V_1 is the volume of eluate, ml; V_2 is the volume of solution of the preparation of the investigation deposited on the chromatogram, ml; V_3 is the volume of solvent used to dissolve the sample of preparation, ml; a is the weight of the sample of preparation under investigation, mg; and h is the amount of moisture in the weighed sample, %.

The samples of ferutin, teferin, and ferutinin were obtained from A. I. Saidkhodzhaev and G. K. Nikonov.

SUMMARY

A chromatospectrophotometric method for determining ferutinin and teferin + ferutin in a preparation has been developed.

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CAULOSIDE G – A NEW TRITERPENE GLYCOSIDE FROM *Caulophyllum robustum* IDENTIFICATION OF CAULOSIDE C

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UDC 547.918+547.914

Continuing a chemical study of the glycosides (caulosides) of *Caulophyllum robustum* [1-4], we now report the results of investigations of the structure of caulosides G(I) (earlier called E [1]) and C.

The permethylated product (II) of cauloside G(I) was synthesized by Purdie's method [5]. Acid hydrolysis of (II) gave the methyl ester of 23-O-methylhederagenin and a mixture of methylated methyl glycosides. The latter, after acetylation were identified by the GLC–MS method [6] as the methyl pyranoside derivatives of: 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4,6-tetra-O-methyl-D-glucose, 3,4-di-O-methyl-L-arabinose, 6-O-acetyl-2,3,4-tri-O-methyl-D-glucose, and 4-O-acetyl-2,3,6-tri-O-methyl-D-glucose.

The reductive cleavage of (II) with lithium tetrahydroaluminate gave a methylated progenin (III) and a methylated oligosaccharide (IV). The hydrolysis of (III) gave 23-methoxyerythrodiol and the methyl pyranoside derivatives of: 2,3,4,6-tetra-O-methyl-D-glucose and 3,4-di-O-methyl-L-arabinose (GLC–MS method). In a hydrolyzate of (IV) by TLC in the presence of markers, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4-tri-O-methyl-D-sorbitol were identified. The reduction of the 2,3,4-tri-O-methyl-D-glucose showed that the latter was attached to the carboxy group of the hederagenin.

The structure of the oligosaccharide (IV) was established on the basis of the mass spectrum of the corresponding acetate (V): the presence of peaks of ions with m/e 640 ($M^+ - 60$) and 567 confirmed that (IV) was a trisaccharide. The peaks of ions with m/e 627, 539, and 117 are due to the fragmentation of a trisaccharide in which 1,5-di-O-acetyl-2,3,4-tri-O-methyl-D-sorbitol is the reduced end [7]. The peaks of ions with m/e 189 (A_1), 157 (A_2), 125 (A_3), and 72 (K_1) are due to the fragmentation of terminal 2,3,4-tri-O-methyl-L-rhamnose.

Hydrolysis of cauloside G by the digestive juice of the snail *Eulota maackii* formed five progenins (VI-X) and hederagenin. From the results of acid hydrolysis and methylation with diazomethane, progenins (VII) and

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(X) contain a carbohydrate chain only at the carboxy group of hederagenin. According to the results of the TLC and PC analyses of the products of acid hydrolysis, the progenins contain the sugars indicated: (VI) – D-glucose, L-arabinose, and L-rhamnose; (VII) – D-glucose and L-rhamnose; (VIII) – D-glucose and L-arabinose; and (X) – D-glucose. According to their R_f values on GLC, progenins (VI) and (IX) are identical with caulosides D and A, respectively. A mixed melting point of (IX) and cauloside A gave no depression of the melting point. The progenins (VI), (VII), (VIII), and (X) were not methylated by diazomethane.

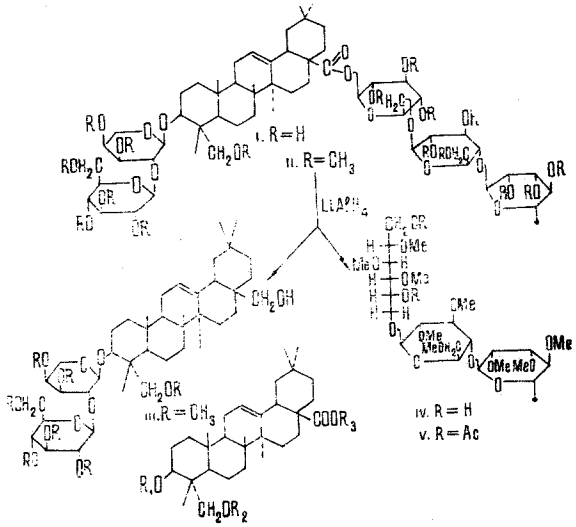
The integral intensity (35 H) of the signals of the acetyl groups in the PMR spectrum of the acetate of the progenin (VII) means that this is a triglycoside. The signals of the anomeric protons of the two glucose residues (δ 5.58 ppm, J = 8 Hz and δ 4.60 ppm, J = 7 Hz) show the presence of β-glycosidic bonds, and a broad signal at δ 4.80 ppm, J = 1.5 Hz, shows an α-glycosidic link of the rhamnose. In the PMR spectrum of the acetate of the progenin (X) the signal of the anomeric proton of the glucose at δ 5.58 ppm, J = 8 Hz shows a β-glycosidic bond, and the integral intensity (18 H) of the signals of the acetyl groups shows that this progenin is a monoglycoside at C_{28} of hederagenin.

The progenin (VIII) is a two-chain glycoside. It contains L-arabinose at the C_3 and D-glucose at the C_{28} atoms of hederagenin. The PMR spectrum of its acetate contains the signals of the anomeric protons (δ 4.41 ppm, J = 7 Hz, and δ 5.58 ppm, J = 8 Hz) showing the α and β configurations of the corresponding glycosidic bonds.

We have shown previously [1] that the alkaline hydrolysis of cauloside G forms a progenin that is identical, according to the absence of a depression of the melting point in a mixture, with cauloside C(XI).

The acid hydrolysis of permethylated cauloside C (XII) gave the methyl ester of 23-O-methylhederagenin, 2,3,4,6-tetra-O-methyl-D-glucose, and 3,4-di-O-methyl-L-arabinose (identification with authentic samples by PC and TLC).

An analysis of the PMR spectrum of the full acetate of cauloside C showed the β-glycosidic bond of the glucose with the arabinose (δ 4.73 ppm, J = 8 Hz), and an α-glycosidic bond (δ 4.42 ppm, J = 6.6 Hz) of the L-arabinose with the hederagenin.



(VI). R_1 = α-L-arabinopyranosyl; R_2 = H; R_3 = α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl.

(VII). $R_1 = R_2$ = H; R_3 = α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl.

(VIII). R_1 = α-L-arabinopyranosyl; R_2 = H; R_3 = β-D-glucopyranosyl.

(IX). R_1 = α-L-arabinopyranosyl; $R_2 = R_3$ = H.

(X). $R_1 = R_2$ = H; R_3 = β-D-glucopyranosyl.

(XI). R_1 = β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl; $R_2 = R_3$ = H.

(XII). R_1 = 2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl-(1 → 2)-2,3,4-tri-O-methyl-α-L-arabinopyranosyl; $R_2 = R_3$ = Me.

Thus, cauloside C is 3-O-(2-β-D-glucopyranosyl-α-L-arabinopyranosyl)hederagenin and is identical with saponin B isolated previously by Murakami et al. [8].

Since the progenin obtained by the alkaline hydrolysis of cauloside G gave no depression of the melting point in admixture with cauloside C (XI), we must ascribe the β configuration to the (1 → 2) bond of the glucose with the arabinose in cauloside G.

Thus, cauloside G is a new hederagenin glycoside and has the structure of 3-O-(2-β-D-glucopyranosyl-α-L-arabinopyranosyl)hederagenin 28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (I).

EXPERIMENTAL

The melting points of the substances (uncorrected) were determined on a Boetius stage, and the optical rotations of the substances on a Perkin–Elmer-141 polarimeter. The GLC analysis of the methylated monosaccharides was performed on a Pye–Unicam-104 instrument with a flame-ionization detector. We used glass columns (1.5 m × 6 mm) filled with the phase 3% OV-17 on Chromosorb W (100-200 mesh). The rate of flow of H_2 and of Ar was 50 ml/min, and the temperature 110-240°C. The following synthetic standards were used for GLC: methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside (T = O), 2,3,4,5,6-penta-O-acetyl-D-galactononitrile (T = 100), and methyl 3,4-di-O-methyl-β-L-arabinopyranoside.

The GLC–MS analysis of the methylated monosaccharides was performed on an LKB-9000S instrument. The PMR spectra were taken on a Bruker HX-90 spectrometer. For TLC we used KSK silica gel (> 300 mesh). The chromatograms were run in the following solvent systems: 1) chloroform–methanol–water (2 : 1 : to saturation); 2) chloroform–methanol–water (25 : 17 : 3); and 3) toluene–ethanol (9 : 1). The substances on the plates were revealed with H_2SO_4 or with a saturated solution of $SbCl_3$ in chloroform.

For column chromatography we used KSK silica gel (115-150 mesh) and the following solvent systems: 4) toluene–butan-1-ol–water (4 : 1; to saturation); 5) chloroform–ethanol; and 6) benzene–ethyl acetate (3 : 1 → 2 : 1).

The monosaccharides were chromatographed on M [slow] paper and in thin layers in the following systems: 7) benzene–pyridine–butan-1-ol–water (1 : 3 : 5 : 3); and 8) n-propanol–water (85 : 15).

The partially and fully methylated monosaccharides were analyzed on FN-12 and 15 papers in system 9 (methyl ethyl ketone saturated with 1% ammonia solution). The substances were revealed with a solution of aniline phthalate in n-butanol.

Isolation of (I). The total glycosidic fraction (15 g) was chromatographed on 1700 g of silica gel previously saturated with 75% of water in system 7. Fractions of similar composition were combined and evaporated. This gave 3 g of cauloside G with mp 218-220°C (n-butanol), $[\alpha]_D^{22}$ −3° (c 1.0; MeOH).

Methylation of (I) and (XI). To a solution of 2.0 g of cauloside G in 30 ml of dimethylformamide were added 12 g of Ag_2O and 30 ml of CH_3I. Methylation was performed in a current of argon for 48 h with two further additions of half the initial amounts of reagents. After the elimination of the precipitate and the excess of CH_3I, the solution was washed with saturated sodium thiosulfate solution, the product was extracted with chloroform, and the solution was dried with Na_2SO_4 and foamed with ethyl acetate, after which 1.750 g of the substance was methylated three times and isolated under the same conditions. Methylation was checked by TLC in system 3. The product of the fourth methylation was chromatographed on silica gel in system 6. The yield of (II) was 0.940 g.

Cauloside C (XI) (1.4 g) was methylated similarly. The permethylate product (XII) was chromatographed on silica gel in system 6 (25 : 1 → 5:1), giving 850 mg of (XII) with mp 98-100°C; $[\alpha]_D^{23}$ + 54.5° (c 0.17; $CHCl_3$).

Found %: C 65.87; H 9.46; OCH_3 24.7. $C_{58}H_{96}O_{18}$.
Calculated %: C 65.2: H 9.46; OCH_3 24.0.

Methanolysis of (II), (III), and (XII). Separately, 0.05 g of (II), 0.02 g of (III), and 0.38 g of (XII) were boiled with a mixture of 42% $HClO_4$ and methanol (1 : 5). The hydrolyzates were diluted with water and the aglycones were filtered off. The aglycones of compounds (II) and (XII) were methylated with CH_2N_2. This gave the methyl ester of 23-O-methylhederagenin. The aglycone of (III) had mp 190-192°C, $[\alpha]_D^{21}$ + 52° (c 0.46; $CHCl_3$).

The residues of the hydrolyzates were neutralized with Dowex 1 × 2 (100-200 mesh, HCO_3^- form), evaporated, and analyzed by PC in system 9.

Preparation of the Methyl Glycosides. A mixture of methylated monosaccharides obtained in the methanolysis of (II) was boiled in absolute methanol in the presence of Amberlite IR-120 (H^+) and was acetylated with a mixture of Ac_2O and pyridine (1 : 2) at room temperature. Methyl α,β-pyranosides of the following sugars were identified by GLC: 2,3,4,6-tetra-O-methyl-D-glucose ($T\beta$ 2.4; $T\alpha$ = 5.2), 2,3,4-tri-O-methyl-L-rhamnose ($T\beta$ = 8.76; $T\alpha$ = 11), 3,4-di-O-methyl-L-arabinose ($T\beta$ = 30; $T\alpha$ = 37), 6-O-acetyl-2,3,4-tri-O-methyl-D-glucose ($T\beta$ = 50; $T\alpha$ = 59); and 4-O-acetyl-2,3,6-tri-O-methyl-D-glucose ($T\beta$ = 54, $T\alpha$ = 78).

Reduction of (II). A solution of 0.045 g of (II) in 5 ml of tetrahydrofuran was treated with small portions of $LiAlH_4$, with the stirring and boiling of the solvent, until the evolution of H_2 ceased (3 h). Then 1 ml of water was added and the mixture was neutralized with 2 N H_2SO_4. Substance (III) was extracted with ether and (IV) with chloroform. This gave 30 mg of (III) and 10 mg of (IV). The oligosaccharide (IV), $[\alpha]_D$ −27° (c 0.73; $CHCl_3$), was acetylated with acetic anhydride in pyridine (1 : 2), and (V) was obtained in the usual way.

Enzymatic Hydrolysis of (I). A solution of 2.0 g of (I) in 100 ml of water was treated with 5 ml of snail digestive juice and the mixture was incubated at 44°C for eight days, with the periodic addition of 1-ml portions of the enzyme preparation. The progenins were extracted with n-butanol and chromatographed on silica gel saturated with 6% of water in system 5 (15 : 1 → 1 : 3). This yielded 12 mg of (VI), mp 208-210°C (MeOH); 96 mg of (VII) – an amorphous substance; 100 mg of (VIII), mp 210-214°C, $[\alpha]_D$ + 34° (MeOH); 80 mg of (IX), mp 225-228°C; 60 mg of (X), mp 220-223°C (MeOH), $[\alpha]_D$ + 35° ($CHCl_3$); and 102 mg of hederagenin.

SUMMARY

From the roots and rhizomes of Caulophyllum robustum Maxim, we have isolated a new triterpene glycoside – cauloside G – which has the structure of 3-O-(2-β-D-glucopyranosyl-α-L-arabinopyranosyl)hederagenin 28-O-α-L-rhamnosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-α-D-glucopyranoside.

It has been established that cauloside C is identical with saponin B isolated previously from the same plant.

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