3. M. N. Mukhametzhanov, V. I. Sheichenko, A. I. Ban'kovskii, and K. S. Rybalko, Khim. Prirodn. Soedin., 405 (1971).
4. H. Yoshioka et al., Tetrahedron, 27 (15), 3317 (1971).

TRITERPENE GLYCOSIDES OF *Scabiosa soongorica*

II. THE STRUCTURE OF SONGOROSIDES C, G, AND I

A. Akimaliev, P. K. Alimbaeva, L. G. Mzhel'skaya, and N. K. Abubakirov

UDC 547.918:547.914.4

In the roots of *Scabiosa soongorica* Schrenk, family Dipsacaceae, we have found 11 triterpene glycosides originally called scabiosides [1]. On a more careful study, it became clear that some of the substances found consisted of mixtures of two compounds. Consequently, it is more correct to consider that the roots of the Dzhungarian scabious contain not less than 17 triterpene glycosides which, in degree of increasing polarity, we have called songorosides (the name scabiosides has been used by Bukharov and Karlin [2]) A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, and R. The main components are the more polar (i.e., richer in sugars) glycosides — songorosides G, I, K, M, O, and R.

From the combined saponins we have so far isolated in the individual state five glycosides — songorosides C, G, I, M, and O. Below we give information on the determination of the structures of songorosides C, G, and I. All three glycosides are derivatives of oleanolic acid (I) and are not saponified by alkali, which shows the absence of acyloside chains in them.

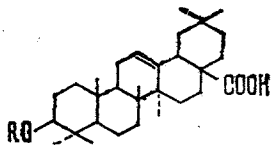
Songoroside C is a bioside with the composition $C_{41}H_{66}O_{11}$. The acid cleavage of glycoside C yielded L-rhamnose and D-xylose. In the hydrolyzate of a permethylate of glycoside C we identified 2,4-di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-L-rhamnose. Consequently, songoroside C has the structure of oleanolic acid 3-O-[O-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranoside] (III).

Songoroside G is a tetraoside with the composition $C_{51}H_{82}O_{19}$, and songoroside I a pentaoside with the empirical formula $C_{56}H_{90}O_{23}$. From the results of acid hydrolysis and gas—liquid chromatography of the silyl derivatives of the methyl glycosides, the sugar moieties of both glycosides consist of L-rhamnose and D-xylose, in a ratio of 1:3 for songoroside G and 1:4 for songoroside I. In the periodate oxidation of songorosides G and I, L-rhamnose and D-xylose remain unchanged.

The exhaustive methylation of songorosides G and I followed by their hydrolysis gave, qualitatively, the same set of methylated sugars, consisting of 2,3,4-tri-O-methyl-D-xylose, 2,4-di-O-methyl-D-xylose, and 2,4-di-O-methyl-L-rhamnose.

To determine the structures of songorosides G (V) and I (VI), we performed stepwise hydrolysis of the glycosides under identical conditions. Among the products of the hydrolysis of each of the glycosides we found an oleanolic acid monoside, $C_{35}H_{56}O_7$, a bioside with the composition $C_{41}H_{66}O_{11}$, and a trioside $C_{46}H_{74}O_{15}$. From the products of hydrolysis of songoroside I, in addition to the compounds mentioned, we isolated a tetraoside $C_{51}H_{82}O_{19}$, identical with songoroside G. The monosides from (V) and (VI) coincided completely in their properties and proved to be oleanolic acid 3-O-β-D-xylopyranoside (II). This compound has been described in the literature previously [3]. It was not found in plants but was described as a product of the degradation of patrinoside D from *Patrinia intermedia* Roem et Schult.

Institute of Physiology and Experimental Pathology, High-Mountain Regions of the Academy of Sciences of the Kirghiz SSR, Frunze. Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 472-476, July-August, 1976. Original article submitted August 20, 1975.



I. R = H, oleanolic acid

II. R = β-*D*-Xyl_p-, songoroside A

III. R = α-*L*-Rha_p(1→3)-β-*D*-Xyl_p-, songoroside C

IV. R = β-*D*-Xyl_p (1→3)-α-*L*-Rha_p (1→3)-β-*D*-Xyl_p-, songoroside E

V. R = β-*D*-Xyl_p (1→3)-β-*D*-Xyl_p (1→3)-α-*L*-Rha_p (1→3)-β-*D*-Xyl_p-, songoroside G

VI. R = β-*D*-Xyl_p (1→3)-β-*D*-Xyl_p(1→3)-β-*D*-Xyl_p (1→3)-α-*L*-Rha_p(1→3)-β-*D*-Xyl_p-, songoroside I

Acid hydrolysis and exhaustive methylation of the biosides showed that they were identical with songoroside C (III). The acid hydrolysis of each trioside yielded L-rhamnose and D-xylose. The exhaustive methylation of the triglycosides led to the same methylated sugars as for songorosides G and I. Thus, the triosides are also identical in structure and are oleanolic acid 3-O-[O-β-D-xylopyranosyl-(1 → 3)-O-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranoside] (IV). We have not isolated songorosides A and E from the plant in the pure state. However, in a chromatographic comparison in several systems it was found that songoroside A corresponds to the monoside (II) and E to the trioside (IV).

On the basis of the experimental facts given, it can be stated that the carbohydrate chains of songorosides G and I have a linear structure and some of the glycosides more polar than songoroside C — the trioside E (IV), the tetraoside G (V), and the pentaoside I (VI) — differ from one another by the presence of an additional molecule of D-xylose. Songoroside G is represented by structure (V), and songoroside I by (VI).

EXPERIMENTAL

For paper chromatography (PC) we used type "S" (medium) paper, and for thin-layer chromatography (TLC) type KSK silica gel and the following solvent systems: 1) chloroform—methanol—water (61:32:7); 2) the same (80:35:7); 3) 1-butanol—ethanol—25% ammonia (7:2:5); 4) 1-butanol—acetic acid—water (4:1:5) (organic phase); 5) 1-butanol—methanol—water (5:3:1); 6) chloroform—ethanol (25:2); 7) benzene—acetone (2:1); 8) chloroform—methanol (8:2).

The free sugars were chromatographed on thin-layer plates impregnated with a 0.3 M solution of sodium dihydrogen phosphate [4].

The sugars were revealed with o-toluidine salicylate, and the genins, the glycosides, and the methylated glycosides with an ethanolic solution of phosphotungstic acid.

The gas—liquid chromatography of the silylated methyl glycosides of the monosaccharides was performed on a UKh-1 chromatograph using a copper column (1 m × 4 mm) containing 5% of silicone phase g 30 m on Diaforit (0.2-0.315 mm) at a column temperature of 176°C with hydrogen as the carrier gas a rate of flow of 55 ml/min.

Isolation and Separation of the Glycosides. The comminuted roots (4 kg) were defatted with chloroform and were then extracted exhaustively with methanol at room temperature (five times) and once with heating. The combined methanolic extract was evaporated in vacuum to a syrupy consistency. The saponins were precipitated by triturating the methanolic extract with acetone. The precipitate was dried in a vacuum desiccator. The yield of saponins on the air-dry weight of the plant was 17.9%. The hemolytic index of the combined material was 1870. The total saponins in portions of approximately 7 g were subjected to preliminary chromatographic separation on a column (height 1.5 m, diameter 4.5 cm) containing silica gel (700 g) in system 1. The individual compositions of the glycosides in the separate fractions were monitored in systems 1 and 2. Fractions were isolated which contained songorosides A, B, and C (0.07 g); B, C, D, and E (0.18 g); E, F, H, I (0.4 g); I, J, K, L, M (1.130 g); M, N, O (0.37 g); and O, P, R (0.37 g).

Songoroside C. The fractions containing songoroside C were combined and rechromatographed on a column of silica gel (1:200) in chloroform—methanol with a gradual increase in

the concentration of the latter from 1 to 10%. The eluate was collected in 0.5-liter portions. Fractions 36-38 yielded an individual glycoside, $C_{41}H_{66}O_{11}$, with mp 218-220°C, $[\alpha]_D^{20}$ + 9 ± 3° (c 1.4; methanol). The yield on the air-dry plant was 0.015%.

Songorosides G and I. The mixture containing glycosides E, F, G, I was chromatographed on a column of silica gel (1:200) in system 3. This permitted the original mixture to be separated into the new fractions F, G, and H, I. Each of them was chromatographed preliminarily on plates (35 × 35 cm) with a fixed layer of silica gel in system 2. The zones with the individual songorosides G and I were removed and eluted with methanol. This gave songoroside G, $C_{51}H_{82}O_{19}$, with mp 251-254°C (from ethanol), $[\alpha]_D^{20}$ − 28.1 ± 2° (c 1.1; methanol) (yield on the plant raw material about 0.05%), and songoroside I, $C_{56}H_{90}O_{23}$, with mp 230-233°C (from ethanol), $[\alpha]_D^{20}$ − 22.8 ± 2° (c 1.6; methanol) (yield 0.06%).

Songorosides M and O. The fraction enriched in glycosides I, J, K, L, and M was chromatographed on a column of silica gel (1:200) in system 3. The result was monitored by TLC in systems 2 and 3. This gave the individual songoroside M with mp 207-209°C, $[\alpha]_D^{20}$ − 30.4 ± 2° (c 1.8; methanol); yield about 1%.

In the manner described above, from the fractions including glycosides M, N, and O we isolated songoroside O with mp 202-204°C, $[\alpha]_D^{20}$ − 41 ± 2° (c 1.7; methanol); yield 1.2%.

Action of Alkali on Songorosides C, G, and I. Each of these glycosides (10 mg) was heated in 2 ml of 10% aqueous ethanolic (1:1) caustic potash at 90°C for 5 h. After the solutions had been cooled and neutralized, only the initial glycosides were found in systems 1 and 2 by TLC.

Acid Hydrolysis of Songoroside C (III) and Its Permethylate. Glycoside (III) (10 mg) was hydrolyzed in 7.5% aqueous methanolic (1:1) sulfuric acid for 5 h. The hydrolyzate was found by TLC in system 5 to contain L-rhamnose and D-xylose, and in system 6 oleanolic acid was detected.

Songoroside C (10 mg) was methylated by Hakomori's method [5]. 2,4-Di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-L-rhamnose were found in the hydrolysis products of the permethylate by TLC in systems 7 and 8 with markers.

Acid Hydrolysis of Songorosides G and I. Separately, 30-mg samples of songorosides G and I were heated with 5 ml of a 7.5% solution of sulfuric acid in aqueous methanol (1:1) at 90°C for 5 h. The precipitate that deposited was filtered off and dried. After recrystallization from ethanol, it had mp 304-306°C, $[\alpha]_D^{20}$ + 78.2 ± 2° (c 1.32; absolute ethanol). The crystals isolated were identified in a mixed melting point test with an authentic sample of oleanolic acid and by TLC in system 6. L-Rhamnose and D-xylose were found in the hydrolyzates of both glycosides after neutralization with AV-17 anion-exchange resin (OH^- form) by PC in system 4 and TLC in system 5.

Periodate Oxidation of Songorosides G and I. Each glycoside (20 mg) was oxidized with a 1% solution of sodium metaperiodate at +6°C for 48 h. The excess of periodate was destroyed with ethylene glycol, and then the reaction mixture was evaporated and extracted with methanol. The methanolic extracts were hydrolyzed with 7.5% sulfuric acid. The residual sugars L-rhamnose and D-xylose were found in the hydrolyzates of both samples after neutralization by TLC in system 5.

Methylation of Songorosides G and I. Songoroside G (50 mg) was methylated by Hakomori's method [5], and the methylation product was hydrolyzed with 7.5% sulfuric acid. In the hydrolyzate of the permethylate of songoroside G by TLC in systems 7 and 8 with markers we identified 2,3,4-tri-O-methyl-D-xylose, 2,4-di-O-methyl-D-xylose, and 2,4-di-O-methyl-L-rhamnose.

Songoroside I (50 mg) was methylated by a similar method. After hydrolysis of the completely methylated glycoside, the same set of methylated sugars was found as for the permethylate of glycoside G.

Stepwise Hydrolysis of Songorosides G and I. Each glycoside (100 mg) was heated with 10 ml of a 0.5% solution of sulfuric acid in aqueous methanol (1:1) at 90°C for 6 h. After cooling, the solution was neutralized with AV-17 anion-exchange resin (OH^- form) and evaporated to dryness. The hydrolysis products were separated preparatively on plates (35 × 35 cm) in system 2. In addition to the aglycone and the unchanged glycosides, we isolated: oleanolic acid D-xylopyranoside (II), $C_{35}H_{56}O_7$, mp 230-234°C, $[\alpha]_D^{20}$ + 35 ± 2° (c 0.69;

methanol), with a yield of 12 mg from the products of hydrolysis of (V) and 6 mg from (VI); songoroside C (III), $C_{41}H_{66}O_{11}$, mp 217-220°C, $[\alpha]_D^{20}$ + 9 ± 3° (c 0.90; methanol), 31 mg from (V) and 11 mg from (VI), and songoroside E, $C_{46}H_{74}O_{15}$ (IV) with mp 224-227°C, $[\alpha]_D^{20}$ − 13 ± 3° (c 1.2; methanol); 34 mg from (V) and 18 mg from (VI).

Literature data [3]: for (II), mp 213-215°C, $[\alpha]_D^{20}$ 30 ± 3° (c 1.1; ethanol).

In addition to the compounds mentioned, the separation of the hydrolysis products of songoroside I gave 24 mg of songoroside G.

Acid Hydrolysis of the Monoside (II), the Bioside (III), and the Trioside (IV). Glycosides (II), (III), and (IV) were each (5 mg) heated separately with 2 ml of a 7.5% solution of sulfuric acid in aqueous methanol (1:1) at 90°C for 5 h. D-Xylose was found in a hydrolyzate of the monoside (II) after neutralization by TLC in system 5, and L-rhamnose and D-xylose were identified similarly in the hydrolyzates of the bioside (III) and the trioside (IV).

Methylation of Glycosides (II), (III), and (IV). The monoside (II) (5 mg) was methylated by Hakomori's method [5]. The methylation product was hydrolyzed with 7.5% sulfuric acid. 2,3,4-Tri-O-methyl-D-xylose was found in the hydrolyzate by TLC in systems 7 and 8 with markers.

The bioside (III) and the trioside (IV) (10 mg each) were hydrolyzed under the same conditions. 2,4-Di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-L-rhamnose were found in the hydrolyzate of (III) by the method given above, and 2,3,4-tri-O-methyl-D-xylose, 2,4-di-O-methyl-D-xylose, and 2,4-di-O-methyl-L-rhamnose in the products of the hydrolysis of a permethylate of compound (IV), with markers.

SUMMARY

By chromatography in a thin layer of silica gel, a methanolic extract of the roots of *Scabiosa soongorica* Schrenk. has been found to contain 17 triterpene glycosides, which have been called songorosides. Songorosides C, G, I, M, and O have been isolated in the individual state. The structures of songorosides C, G, and I have been established — they are di-, tetra-, and pentaosides of oleanolic acid.

The sugar chain in the glycosides mentioned is attached to the hydroxy group at C_3 of oleanolic acid and has a linear structure. The sugars are attached by 1 → 3 bond, and in songoroside I they appear in the following sequence: aglycone-D-xylose—L-rhamnose—D-xylose—D-xylose—D-xylose. The bioside (songoroside C) and the tetraoside (songoroside G) can be considered as biochemical precursors of songoroside I. Songoroside A is oleanolic acid D-xylopyranoside, and songoroside E is the trioside intermediate between songorosides C and G.

LITERATURE CITED

1. P. K. Alimbaeva and A. Akimaliev, Farmatsiya, No. 3 (1975).
2. V. G. Bukharov and V. V. Karlin, Khim. Prirodn. Soedin., 211 (1970).
3. V. G. Bukharov, V. V. Karlin, and V. A. Talan, Khim. Prirodn. Soedin., 84 (1969).
4. Yu. S. Ovodov et al., J. Chromatogr., 26, III (1967).
5. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).