolysis of Cauloside B. A 150-mg sample of the cauloside was hydrolyzed in 20 ml of mixture 9. After chromatographic purification in system 7 (1 : 5), the aglycone had mp 277-280°C [from methanol–ethyl acetate (1 : 0)].* Found, %: C 73.64, 73.79; H 10.31, 10.17. $C_{30}H_{48}O_5$. Calculated %: C 73.77;

* As in Russian original – Publisher.

H 9.90. Mass spectrum: M^+ 488. The strongest peaks are those with m/e 264 (diene fragment a), 246 (a − 18), and 202 (a − 18 − 44). IR spectrum (KBr, cm^{-1}): 1710 (COOH). The hydrolyzate was shown by paper chromatography in system 3 to contain arabinose. Hydrolysis with mixture 8 led to the aglycone, with mp 305-308°C. IR spectrum (KBr, cm^{-1}): 1755 (γ-lactone).

Alkaline Hydrolysis of Caulosides D and E. Caulosides D and E (20-mg samples of each) were heated with 10% KOH solution in sealed tubes for 3 h. The solutions were cooled and neutralized with KU-2 cation exchange resin (H^+ form). Thin-layer chromatography in system 1 showed the absence of the initial caulosides D and E from the hydrolyzate. The progenins of caulosides D and E obtained as a result of the hydrolysis were identical chromatographically with caulosides A and C, respectively.

CONCLUSIONS

Glycosides which we have called "caulosides" A, B, C, D, and E have been isolated from the perennial relict plant Caulophyllum robustum.

The aglycone of caulosides A, C, D, and E is hederagenin. It has been found that the carbohydrate chains of caulosides D and E contain glucose, arabinose, and rhamnose; those of C, glucose and arabinose; and those of A and B, arabinose. Caulosides D and E contain carbohydrate chains attached by an O-acyl glycoside bond.

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