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TRITERPENE GLYCOSIDES OF *Salsola micranthera*.

I. STRUCTURES OF SALSOLOSIDES C AND D

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New triterpene glycosides — salsolosides C and D — have been isolated from the epigeal part of *Salsola micranthera* Botsch. On the basis of chemical transformations and physicochemical measurements, salsoloside C has been assigned the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuropyranoside], and salsoloside D has the structure of hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuropyranoside].

Several new glycosides of the triterpene series have been isolated from the plant *Climacoptera transoxana* (Iljin) Botsch, family Chenopodiaceae [1]. We have also investigated for its glycoside content the epigeal part of the annual plant *Salsola micranthera* Botsch. from the same family. The raw material was collected in southeastern Turkmenia, in the environs of the village of Tashlyk.

When the purified combined glycosides from its methanolic extract were subjected to thin-layer chromatography (TLC), seven compounds were detected which have been named salsolosides A, B, C, D, E, F, and G. In the present paper we give information on the determination of the structures of salsolosides C (I) and D (II).

The acid hydrolysis of glycosides (I) and (II) separately led to oleanolic acid (III) and hederagenin (IV), respectively.

Analysis of the carbohydrate fractions of the hydrolysates by TLC and PC showed that the sets of monosaccharides in the two glycosides were identical, consisting of D-glucuronic acid, D-glucose, and D-xylose. It was found with the aid of gas–liquid chromatography (GLC) [2] that the monosaccharides were present in a ratio of 1:1:1.

The alkaline hydrolysis of salsolosides C (1) and D (II) led to the formation of the progenins (V) and (VI). D-Glucuronic acid and D-xylose were found in an acid hydrolysate of glycoside (V) and (VI). Consequently, the D-glucose forms the acyloside moiety of the compounds (I) and (II) under consideration.

Stepwise acid hydrolysis of salsolosides C (I) and D (II) led to the glucuronosides (VII) [3] and (VIII) [4] of known structure. Consequently, the D-glucuronic acid residue is attached directly to the genins at C-3.

To determine the position of attachment of the D-xylose residue, glycosides C (I) and D (II) separately were methylated by Hakomori's method [5]. This gave the permethylates (IX) (M^+ 1066) and (X) (M^+ 1096).

The mass spectra of the permethylates (IX) and (X) showed the characteristic peaks of ions corresponding to fragments of the carbohydrate moieties and the genins. The peaks of

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ions with m/z 802 and 832 and m/z 656 and 686 confirmed the nature of the substitution at C-3 and C-28 in the aglycone residues. The peak of an ion with m/z 393 must be assigned to the carbohydrate chain at C-3. It must be mentioned that an ion of the same size arises directly from oleanolic acid, and therefore in the case of the permethylate (IX) this peak was stronger. Fragments with m/z 219 and 175 confirmed the presence in the molecules of the glycosides (I) and (II) of two terminal sugar residues — D-glucose and D-xylose.

In addition to the main permethylates, methylated products (XI) (M^+ 862) and (XII) (M^+ 892) were isolated in the individual state. The acid hydrolysis of these substances led to 2,3,4-tri-O-methyl-D-xylose and a dimethyl derivative of D-glucuronic acid. The genins of compounds (XI) and (XII) proved to be methyl oleanolate (XIII) and the methyl ester of 23-methylhederagenin (XIV), respectively. It is obvious that the permethylates (XI) and (XII) were formed as a consequence of the splitting out of the acyloside moiety, i.e., the D-glucose residue at the C-28 carboxy group, during the reaction.

The permethylates (IX) and (X) were subjected to reduction with lithium tetrahydroaluminate followed by acid hydrolysis. As the genins in the reduction products erythrodiol (XV) and 28-hydroxy-23-methoxy-β-amyrin (XVI) were identified. With the aid of TLC, 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-D-sorbitol, and a dimethyl derivative of D-glucose were revealed in the carbohydrate fractions of the hydrolysates.

Salsolosides C (I) and D (II) were subjected to periodate oxidation followed by acid hydrolysis. No free monosaccharides were detected in the products of the oxidative degradation of glycosides (I) and (II). This fact shows the absence of 1 → 3 bonds in the sugar chain. At the same time, the di-O-methyl derivative of D-glucose obtained in the reductive degradation of the permethylate (IX) and (X) did not react with the Bonner reagent [6], which, in its turn, excludes the presence of 1 → 2 bonds between the D-xylose and D-glucuronic acid residues. Consequently, the di-O-methyl derivative of D-glucose was 2,3-di-O-methyl-D-glucose.

A calculation of molecular rotation differences [7] showed the β configuration of the glycosidic centers. This conclusion was confirmed by the PMR spectra of the permethylates (IX) and (X), in which the doublet signals of the three anomeric protons (J = 7.5 Hz) were clearly traced at 4.28, 4.63, and 5.26 ppm and at 4.17, 4.61, and 5.32 ppm, respectively [8].

Thus salsolosides C (I) and D (II) form a pair of bisdesmosidic glycosides differing only by their genins. Salsoloside C has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuronopyranoside], and salsoloside D is hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuronopyranoside].

Salsoloside D proved to be a structural isomer of copteroside D, which has been isolated from *Climacoptera transoxana* [9]. Both glycosides contain hederagenin as the aglycone and each has the same set of sugar residues — of D-glucuronic acid, D-glucose, and D-xylose in a ratio of 1:1:1. The difference between them consists only in the fact that in copteroside D the D-xylose residue is attached to the D-glucuronic acid residue through the C-2 carbon atom, and in salsoloside D through the C-4 atom.

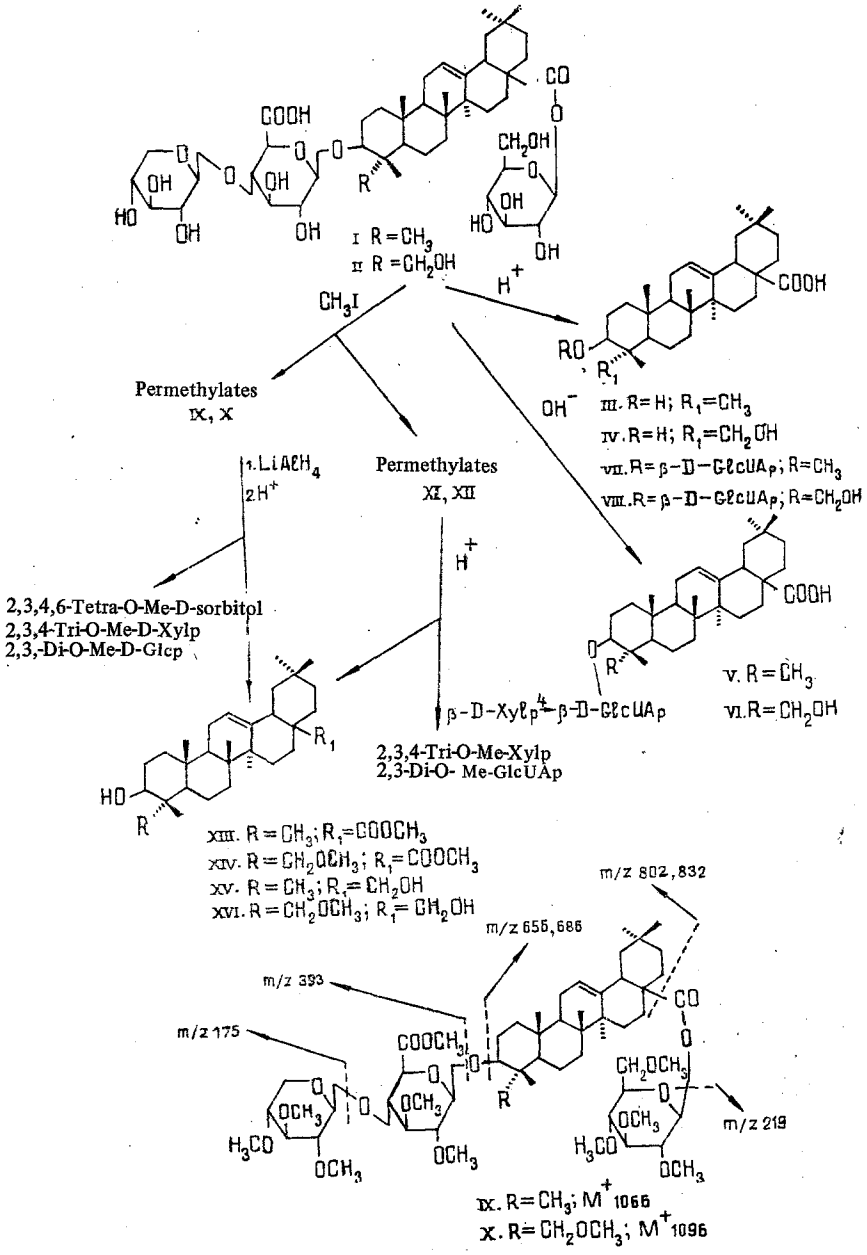
EXPERIMENTAL

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

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

Isolation of the Combined Glycosides. The air-dry raw material (1.7 kg) was extracted three times with chloroform and then five times with hot methanol. The evaporated methanolic extract was dissolved in 1 liter of water and extracted with n-butyl alcohol (10 × 200 ml). The butanolic extracts were evaporated to dryness. The dry residue (40 g, yield 2.35%) was dissolved in methanol, and the glycosides were precipitated with acetone.

The combined glycosides purified in this way (35 g) were transferred to a column of silica gel. Elution was performed with system 1. The process was monitored by TLC in systems 1 and 2. Fractions containing the compounds AB, BCD, CD, DE, E, and EFG were obtained.



Isolation of the Individual Glycosides. After rechromatography of the fractions containing glycosides C and D in systems 1a and 1b, successively, the individual substances were isolated. Salsoloside C (the yield calculated on the air-dry raw material amounted to 0.21%) — $C_{47}H_{74}O_{18}$, mp 214-220°C, $[\alpha]_D^{20}$ + 16.0 ± 3° (c 1.0; methanol). Salsoloside D (yield 0.44%) — $C_{47}H_{74}O_{19}$, mp 228-230°C, $[\alpha]_D^{20}$ + 18.6 ± 3° (c 1.2; methanol).

Oleanolic Acid (III) and Oleanolic Acid 3-O-β-D-Glucuronopyranoside (VII) from Salsoloside C (I). Glycoside C (105 mg) was hydrolyzed with a 6% solution of sulfuric acid at 95°C for 6 h. After the reaction products had been separated on a column of silica gel in system 3b, 22 mg of oleanolic acid was isolated and was identified from its physicochemical constants and by a TLC comparison with an authentic sample (system 3a).

Glycoside C (200 mg) was heated in 30 ml of 1% sulfuric acid at 100°C for 3 h. After the appropriate working up, 40 mg of substance (VII) was isolated by column chromatography in system 1b, and it was identified from its physicochemical constants and with the aid of TLC (systems 1 and 2) with a sample of oleanolic acid glucuronoside [4].

Alkaline Hydrolysis of Salsoloside C (I). Glycoside C (120 mg) was saponified with an 8% solution of KOH (20 ml) with heating for 5 h. After neutralization with dilute sulfuric acid, the reaction mixture was extracted with butanol. The butanolic extract was washed with water

and evaporated. The dry residue was chromatographed on a column of silica gel. This gave 75 mg of the oleanolic acid bioside (V), $C_{41}H_{64}O_{13}$, mp 210-214°C (from ethanol, $[\alpha]_D^{20}$ +18 ± 2° (c 1.0; methanol). Acid hydrolysis of the bioside led to D-glucuronic acid and D-xylose (TLC, system 6).

Permethylate (IX) from Salsoloside C (I). Glycoside (I) (300 mg) was dissolved in 40 ml of dimethyl sulfoxide, and 300 mg of sodium hydride was added to the solution in small portions over an hour with continuous stirring. The reaction was carried out at room temperature. Then 5 ml of methyl iodide was added dropwise and the mixture was stirred for another 3 h. After the appropriate working up, the residue was chromatographed on a column with elution by benzene. This gave 210 mg of the amorphous permethylate $C_{57}H_{94}O_{18}$ $[\alpha]_D^{20}$ +10.0 ± 2° (c 1.1; methanol). In the IR spectrum there was no absorption in the hydroxy group region. Mass spectrum, m/z (%): M^+ 1066 (0.3), 802 (15.8), 656 (2.9), 393 (23.7), 248 (36.8), 219 (31.5), 175 (100). PMR spectrum ($CDCl_3$, δ, ppm): 4.28 (anomeric proton, d, J = 7.5 Hz), 4.63 (anomeric proton, d, J = 7.7 Hz); 5.23 (H at C-12, m); 5.26 (anomeric proton, d, J = 7.5 Hz).

In addition to the main permethylate (IX), a by-product of methylation (XI) (50 mg) was isolated: $C_{48}H_{78}O_{13}$, $[\alpha]_D^{20}$ +7.5 ± 2° (c 0.8; methanol). Mass spectrum, m/z (%): M^+ 862 (0.3), 671 (0.22), 453 (52.2), 393 (17.4), 262 (47.8), 175 (100).

The permethylate (XI) (40 mg) was hydrolyzed in 10 ml of 6% sulfuric acid in methanol (70°C, 5 h). Methyl oleanolate (XIII) (TLC, system 3a) and 2,3,4-tri-O-methyl-D-xylose and 2,3-di-O-methyl-D-glucuronic acid (TLC, system 3c) were detected.

Reductive Cleavage of the Permethylate (IX). Compound (IX) (180 mg) was reduced with lithium tetrahydroaluminate (160 mg) by the usual procedure. The reaction product was hydrolyzed with 6% sulfuric acid in methanol. Erythrodiol (XV) was identified as the genin. In the filtrate, after appropriate working up, 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-D-sorbitol, and 2,3-di-O-methyl-D-glucose were identified by TLC in systems 3c and 4c in the presence of markers. The last-mentioned compound did not react with the Bonner reagent.

Periodate Oxidation of Salsoloside C (I). Glycoside (I) (50 mg) was oxidized with sodium periodate (1% solution, 20 ml) for 48 h. Then a few drops of ethylene glycol was added to the reaction mixture and the reaction product was hydrolyzed with 6% sulfuric acid. No free monosaccharides were detected in the neutralized hydrolysate.

Hederagenin (IV) and Hederagenin 3-O-β-D-Glucuronopyranoside (VII) from Salsoloside D (II). Glycoside (II) (120 mg) was hydrolyzed with sulfuric acid under the conditions described for salsoloside C (I). This gave 42 mg of hederagenin. D-Glucuronic acid, D-glucose, and D-xylose were detected in the hydrolysate (TLC, system 6). When 350 mg of salsoloside D was hydrolyzed with 1% H_2SO_4 for 6 h, 87 mg of hederagenin 3-O-β-D-glucuronopyranoside was obtained.

Alkaline Hydrolysis of Salsoloside D (II). Glycoside (II) (152 mg) was hydrolyzed with 8% KOH solution at 100°C for 5 h. This gave 117 mg of the hederagenin bioside (VI), $C_{41}H_{64}O_{14}$, mp 222-224°C (from ethanol). $[\alpha]_D^{20}$ +17.0 ± 2° (c 1.3; methanol). The acid hydrolysis of bioside (VI) gave hederagenin, D-glucuronic acid, and D-xylose.

The Permethylate (X) from Salsoloside D. Glycoside (II) (400 mg) was methylated under the conditions given for salsoloside C. This produced 290 mg of the permethylate (X), $C_{58}H_{96}O_{19}$ $[\alpha]_D^{20}$ +9 ± 3° (c 1.1; methanol). Mass spectrum, m/z (%): M^+ 1096 (0.13), 832 (20.0), 686 (2.7), 393 (15.3), 248 (13.3), 219 (16.7), 187 (100), 175 (86.7). PMR spectrum ($CDCl_3$, δ, ppm): 4.17 (anomeric proton, d, J = 7.5 Hz); 4.61 (anomeric proton, d, J = 7.5 Hz), 5.20 (H at C-12, m); 5.32 (anomeric proton, d, J = 7.5 Hz).

The methylation by-products (XII) (42 mg) had the formula $C_{49}H_{80}O_{14}$, $[\alpha]_D^{20}$ +13 ± 2° (c 0.8; methanol). Mass spectrum, m/z (%): M^+ 892 (0.7), 701 (0.5), 483 (72.5), 393 (12.5), 262 (100), 175 (75).

Acid hydrolysis of the permethylate (XII) led to 2,3,4-tri-O-methyl-D-xylose and 2,3-di-O-methyl-D-glucuronic acid (TLC, system 3c). The methyl ester of 23-methylhederagenin (XIV) was detected as the genin.

Reductive Cleavage of the Permethylate (X). The permethylate (X) (200 mg) was reduced with lithium tetrahydroaluminate. In the cleavage products, 28-hydroxy-23-methoxy-β-amyrin (XVI) was detected as the genin. The methylated carbohydrates proved to be 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-D-sorbitol, and 2,3-di-O-methyl-D-glucose (TLC, systems 3c and 4).

Periodate Oxidation of the Salsoloside (II). The glycoside (100 mg) was oxidized in 30 ml of 1% sodium periodate solution. After hydrolysis, no free sugars were detected among the oxidation products.

SUMMARY

New triterpene glycosides — salsolosides C and D, which form a pair of bisdesmosidic glycosides — have been isolated from the epigeal part of *Salsola micranthera* Botsch. (family Chenopodiaceae).

Salsoloside C has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuronopyranoside], and salsoloside D is hederagenin 28-O-β-D-glucopyranoside 3-O-[O-β-D-xylopyranosyl-(1 → 4)-β-D-glucuronopyranoside].

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TRANSFORMED STEROIDS.

133. SYNTHESIS OF 20-DEOXYSTEROIDS WITH A TETRAHYDROPYRAN RING E

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The synthesis of 20-deoxy compounds — the precursors of the steroid pyranols and pyranones synthesized by the authors previously — has been effected from a steroid 20-ketotetrahydropyran by the hydrogenolysis of the corresponding ethylene dithioketal with Raney nickel. The ^{1}H and ^{13}C NMR spectra have been studied in detail. Transformations of rings A and B via the epoxide or the 3,5α-cyclosteroid have led to 3-acetoxy-16β,23-epoxy-5αH-21,24-dinorchol-5-en-6-one and 3-acetoxy-5α-hydroxy-16β,23-epoxy-21,24-dinorchol-5-en-6-one.

It is proposed [1] to correlate the degree of the inhibitory action of steroid aglycones on Na^+,K^+-dependent ATPase with the position in space of the 23-carbonyl group. At the same time, we have shown previously [2] that a number of steroids bearing a carbonyl group in a different position or having a hydroxy or acetate function in place of it also exhibit ATPase-inhibiting properties. This induced us to undertake the synthesis of steroid E-tetrahydropyrans having no oxygen functions in ring E.

The initial compound for this purpose was the 20-ketotetrahydropyran (I) [3], the carbonyl group in which can be eliminated either through the tosylhydrazone by reduction with $NaBH_4$ [4] or by desulfurizing the corresponding ethylene dithioketal [5]. The reduction of the tosylhydrazone (Ia) took place with low yield and led only to a mixture of products. The ethylene dithioketal (II) was obtained with high yield by treating (I) with ethanedithiol in the presence of perchloric acid. The prolonged boiling of (II) in absolute ethanol in the presence of Raney nickel previously saturated with hydrogen led, again with a high yield, to the 20-deoxy compound (III). The tetrahydropyranyl steroid (III) is the parent compound of

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