TRITERPENE GLYCOSIDES OF *Climacoptera transoxana*.

III. THE STRUCTURES OF COPTEROSIDES E AND F

Ch. Annaev, M. I. Isaev,
and N. K. Abubakirov

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New triterpene glycosides have been isolated from the epigeal part of *Climacoptera transoxana* (Iljin) Botsch. — copterosides E and F. According to chemical transformations and physicochemical characteristics, copteroside E has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-{[O-β-D-xylopyranosyl-(1→2)]-[O-β-D-xylopyranosyl-(1→4)]-β-D-glucuronopyranoside} and copteroside F that that of hederagenin 28-O-β-D-glucopyranoside 3-O-{[O-β-D-xylopyranosyl-(1→2)]-[O-β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside}.

Continuing a study of the triterpene glycosides of *Climacoptera transoxana* (Iljin) Botsch. family *Chenopodiaceae*, we have isolated from a methanolic extract of the epigeal part of this plant substances E and F [1, 2], which we have called copterosides E (I) and F (II), respectively. In the present paper we give proofs of the structures of these glycosides.

The acid hydrolysis of copterosides E (I) and F (II) led to the formation of oleanolic acid (III) and hederagenin (IV), respectively.

In hydrolysates of boths these glycosides, D-glucuronic acid, D-glucose, and D-xylose were detected by PC and TLC. GLC [3] showed the presence of the same monosaccharides in the same ratios — 1:1:2.

As has been shown previously [1], the glycosides (I) and (II) under consideration contain O-acylosidic carbohydrate components. The alkaline hydrolysis of copterosides E (I) and F (II) gave the corresponding progenins (X) and (XI).

The acid hydrolysis of the glycosides (X) and (XI) followed by the analysis of the carbohydrate fractions of the hydrolysates by TLC and PC showed that they contained no D-glucose.

Progenins (X) and (XI) were methylated by Hakomori's method [4]. The molecular ions in the mass spectra of the permethylates obtained (XII) (M^+ 1022) and (XIII) (M^+ 1052) showed that these glycosides each contains three sugar residues, i.e., D-glucuronic acid and D-xylose in a ratio of 1:2. Consequently the O-acylosidic moieties of copterosides E and F are represented by D-glucose alone.

Copterosides E (I) and F (II) were also methylated by Hakomori's method, which gave the permethylates (VIII) (M^+ 1226) and (IX) (M^+ 1256), respectively. The permethylates (VIII) and (IX) were subjected to acid hydrolysis. Oleanolic acid (III) and 23-O-methylhederagenin (IV), respectively, were isolated from the genin fractions of the hydrolysis products.

Analysis of the carbohydrate fractions of the hydrolysates of the permethylates with the aid of TLC in various solvent systems showed that they were identical and consisted of three components — O-methyl derivative of D-glucuronic acid, D-glucose, and D-xylose. The last two were identified with the aid of TLC in the presence of authentic samples as 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose. The formation of 2,3,4-tri-O-methyl-D-xylose shows that the two D-xylose molecules are terminal, and the D-glucuronic acid residue is a center of branching. This was confirmed by the formation of the 3-O-β-D-glucuronopyranosides of oleanolic acid (VI) and of hederagenin (VII) [1], respectively, both in the Smith degradation [5] of copteroside E (I) and F (II) and also in the stepwise hydrolysis of the progenins (X) and (XI).

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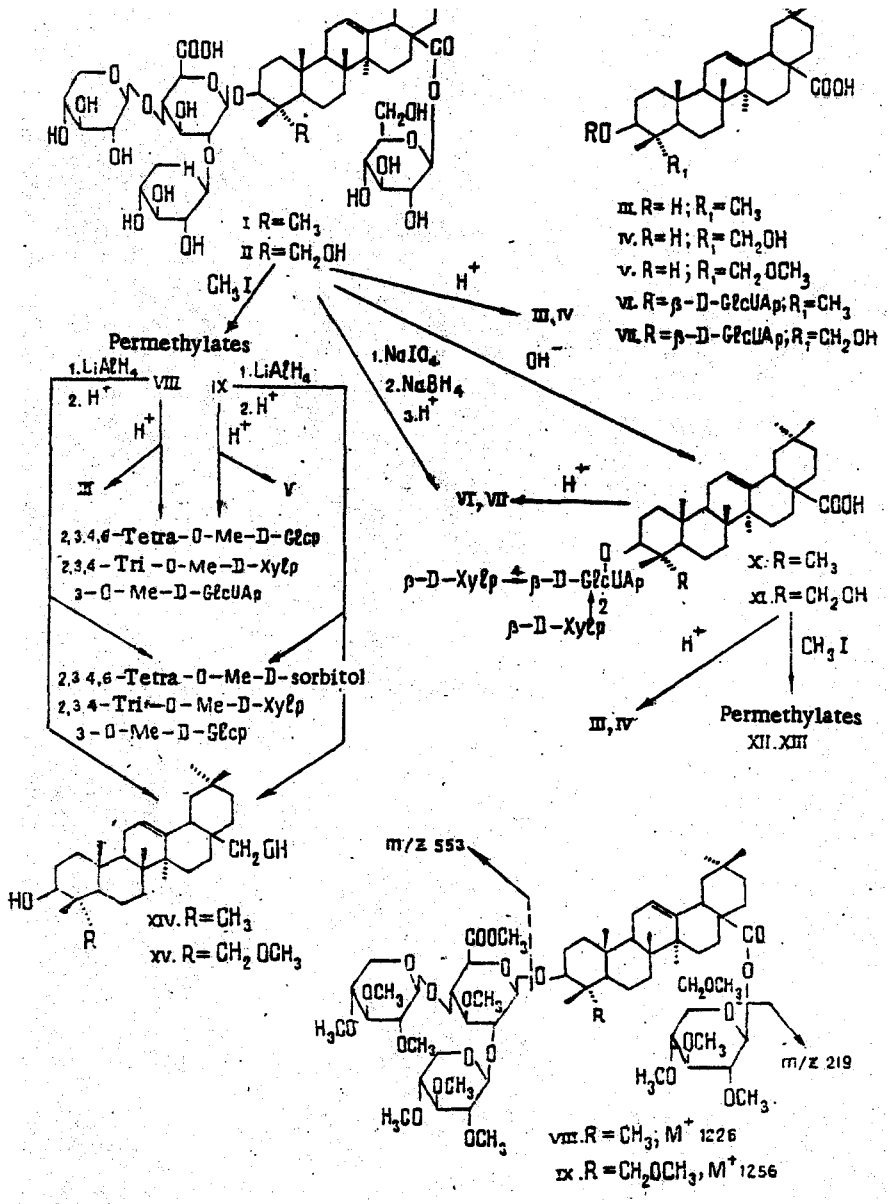

Consequently, the third methylated sugar obtained from the permethylates (VIII) and (IX) must be a mono-O-methyl derivative of D-glucuronic acid.

To determine the structure of this mono-O-methyl derivative, the permethylates (VIII) and (IX) were subjected to reduction with lithium tetrahydroaluminate, followed by acid hydrolysis. In the genin fraction of the products of reductive cleavage obtained from the permethylate (VIII) erythrodiol (XIV) was detected, and in the permethylate of (IX) 28-hydroxy-23-methoxy-β-amyrin (XV). The carbohydrate fractions of the products proved to be identical in the two cases, consisting of 2,3,4,6-tetra-O-methyl-D-sorbitol, 2,3,4-tri-O-methyl-D-xylose, and a mono-O-methyl-D-glucose.

The last substance, isolated in the individual form, gave a positive Bonner reaction [6], showing the presence of an α-diol grouping. However, the methyl glycoside obtained by methylating the mono-O-methyl-D-glucose was not oxidized by sodium periodate and did not react with the Bonner reagent. These facts unambiguously determine the mono-O-methyl derivative of D-glucose as 3-O-methyl-D-glucose, and, correspondingly, the mono-O-methyl derivative of D-glucuronic acid is therefore 3-O-methyl-D-glucuronic acid.

Additional information on the structures of copterosides E (I) and F (II) was obtained by analyzing the mass spectra of the corresponding permethylates (VIII), (IX), (XII), and (XIII). The presence of the peak of an ion with m/z 553 in the mass spectrum of each of the permethylates (VIII), (IX), (XII), and (XIII), corresponding to the methylation of a carbo-

hydrate chain at C-3 of the aglycone including one D-glucuronic acid and two D-xylose residues, and the peak of an ion with m/z 219 in the mass spectrum of each of the permethylates (VIII) and (IX), corresponding to a completely methylated hexose [7] permitted the assumption that the D-glucose was attached to the carboxyl of each aglycone.

A calculation of molecular rotation differences by Klyne's method [8] showed that all the anomeric centers had the β configuration.

Thus, copteroside E (I) and F (II) form a pair of bisdesmosidic glycosides differing from one another only by the genins.

Copteroside E has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-{[O-β-D-xylopyranosyl-(1→2)]-[O-β-D-xylopyranosyl-(1→4)]-β-D-glucuronopyranoside, and copteroside F is hederagenin 28-O-β-D-glucopyranoside 3-O-{[O-β-D-xylopyranosyl-(1→2)]-[O-β-D-xylopyranosyl-(1→4)]-β-D-glucuronopyranoside}.

EXPERIMENTAL

General Observations. For general observations, see [1]. The gas–liquid chromatography of the silylated methyl glycosides was performed on Chrom 5, chromatograph with a 1.27 m × 3 mm column containing 20% of Termol-2 on Chromaton N-AW, 200°C, rate of flow of helium 40 ml/min.

The following solvent systems were used: 1) chloroform–methanol–water [a) (65:35:8); b) (76:27:6)]; 2) butanol–ethanol–25% ammonia (7:2:5); 3) chloroform–methanol [a) (20:1); b) (50:1); c) (15:3)]; 4) benzene–acetone [a) (2:1); b) (10:1)]; 5) butan-1-ol–acetic acid–water (4:1:5); and 6) butan-1-ol–methanol–water (5:3:1).

Isolation of Copterosides E (I) and F (II). Fractions consisting of mixtures of compounds D and E (2.2 g), D, E, and F (3.5 g), and E and F (4.76 g) [1, 2] were chromatographed separately on columns of silica gel with elution by system 1a. After repeated chromatography of fractions enriched with individual compounds, 1.6 g of glycoside E (I) was obtained: $C_{52}H_{82}O_{22}$, mp 236-240°C (decomp.) (from aqueous acetone), $[\alpha]_D^{20}$ +10 ± 2° (c 1.4; water–methanol (1:2)); ν_{max}^{KBr}, cm^{-1}: 3600-3200 (OH); 1740 (C=O of an ester group); and 3.2 g of glycoside F (II), $C_{52}H_{82}O_{23}$, mp 230-232°C (decomp., from aqueous acetone), $[\alpha]_D^{20}$ +28 ± 2° (c 1.14; water–methanol (1:1)); ν_{max}^{KBr}, cm^{-1}: 3600-3200 (OH); 1730 (C=O of an ester group).

The yield of copteroside E (I) was 0.32% and that of copteroside F (II) 0.67% on the weight of the air-dry raw material.

Oleanolic Acid (III) from (I). Copteroside E (50 mg) was hydrolyzed with a 6% solution of sulfuric acid at 100°C for 6 h. The precipitate that deposited was separated off, washed several times with water, and transferred to a column of silica gel, and elution with system 3b yielded in the individual state 22 mg of oleanolic acid (III) with mp 304-306°C (from ethanol), $[\alpha]_D^{20}$ +78 ± 2° (c 1.3; ethanol).

The filtrate was neutralized with barium carbonate and evaporated. D-glucose, D-glucuronic acid, and D-xylose were found in the residue by PC (system 5) and TLC (system 6). The ratio of the sugars according to GLC was 1:1:1.93.

Alkaline Hydrolysis of Copteroside E (I). Copteroside E (I) (0.8 g) was saponified with an 8% solution of KOH at 100°C for 4 h. The reaction mixture was neutralized with dilute sulfuric acid and extracted with butanol. The butanolic extract was washed with water and evaporated to dryness. The residue was first chromatographed on a column of silica gel with elution by system 1a and then on Sephadex G-10 with elution by water. This gave 510 mg of the trioside (X), $C_{46}H_{72}O_{17}$, mp 218-222°C (methanol–acetone (1:2)), $[\alpha]_D^{20}$ 0 ± 3° (c 1.1; methanol).

Acid Hydrolysis of Glycoside (X). Trioside (X) (40 mg) was hydrolyzed with 6% sulfuric acid on the water bath for 6 h. After the elimination of the oleanolic acid and neutralization, D-glucuronic acid and D-xylose were detected in the hydrolysate by TLC in system 6.

Partial Hydrolysis of the Glycoside (X). Glycoside (X) (300 mg) was hydrolyzed with 0.5% aqueous sulfuric acid on the water bath for 8 h. The reaction mixture was diluted with water and exhaustively extracted with butanol. The butanolic extract was washed with water and evaporated. The residue was chromatographed on a column of silica gel. Elution with system 3b gave oleanolic acid (41 mg).

Continuing the elution of the column with system 1b, 32 mg of oleanolic acid glucuronoside (VI) was obtained with mp 214-217°C (from ethanol), $[\alpha]_D^{20}$ +30.2 ± 2° (c 1.2; ethanol), also identified from its R_f values on TLC in systems 1 and 2 in the presence of an authentic sample [9].

The Permethylate (XII) from (X). The Hakomori methylation [4] of 400 mg of glycoside (X) gave 310 mg of the permethylate (XII), $C_{55}H_{90}O_{17}$, mp 104-106°C (from methanol), $[\alpha]_D^{20}$ −21 ± 2° (c 0.95; methanol). Mass spectrum, m/z (%): M^+ 1022(0.49), 962(0.14), 553(1.4), 453(40), 262(46), 203(51), 175(100).

Smith Degradation of Copteroside E (I). A solution of 400 mg of substance (I) in 50 ml of 5% aqueous periodic acid was left at room temperature for 48 h. Then the reaction products were extracted with chloroform, and the chloroform extract was washed with water and evaporated. The residue was dissolved in 50 ml of methanol, 0.4 g of sodium tetrahydroborate was added, and the mixture was left at room temperature for 24 h. Then it was acidified with dilute hydrochloric acid to pH ≈ 1 and was left for another 24 h at the same temperature, after which the reaction products were extracted with butanol, and the butanolic extract was washed with water and evaporated to dryness. The residue was chromatographed on a column of silica gel with elution by system 1b. This gave 80 mg of oleanolic acid 3-O-β-D-glucosiduronic acid (VI), shown to be identical with an authentic sample.

The Permethylate (VIII) from (I). The Hakomori methylation of 800 mg of glycoside (I), after the appropriate working up of the products and their chromatography on a column of silica gel with elution by system 4b yielded the amorphous permethylate (VIII), $C_{64}H_{106}O_{22}$, $[\alpha]_D^{20}$ +1.3 ± 2° (c 1.5; methanol). The IR spectrum lacked the absorption due to hydroxy groups. Mass spectrum, m/z (%): M^+ 1226(0.05), 962(1.0), 553(0.25), 657(0.8), 467(10), 248(16), 219(13.5), 218(30), 203(61), 175(43), 143(100).

Acid Hydrolysis of the Permethylate (VIII). The permethylate (VIII) (200 mg) was hydrolyzed with 7% methanolic sulfuric acid at the boiling point of the reaction mixture for 6 h. After the usual working up, 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-xylose were identified in the carbohydrate fraction of the hydrolysate by TLC in systems 4a and 3c in the presence of authentic samples and with the aid of GLC. In addition to the methylated sugars mentioned, TLC revealed the presence of a more polar component — 3-O-methyl-D-glucuronic acid.

Oleanolic acid (III) was identified as the aglycone.

Reductive Cleavage of the Permethylate (VIII). A solution of 300 mg of the permethylate (VIII) in 50 ml of absolute tetrahydrofuran was treated with 0.25 g of lithium tetrahydroaluminate. The mixture was stirred at the boiling point for 8 h. Then to decompose the excess of reducing agent 10 ml of acetic acid and 50 ml of dilute sulfuric acid were added. After the organic layer had been separated off, the aqueous layer was extracted with ether. The organic extracts were combined and evaporated. The residue was hydrolyzed, and in the genin fraction of hydrolysate erythrodiol (XIV) was detected, with mp 234-236°C (from acetone).

In the filtrate, after appropriate treatment, 2,3,4,6-tetra-O-methyl-D-sorbitol and 2,3,4-tri-O-methyl-D-xylose were identified by TLC in the presence of markers. A polar component, 3-O-methyl-D-glucose, was isolated with the aid of a column and elution with system 3c. It gave a positive Bonner reaction [6]. The mono-O-methyl-D-glucose was subjected to methylation with absolute methanol containing 6% of sulfuric acid. The methyl mono-O-methyl-D-glucoside so obtained was not oxidized by sodium periodate in the course of two days.

Hederagenin (IV) from (II). Copteroside F (II) (100 mg) was hydrolyzed with a 6% solution of sulfuric acid. After the usual working up and purification, 20 mg of a genin was obtained which was identified by TLC in system 3a as hederagenin. The set of carbohydrates consisted of D-glucose, D-glucuronic acid, and D-xylose. The ratio of the monosaccharides according to GLC was 1:1:2.01, respectively.

Alkaline Hydrolysis of Copteroside F (II). Glycoside (II) (1.2 g) was hydrolyzed with 8% KOH solution on a water bath for 4 h. The reaction product was purified on Sephadex G-10, giving 820 mg of the trioside (XI), $C_{46}H_{72}O_{18}$, mp 242-244°C (from aqueous acetone); $[\alpha]_D^{20}$ +21 ± 2° (1.6; methanol).

Acid Hydrolysis of the Glycoside (XI). The trioside (XI) (100 mg) was hydrolyzed with 6% sulfuric acid on a water bath for 7 h. Hederagenin was identified by TLC in system 3a, and D-xylose, and D-glucuronic acid in system 6.

Hederagenin 3-O-β-D-Glucuronopyranosiduronic Acid (VII) from (XI). The stepwise acid hydrolysis of (XI) (0.5 g) with 0.5% sulfuric acid led to the glucuronoside (VII) (150 mg), which was identified by TLC in systems 1 and 2 [1, 9].

The Permethylation (XIII) from (XI). The Hakomori methylation of 0.5 g of glycoside (XI) gave the permethylate (XIII), $C_{56}H_{92}O_{18}$, $[\alpha]_D^{20}$ +25 ± 2° (c 0.8; methanol). The IR spectrum lacked the characteristic absorption of hydroxy groups. Mass spectrum, m/z (%): M^+ 1052(0.02), 992(0.03), 553(0.46), 483(6.0), 262(30), 203(100), 175(56).

Smith Degradation of Copteroside F (II). The destructive cleavage of 500 mg of glycoside (II) was carried out by the method described for copteroside E (I). The final product (50 mg) proved to identical with hederagenin glucosiduronic acid.

The Permethylate (IX) from (II). Copteroside F (II) (1.2 g) was methylated by Hakomori's method. This gave 900 mg of the permethylate (IX), $C_{63}H_{108}O_{23}$, $[\alpha]_D^{20}$ +12 ± 2° (c 0.83; methanol). The band of the absorption of hydroxy groups was absent from the IR spectrum. Mass spectrum, m/z (%): M^+ 1256(0.05), 992(0.33), 686(0.3), 553(1.0), 467(7), 248(26), 219(40), 218(33), 203(50), 175(96), 143(100).

23-O-Methylhederagenin (V) from (IX). The permethylate (IX) (200 mg) was hydrolyzed with a 7% solution of sulfuric acid in methanol. After appropriate working up, in the genin fraction of the reaction products 23-O-methylhederagenin was identified by TLC (system 3a) in the presence of an authentic sample.

In the filtrate 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-D-xylose, and 3-O-methyl-D-glucuronic acid were identified by GLC in systems 4a and 3c.

Reductive Cleavage of the Permethylate (IX). The permethylate (IX) (200 mg) was subjected to reductive cleavage by the method described above. In the cleavage products 28-hydroxy-23-methyoxy-β-amyrin (XV), mp 188-190°C (from ethanol) was detected as the genin. The methylated carbohydrates proved to be 2,3,4-6-tetra-O-methyl-D-sorbitol, 2,3,4-tri-O-methyl-D-xylose, and 3-O-methyl-D-glucose.

SUMMARY

The epigeal part of *Climacoptera transoxana* (Iljin) Botsch. has yielded two new triterpene glycosides — copteroside E and F, which form a pair of bisdesmosidic glycosides.

Copteroside E has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-{[O-β-D-xylopyranosyl-(1→2)]-[O-β-D-xylopyranosyl-(1→4)]-β-D-glucuronopyranoside} and copteroside F is hederagenin 28-O-β-D-glucopyranoside 3-O-{[O-β-D-xylopyranosyl-(1→2)]-[O-β-D-xylopyranosyl-(1→4)]-β-D-glucuronopyranoside}.

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