TRITERPENE GLYCOSIDES OF *Climacoptera transoxana*.

IV. STRUCTURES OF COPTEROSIDES G AND H

Ch. Annaev and N. K. Abubakirov

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From the epigeal part of the plant *Climacoptera transoxana* (Iljin) Botsch. have been isolated new triterpene glycosides — copterosides G and H, which are bisdesmosidic glycosides. On the basis of chemical transformations and spectral characteristics, copteroside G has been assigned the structure of gypsogenic acid 28-O-β-D-glucopyranoside 3-O-β-D-glucuronopyranoside, while copteroside H is gypsogenic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 2)-β-glucuronopyranoside].

We have previously [1] reported on the presence in the combined glycosides from *Climacoptera transoxana* (Iljin) Botsch. (family Chenopodiaceae) of compounds G (I) and H (VIII). Glycoside G predominates quantitatively. According to the results of acid hydrolysis, the genin of both glycosides is gypsogenic acid (II). Copteroside G (I) proved to be a bioside of gypsogenic acid containing D-glucose and D-glucuronic acid residues as the sugar components. A ratio of the sugars of 1:1 was confirmed by GLC analysis [2]. Copteroside H (II) differed from copteroside G (I) by the presence of an additional D-xylose residue.

Glycosides (I) and (VIII) were subjected to the action of alkali. Acid hydrolysates of the progenins (III) and (X) obtained on alkaline saponification, did not contain D-glucose. Consequently, this constituted the acylosidic moiety of each of compounds (I) and (VIII).

The hydrolysis of copterosides G (I) and H (VIII) (separately) with dilute sulfuric acid led to the formation of the progenin (III), coinciding in its physicochemical constants with the substance obtained on the alkaline saponification of glycoside G (I). As will be shown below, this is gypsogenic acid 3-O-β-D-glucuronopyranoside.

To determine the dimensions of the oxide rings of the D-glucose and D-xylose residues, copterosides G (I) and H (VIII) were methylated by Hakomori's method [3]. The permethylates (VI) (M^+ 950) and (IX) (M^+ 1110) were obtained. The permethylate (VI) was hydrolyzed with sulfuric acid. As the genin a product (VII) was isolated, the mass spectrum of which contained the peak of the molecular ion with a mass of 500 and the peaks of the main fragments of retrodiene fragmentation with m/z 248 and 203. We are justified in assigning to compound (VII), which is here described for the first time, the structure of gypsogenic acid 23-monomethyl ester. The methylated sugars proved to be 2,3,4-tri-O-methyl-D-glucuronic acid and 2,3,4,6-tetra-O-methyl-D-glucose (scheme 1).

In order to determine the positions of attachment of the D-xylose and the D-glucuronic acid residues in copteroside H (VIII), the permethylate (IX) (scheme 2) was reduced with lithium tetrahydroaluminate and hydrolyzed. The mass spectrum of the genin (XI) of the reduced product had the peak of the molecular ion with m/z 458. The presence of fragments with m/z 234, 223, 216, and 203 permitted substance (XI) to be identified as 23,28-dihydroxy-β-amyrin. The sugar fraction of the hydrolysate consisted of 2,3,4,6-tetra-O-methyl-D-sorbitol, 2,3,4-tri-O-methyl-D-xylose, and a dimethyl derivative of D-glucose. The last-mentioned sugar gave a positive reaction for the presence of an α-diol grouping [4]. It was established with the aid of GLC that this compound was 3,4-di-O-methyl-D-glucose. (See scheme 1.)

Consequently, the D-xylose and D-glucose residues in glycoside (VIII) are terminal residues: the D-xylose residue is attached to the D-glucuronic acid residue by a 1 → 2 bond, and the D-glucose residue is attached to the C-28 carboxy group (scheme 2).

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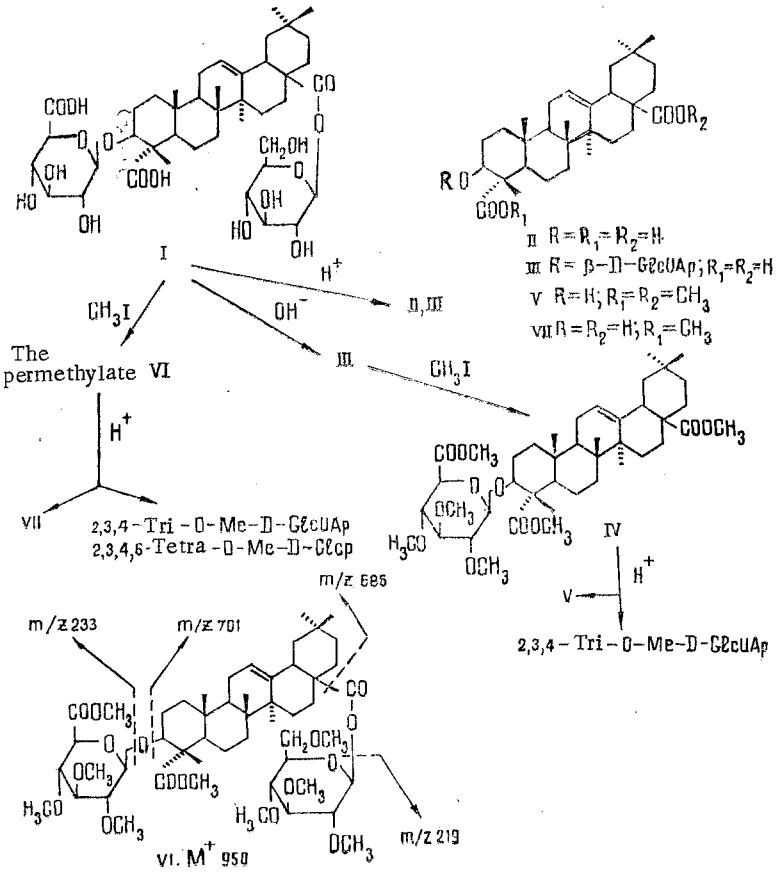


Scheme 1.

In the mass spectrum of the permethylate (VI) there are the characteristic peaks of ions with m/z 701, 686, 233, and 219, confirming the suggested structure (I) (scheme 1).

In the spectrum of the permethylate (IX), there is the characteristic peak of an ion with m/z 393, corresponding to the carbohydrate chain at C-3, and the peaks of fragments with m/z 846 and 701 show the nature of the substitution at C-3 and C-28 of the aglycone. The terminal nature of the D-glucose and D-xylose residues is shown by the peaks of ions with m/z 219 and 175.

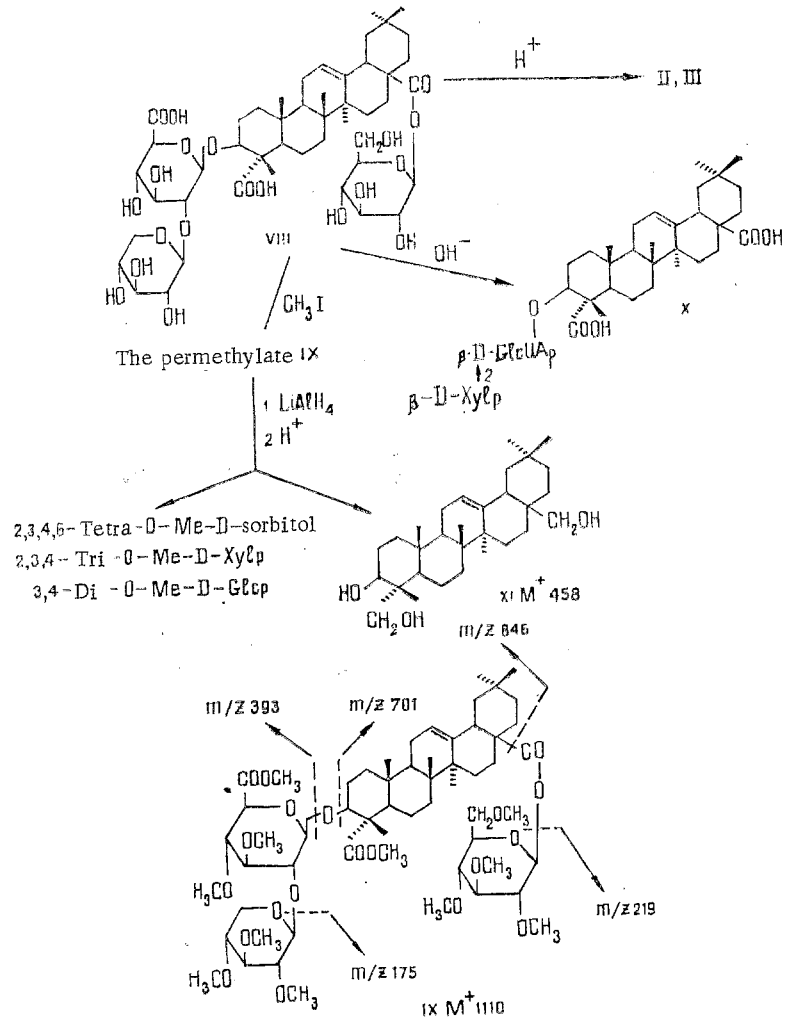
Since this is the first time that gypsogenic acid glucuronopyranoside (III) has been described, we confirmed its structure by the production of the permethylate (IV) (scheme 1). The acid hydrolysis of compound (IV) (M^+ 746) gave 2,3,4-tri-O-methyl-D-glucuronic acid and dimethyl gypsogenate (V) (M^+ 514). The PMR spectrum of the permethylate (IV) showed at 4.15 ppm the signal of an anomeric proton in the form of a doublet with J = 7.5 Hz. This fact indicates the β configuration of the glycosidic bond [5].

Molecular rotation difference calculations showed the β configuration of all the other glycosidic centers of both glycosides.

Copteroside G has the structure of gypsogenic acid 28-O-β-D-glucopyranoside 3-O-β-D-glucuronopyranoside, while copteroside H is gypsogenic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranoside].

Copterosides G (I) and H (VIII) are the most polar glycosides of the plant. After their structure had been determined it was found that their polarity was due not to a lengthening of the carbohydrate chain but to an additional carboxy group in the aglycone.

Thus, from the plant *Climacoptera transoxana* have been isolated glycosides of three related aglycones: oleanolic acid (copteroside E), hederagenin (copterosides B, C, D, and F), and gypsogenic acid (copterosides G and H). It was to be expected that glycosides of gypsogenin would also be detected, since gypsogenic acid is usually a product of the autooxidation of gypsogenin at the aldehyde group. It is not excluded that when the plant is studied in other vegetation stages glycosides of the missing link will also be found.



Scheme 2

EXPERIMENTAL

General Observations. The following solvent systems were used: 1) chloroform—methanol—water (65:35:8); 1-butanol—ethanol—25% ammonia (7:2:5); 3) chloroform—methanol [a — (2:1); b — (9:1)]; 4) benzene—acetone (2:1); 5) 1-butanol—acetic acid—water (4:5:1); 6) 1-butanol—methanol—water (5:3:1).

The gas-liquid chromatography of the methyl glycosides of the methylated sugars was performed on a Chrom-5 chromatograph with a 1 m × 3 mm column containing Celite bearing 20% of poly(butane-1,4-diyl succinate) at a temperature of 160°C with a rate of flow of the carrier gas of 50 ml/min.

PMR spectra were taken on a JNM-4H-100 (100 MHz instrument) with HMDS as internal standard.

In all cases, the completeness of methylation was confirmed by the disappearance of the absorption band of hydroxy groups from the IR spectra. Other information is given in a previous paper [1].

Isolation of Copterosides G (I) and H (VIII). The fractions containing glycosides G and H were rechromatographed separately on columns of silica gel in system 1. The individual glycosides were obtained. Copteroside G (I) (yield on the weight of air-dry raw material, 0.41%), $C_{42}H_{64}O_{16}$, mp 214-218°C (from methanol), $[\alpha]_D^{20}$ +28.0 ± 2° (c 1.2); methanol).

Copteroside H (VIII), yield 0.12%, $C_{47}H_{72}O_{20}$, mp 240-244°C (from methanol), $[\alpha]_D^{20}$ +32 ± 2, (c 0.95); methanol—water (1:1)). All the gypsogenic acid glycosides described in this paper melted with decomposition.

Gypsogenic Acid (II) from Glycosides (I) and (VIII). Glycosides (I) (100 mg) and (VIII) (45 mg), separately, were hydrolyzed with 6% sulfuric acid at 100°C for 6 h. The reaction mixtures were diluted with water. The precipitate that deposited were separated off and washed with water. On columns of silica gel, chloroform, and system 3b eluted, respectively, 25 mg and 12 mg of the pure substance (II) with mp > 350° (from aqueous methanol), $[\alpha]_D^{20}$ +96 ± 2° (c 0.8; pyridine). According to the literature [6]: mp 380°C. The IR spectrum of the compound that we have obtained coincided with that of an authentic sample of gypsogenic acid.

The acid hydrolysates were neutralized with $BaCO_3$. In the evaporated residues D-glucose and D-glucuronic acid were detected by PC (system 5) and TLC (system 6), and in the hydrolysate and glycoside (VIII) D-xylose was also detected.

Alkaline Hydrolysis of Copteroside G (I). Glycoside G (200 mg) was saponified with an 8% solution of KOH at 100°C for 5 h. After neutralization with 5% sulfuric acid solution, the reaction mixture was extracted with n-butanol. The butanolic extracts were washed with water and evaporated to dryness. On a column of silica gel with elution by system 1, 132 mg of the individual substance (III) was isolated. On subsequent hydrolysis with 6% H_2SO_4 this substance gave gypsogenic acid (II) and D-glucuronic acid.

Gypsogenic Acid 3-O-β-D-Glucuronopyranoside (II) from Glycosides (I) and (VIII). Glycosides (I) (500 mg) and (VIII) (240 mg) were hydrolyzed with 1% sulfuric acid solution at 100°C for 6 h. The reaction mixtures were diluted with water and exhaustively extracted with n-butanol. The butanolic extracts were washed with water and concentrated. On columns of silica gel with elution successively by solvent systems 3a and 1, gypsogenic acid glucuronoside (III) was isolated in an amount of 150 mg and 80 mg, respectively, mp 26-228°C (from aqueous ethanol), $[\alpha]_D^{20}$ +33.0 ± 2°C (c 1.3; ethanol). On TLC in systems 1 and 2, the acid (III) did not differ from the substance isolated by the alkaline saponification of copteroside G (I).

The glucuronoside (III) (200 mg) was methylated by Hakamori's method (see the following experiment). The permethylate (IV) [dimethyl gypsogenate 3-O-(methyl 2,3,4-tri-O-methyl-D-glucopyranosiduronate)], $C_{42}H_{66}O_{11}$, had mp 162°C (from acetone), $[\alpha]_D^{20}$ +23 ± 2° (c 0.9); benzene).

Mass spectrum, m/z (%): M^+ 746 (0.53), 686 (2.3), 518 (5.1), 497 (32), 262 (41), 233 (39.3), 203 (100).

PMR spectrum ($CDCl_3$, δ, ppm): 0.64-1.12 (the signals of protons from six methyl groups, in the form of singlets), 3.38-3.71 (the signals of the protons of six methoxy groups), 4.15 (anomeric proton in the β-D-glucuronic acid residue, d, J = 7.5 Hz).

The permethylate (IV) (80 mg) was hydrolyzed with a 6% solution of sulfuric acid in methanol at the boil for 5 h. Dimethyl gypsogenate (V) was isolated. $C_{32}H_{50}O_5$, mp 240-242°C (from ethyl acetate). According to the literature [6]: mp 249-250°C. Mass spectrum, m/z (%): 514 (M^+) (5.5), 262 (58.0), 203 (100.0).

The Permethylate (VI) from Copteroside G (I). Glycoside (I) (500 mg) was methylated by Hakomori's method (40 ml of dimethyl sulfoxide, 400 mg of NaH, and 6 ml of CH_3I, at room temperature). This gave 420 mg of the permethylate (VI), amorphous, $C_{51}H_{82}O_{16}$, $[\alpha]_D^{20}$ +17 ± 2° (c 0.7; methanol).

Mass spectrum, m/z (%): M^+ 950 (0.25), 730 (0.7), 701 (1.2), 686 (71.4), 248 (20.4), 233 (24.4), 219 (30), 187 (100), 201 (61).

The permethylate (VI) (220 mg) was hydrolyzed in 20 ml of 6% sulfuric acid in methanol at the boil for 5 h. As the genin was obtained the 23-monomethyl gypsogenate (VII), with mp 195-197°C (from acetone). ν_{max}^{KBr}, cm^{-1}: 3520 (OH), 1710, 1730, 1255 (C=O of carbonyl and ester groups).

Mass spectrum, m/z (%): M^+ 500 (24.8), 251 (25), 248 (50), 203 (100).

In the hydrolysate, 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4-tri-O-methyl-D-glucuronic acid were detected with the aid of TLC (system 4).

Alkaline Hydrolysis of Copteroside H (VIII). The glycoside (VIII) (120 mg) was saponified under the conditions described for copteroside G. This led to the isolation of 90 mg of a gypsogenic acid glycoside (X) with mp 208-212°C (from ethanol), $[\alpha]_D^{20}$ +18 ± 2° (c 1.1;

methanol). When 50 mg of this substance was hydrolyzed with 6% sulfuric acid at 100°C for 7 h, D-glucuronic acid and D-xylose were detected by TLC in system 6.

The Permethylate (IX) from Copteroside H (VIII). The glycoside (200 mg) was methylated by Hakomori's method (40 ml of dimethyl sulfoxide, 150 mg of sodium hydride, and 4 ml of CH_3I). This gave 145 mg of the permethylate (IX) in amorphous form, $C_{58}H_{94}O_{20}$, $[\alpha]_D^{20}$ +10 ± 2° (c 0.5; methanol). Mass spectrum, m/z (%): M^+ 1110 (0.02), 846 (16.6), 701 (0.6), 483 (41.6), 393 (0.4), 248 (30.5), 219 (25.0), 175 (91.6), 101 (100.0).

Reductive Cleavage of the Permethylate (IX). Compound (IX) (100 mg) was reduced with lithium tetrahydroaluminate (80 mg) by the usual procedure. The product of the reaction was hydrolyzed with a 6% solution of sulfuric acid in methanol. According to TLC in system 4, the sugar fraction consisted of 2,3,4,6-tetra-O-methyl-D-sorbitol, 2,3,4-tri-O-methyl-D-xylose, and 3,4-di-O-methyl-D-glucose. The last-mentioned methylated sugar was also identified with the aid of GLC [7], and its reaction for an α-diol group was positive.

The genin of the reduced product was characterized as 23,28-dihydroxy-β-amyrin (XI), mp 198-200°C (hexane—acetone (10:2)) mass spectrum, m/z (%): M^+ 458 (3.0), 234 (40.0), 223 (5.0), 216 (25.0), 203 (100.0).

SUMMARY

From the epigeal part of *Climacoptera transoxana* (Iljin). Botsch. have been isolated two new triterpene glycosides — copterosides G and H, which are bisdesmosidic glycosides.

Copteroside G had the structure of gypsogenic acid 28-O-β-D-glucopyranoside 3-O-β-D-glucuronopyranoside, and copteroside H is gypsogenic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranoside].

LITERATURE CITED

1. Ch. Annaev, M. Isamukhamedova, and N. K. Abubakirov, Khim. Prir. Soedin., 113 (1982).
2. T. T. Gorovits, Khim. Prir. Soedin., 263 (1970).
3. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
4. T. G. Bonner, Chem. Ind. (London), 345 (1960).
5. J. M. van der Veen, J. Org. Chem., 28, 564 (1963).
6. L. Ruzicka, G. Giacomello, and Ad. Grob, Helv. Chim. Acta, 21, 83 (1938).
7. G. O. Aspinall, J. Chem. Soc., 1676 (1963).

TRITERPENE GLYCOSIDES OF *Salsola micranthera*.

II. THE STRUCTURE OF SALSOLOSIDE E

Ch. Annaev, M. Isamukhamedova, and N. K. Abubakirov

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A new triterpene glycoside — salsoloside E — has been isolated from the epigeal part of the plant *Salsola micranthera* Botsch. family Chenopodiaceae. On the basis of chemical transformations and physicochemical characteristics its structure has been established as oleanolic acid 28-O-β-D-glucopyranoside 3-O-{[O-β-D-glucopyranosyl-(1 → 2)][O-β-D-xylopyranosyl-(1 → 4)]-β-D-glucuronopyranoside}.

From the combined glycosides of the epigeal part of *Salsola micranthera* Botsch. (family Chenopodiaceae) we have isolated an individual glycoside — salsoloside E (I) [1]. This is quantitatively the main glycoside of the plant. Gas—liquid chromatography [2] showed the presence in the sugar moiety of salsoloside E of D-glucuronic acid, D-glucose, and D-xylose residues in a ratio of 1:2:1. The aglycone of the new compound is oleanolic acid (II). In an acid hydrolysate of compound (V) obtained by the alkaline saponification of glycoside E, the same monosaccharides were detected with the aid of PC and TLC.

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