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TRITERPENE GLYCOSIDES OF *Climacoptera transoxana*. I.

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Two tripene glycosides — copterosides B and C — have been isolated from the epigeal part of *Climacoptera transoxana* (Iljin) Botsch. On the basis of chemical transformations and physicochemical characteristics, copteroside B has been identified as hedaragenin 3-O-β-D-glucopyranosiduronic acid while copteroside C has the structure of hederagenin 3-O-[O-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranoside].

Plants of the family Chenopodiaceae have been little studied for their saponin content. There is information on the presence of glycosides of oleanolic acid and of hederagenin in the common beet (*Beta vulgaris*) [1, 2] and pricklyseed spinach (*Spinacia oleracea*) [3].

We have investigated the triterpene glycosides of the annual plant *Climacoptera transoxana* (Iljin) Botsch. The material has been studied previously for its flavonoid content [4] and in the same paper the presence of triterpene glycosides in the plant was mentioned. The raw material was collected at the end of the vegetation period in the steppe salt marches in southern-eastern Turkmenia (Kerkinskii district).

When a methanolic extract of the plant was chromatographed in a thin layer (TLC) in various solvent systems, we detected no fewer than eight substances of glycosidic nature. They were called, in order of increasing polarity, copterosides A, B, C, D, F, G, and H. In addition to glycosides, the presence of compounds of low polarity (free aglycones — hederagenin and oleanolic acid) and also of more polar substances of genin nature was detected. The same aglycones were formed on the acid hydrolysis of the combined glycosides. The carbohydrates in the hydrolysate consisted of D-glucose, D-glucuronic acid, and D-xylose.

The results of alkaline hydrolysis of the combined material showed that glycosides D, F, G, and H each had an acyloside chain.

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Copterosides B and C were the least polar glycosides of the total. To determine their structures, each of them separately was hydrolyzed with 5% sulfuric acid. Hederagenin was found as the genin in both glycosides.

Analysis of the hydrolysates by TLC, PC, and GLC [5] showed that the carbohydrate moiety of compound B consisted of one D-glucuronic acid residue and that of compound C of one D-glucuronic acid residue and one D-xylose residue.

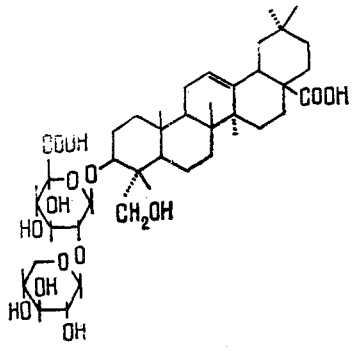
Glycoside B proved to be a hederagenin glucosiduronic acid [6]. On stepwise acid hydrolysis, glycoside C formed the same glucosiduronic acid. The substance did not change in an attempt at alkaline saponification.

The Hakomori methylation of glycoside B and C [7] gave the corresponding permethylates. After the methanolysis of both permethylates, the methyl ester of 23-O-methylhederagenin was identified as the genin.

According to TLC after the destruction of the methyl glycosides, the carbohydrate moieties of the permethylates contained 1 and 2 components, respectively. The methylated sugar formed from the permethylate of copteroside B proved to be 2,3,4-tri-O-methyl-D-glucuronic acid. The less polar component of the carbohydrate part of the permethylate of copteroside was identified by TLC in the presence of an authentic sample [8] as 2,3,4,-tri-O-methyl-D-xylopyranose.

To identify the most polar methylated sugar, copteroside C was subjected to periodate oxidation followed by acid hydrolysis. No free monosaccharides were detected in the reaction mixture. At the same time, on TLC the methylated carbohydrate under investigation gave a positive Bonner reaction [9], showing the presence of an α-diol grouping. Taken together, the facts given permit the methylated sugar under consideration to be identified as 3,4-di-O-methyl-D-glucuronic acid. This conclusion was confirmed by the fact that 2,3,4-tri-O-methyl-D-xylopyranose and 3,4-di-O-methyl-D-glucopyranose were identified in the carbohydrate part of the reduced permethylate with the aid of GLC [10].

A molecular rotation difference calculation showed the β configuration of the glycosidic centers of D-glucuronic acid and the D- xylose residues [11]. According to the facts given, copteroside C has the structure



EXPERIMENTAL

Type KSK silica gel (50-100 μm), type C ("medium") paper, and Silufol plates (Czechoslovakia) were used for chromatography, together with the following solvent systems: 1) chloroform—methanol—water (65:35:3 → 8); 2) butanol—ethanol—25% ammonia (10:2:5); 3) chloroform—methanol: a) (20:1), b) (50:1); 4) benzene—acetone (2:1); 5) butanol—acetic acid—water (4:1:5); and 6) butanol—methanol—water (5:3:1).

The genins, the glycosides, and their derivatives were detected on TLC with a 25% methanolic solution of tungstophosphoric acid, and the sugars and their derivatives with o-toluidine salicylate on plates impregnated with a 0.3 M aqueous solution of NaH_2PO_4, followed by heating (100-120°C for 5-10 min).

The gas—liquid chromatography (GLC) of the silylated methyl glycosides was performed on a Chrom-5 chromatograph with a 3.7 m × 3 mm column containing 5% of the silicone phase SE-30 on Chromaton at 190°C with helium as the carrier gas at a rate of flow of 50 ml/min.

IR spectra were recorded on a UR-20 instrument. Mass spectra were obtained on a MKh-1310 spectrometer at an ionizing voltage of 50 V and a temperature of 130-170°C.

Isolation of the Total Glycosides. The air-dry raw material (1.1 kg) was extracted three times with chloroform and then, exhaustively, with hot methanol. The evaporated methanolic extract (total yield of extractive substances 28%) was dissolved in 1 liter of water and extracted with n-butanol (10 × 200 ml). The butanolic extract was evaporated to dryness, the dry residue (28.5 g or 2.6%) was dissolved in methanol with the addition of a small amount of water, and the glycosides were precipitated with acetone. The yield of purified total mixture was 23 g.

Of the total glycosides, 13 g was transferred to a column containing 1.1 kg of silica gel and elution was carried out in system 1, the process being monitored by TLC in system 2. This gave fractions consisting of mixtures of the compounds indicated: AB, ABC, BCG, DE, DEF, and FGH.

Hydrolysis of the Total Glycosides. 1. Acid Hydrolysis. The total glycosides (210 mg) were hydrolyzed with 5% sulfuric acid at 100°C for 10 h. The reaction mixture was diluted with water and the precipitate that deposited was filtered off. Hederagenin and oleanolic acid were detected in the precipitate by TLC in system 3a. Column chromatography with elution by system 3b yielded in the individual form hederagenin, with mp 326-328°C (from ethanol), $[\alpha]_D^{20} + 78 \pm 2^\circ$ (*c* 0.8 ; pyridine); ν_{max}^{KBr}, cm^{-1}: 3465 (OH); 1708 (C=O of a carboxy group). Mass spectrum, m/z: M^+ 472, 248, 223, 203 [12]. Hederagenin methyl ester was obtained by the methylation of the aglycone with an ethereal solution of diazomethane at room temperature for 4 h. The ester had mp 230-232°C (from ethanol), $[\alpha]_D^{20} + 74 \pm 2^\circ$ (*c* 0,9; chloroform). Mass spectrum, m/z: M^+ 486, 362, 223, 203.

After the separation of the precipitate, the acid hydrolysate was neutralized with $BaCO_3$ and evaporated. The residue was found by PC (system 5) and TLC (system 6) to contain D-glucose, D-glucuronic acid, and D-xylose.

2. Alkaline Hydrolysis. The total glycosides (50 mg) were hydrolyzed with 5% aqueous KOH at 100°C for 5 h. The reaction mixture was neutralized with a dilute solution of sulfuric acid and extracted with n-butanol, and the butanolic extract was washed with water and evaporated to dryness. In comparison with the initial total material, glycosides D, E, F, G, and H were no longer detected on TLC (systems 1 and 2).

Isolation of Individual Glycosides. After the repeated rechromatography of the fraction containing glycosides B and C in system 1, individual substances were isolated. The yield of copteroside B calculated on the air-dry raw material was 0,091%, and that of copteroside C 0.15%.

Hederagenin 3-O-β-D-glucopyranosiduronic acid (copteroside B), $C_{36}H_{36}O_{10}$, had mp 190-193°C (from ethanol), $[\alpha]_D^{20} + 27{,}0 \pm 2^\circ$ (*c* 1,0 ; ethanol). According to the literature [6]: mp 198-200°C (from ethanol), $[\alpha]_D^{20} + 29 \pm 3^\circ$ (*c* 1.1 ; ethanol). The compound was identified by comparison with an authentic sample of hederagenin 3-O-β-D-glucopyranosiduronic acid from *Ladyginia bucharica* with the aid of TLC in systems 1 and 2.

The hederagenin glucosiduronic acid (30 mg) was hydrolyzed with 7% sulfuric acid in ethanol at 100°C for 6 h. After appropriate working up, hederagenin was obtained. D-Glucuronic acid and its lactone were detected in the hydrolysate with the aid of GLC in system 6.

With stirring, 80 mg of sodium hydride was added to a solution of 100 mg of the glucosiduronic acid in 40 ml of dimethyl sulfoxide. After an hour, 6 ml of methyl iodide was added dropwise, and the mixture was stirred for 3 h. After this, it was poured into a 5% solution of sodium hyposulfite and exhaustively extracted with chloroform. The chloroform extract was washed with water and dried with anhydrous sodium sulfate. Chromatography on a column with elution by benzene yielded an amorphous permethylate. The completeness of methylation was checked by the absence of hydroxylic aborption in the IR spectrum taken in Nujol, and also by mass spectrometry. The compound had $[\alpha]_D^{20} + 33 \pm 2^\circ$ (*c* 1,2; benzene); ν_{max}^{Nujol}, cm^{-1}: 1760, 1740 (C=O of ester groupings). Mass spectrum, m/z: M^+ 732, 673, 483, 451, 262, 233, 203.

The permethylate was hydrolyzed with 7% sulfuric acid in methanol at the boiling point of the reaction mixture for 5 h, and then the solution was diluted with water and the methanol was evaporated off. The precipitate that deposited was filtered off. The filtrate was heated at 100°C for another 4 h. Then the solution was neutralized with $BaCO_3$ and filtered, and the water was distilled off. 2,3,4-Tri-O-methyl-D-glucuronic acid was identified in the residue by TLC in system 4. The methyl ester of 23-O-methylhederagenin was identified in the genin fraction; it had mp 188-190°C (from ethyl acetate); $[\alpha]_D^{20} + 67 \pm 2^\circ$ (*c* 2,2; chloroform). Mass spectrum, m/z: M^+ 500, 262, 237, 220, 203. According to the literature [13]: mp 228-230°C, $[\alpha]_D^{20} + 68{,}6 \pm 2^\circ$ (*c* 2,04; chloroform).

Copteroside C, $C_{41}H_{64}O_{14}$, had mp 218-221°C (from ethanol), $[\alpha]_D^{20} + 16 \pm 2^\circ$ (*c* 0,8; methanol).

The glycoside (30 mg) was hydrolyzed under the conditions described for hederagenin glucosiduronic acid. This yielded hederagenin and a carbohydrate fraction in which D-glucuronic acid and D-xylose were detected (TLC, system 6).

Glycoside C (150 mg) was hydrolyzed with 0.5% aqueous sulfuric acid at 90°C for 6 h. The reaction mixture was diluted with water and the reaction products were extracted with n-butanol. In the concentrated butanolic extract, hederagenin, hederagenin glucosiduronic acid, and the initial glycoside were detected by TLC in systems 1 and 2.

Periodate Oxidation of Copteroside C. The glycoside (52 mg) was oxidized in 50 ml of a 1% solution of sodium periodate at room temperature for 48 h. Then 0.5 ml of ethylene glycol was added to the reaction mixture to decompose the excess of periodate and the reaction product was hydrolyzed with 5% sulfuric acid. No free monosaccharides were detected in the $BaCO_3$-neutralized hydrolysate.

Hakomori Methylation of Copteroside C. The glycoside (100 mg) was methylated under the same conditions as hederagenin glucosiduronic acid. This gave an amorphous permethylate, $[\alpha]_D^{20} + 19 \pm 2^\circ$ (*c* 0,96; methanol). ν_{max}^{Nujol}, cm^{-1}: 1760 (C=O of ester groupings). Mass spectrum, m/z: M^+ 892, 701, 483, 437, 393, 451, 262, 203, 189, 175. Hydrolysis of the permethylate gave 3,4-di-O-methyl-D-glucuronic acid and 2,3,4-tri-O-methyl-D-xylose, which were identified by TLC in system 4. The methyl ester of 23-O-methylhederagenin, identified by TLC and a mixed melting point, was obtained as the genin.

A solution of 70 mg of the permethylate in 40 ml of tetrahydrofuran was treated with 70 mg of $LiAlH_4$. The reaction mixture was heated to boiling for 8 h, and then ethyl acetate was added to decompose the unchanged reducing agent. After this, 10 ml of 2% sulfuric acid was added to the reaction mixture and the organic layer was separated off, washed with water, and concentrated. The residue was hydrolyzed with 6% sulfuric acid, and then the reaction mixture was diluted with water and the precipitate that deposited was filtered off. It was established by the TLC method in system 3a with a marker that the aglycone of the reduced permethylate was 28-hydroxy-23-methoxy-β-amyrin.

The filtrate after neutralization was evaporated to dryness. 2,3,4-Tri-O-methyl-D-xylopyranose and 3,4-di-O-methyl-D-glucopyranose were identified in the residue with the aid of GLC.

SUMMARY

Two triterpeneglycosides have been isolated from the epigeal part of *Climacoptera transoxana* (Iljin) Botsch. – copteroside B and C. Copteroside B has been identified as hederagenin 3-O-β-D-glucopyranosiduronic acid. Copteroside C has the structure of hederagenin 3-O-[O-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranoside].

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SPECTROPHOTOMETRIC DETERMINATION OF SPHAEROPHYSINE BENZOATE BY THE REACTION WITH DINITROBINDONE

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A method for the quantitative determination of sphaerophysine benzoate that is highly sensitive and simple in performance has been developed which may find wide use in pharmaceutical analysis.

Sphaerophysine is an alkaloid isolated from *Sphaerophysa* Pall. DC [salt globe pea]. In medicine, sphaerophysine in the form of its benzoate is used to lower arterial pressure, increase tonus, and to enhance the contraction of the musculature of the uterus [1].

In spite of its wide use, sphaerophysine benzoate has been studied inadequately. Methods of acid–base titration in a nonaqueous medium [2] and of polarographic [3] and bromatometric [4] determination have been described. These methods are characterized by low sensitivity, inconvenience in performance, and lengthiness of the analysis.

Our aim was to develop a highly sensitive method simple in use for the quantitative determination of sphaerophysine benzoate. We have used the reaction with dinitrobindone. It is established that dinitrobenzone reacts with sphaerophysine benzoate in dioxane to form an orange-red product. The intensity of the coloration is directly proportional to the sphaerophysine content of the sample under investigation and obeys Beer's law within the range of concentrations of 1.2-2.4 mg of substance in 100 ml of solution. The reaction was carried out at room temperature using a 0.5% solution of dinitrobindone. Dioxane of ch.d.a. ["pure for analysis"] grade was used as the solvent for the reagent and the compound being determined. It is assumed that in the reaction a salt of dinitrobindone with the sphaerophysine cation is formed.

We may give the following spectral characteristics for the reaction that we have developed:

Analytical index	Numerical value
Absorption maximum, nm	492
Molar absorption coefficient	19,550
Specific absorption, $cm^2/\mu g$	0.04417
Sandell coefficient	0.02264
Koch and Koch-Dedic coefficient	1.13
Limits of detection, μg/ml	1.13

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