TRITERPENE GLYCOSIDES OF THE LEAVES OF Eleutherococcus senticosus

I. ISOLATION AND GENERAL CHARACTERISTICS

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Preparations from the leaves of Eleutherococcus senticosus possess physiological activity [1, 2], and therefore they are of interest for chemical study. An investigation of a methanolic extract of the leaves that we have performed has shown that they do not contain the phenol glycosides found in the roots of the plant [3], but they contain at least three glycosides of oleanolic acid. Preliminary information on the isolation of the total glycosidic fraction from the leaves has been published previously [4]. The present paper describes the isolation of the oleanolic acid glycosides, which have been called in order of increasing polarity eleutherosides H, L, and M. The acid hydrolysis of these compounds gave oleanolic acid.

Eleutheroside H is a mixture of two glycosides, I and K, with similar polarities, which it was possible to separate in the form of the corresponding methylated derivatives. Furthermore, glycosides I and K have been obtained by the alkaline hydrolysis of eleutherosides L and M, respectively, which may indicate a biogenetic connection between the two groups of glycosides.

The acid hydrolysis of eleutherosides I and K formed L-rhamnose and L-arabinose. The molecular weights, determined from the yield of genin, and the elementary analyses showed that these glycosides are biosides. The periodate oxidation of eleutherosides I and K led to the complete decomposition of the monosaccharide residues, which shows the absence of 1,3-glycosidic linkages in the carbohydrate chains of the glycosides. The treatment of the eleutherosides with diazomethane and subsequent hydrolysis of the compounds formed gave methyl oleanolate. Consequently, the carbohydrate chain is attached to the C-3 hydroxyl of the genin.

The analyses of eleutherosides L and M are similar. The acid hydrolysis of these glycosides formed D-glucose, L-rhamnose, and L-arabinose in a molar ratio of 2:2:1 (GLC) (Fig. 1). The molecular weights of these compounds show the presence in their carbohydrate chain of five monosaccharide residues. The alkaline hydrolysis of eleutherosides L and M gave eleutherosides I and K, respectively, and an oligosaccharide consisting of glucose and rhamnose residues. The alkaline hydrolysis of eleutherosides L and M in the presence of potassium borohydride with subsequent hydrolysis of the oligosaccharides obtained formed sorbitol, in addition to the monosaccharides mentioned above. It follows from this that eleutherosides L and M contain two carbohydrate chains, one attached to the hydroxyl at C-3 and consisting of L-arabinose and L-rhamnose residues, and the other connected by an O-acylglycosidic bond through a glucose residue. The latter includes D-glucose and L-rhamnose residues. The periodate oxidation of eleutherosides L and M led to the complete decomposition of the monosaccharide residues, which shows the absence of 1,3 linkages and of branching in their carbohydrate chains.

EXPERIMENTAL

Paper chromatography (PC) was performed on Filtrak FN-12 paper in the following solvent systems: 1) butan-1-ol–pyridine–water (6:4:3); 2) ethyl acetate–acetic acid–water (2:1:3). For thin-layer chromatography (TLC), the following systems were used: 3) chloroform–methanol (9:1); 4) chloroform–meth-

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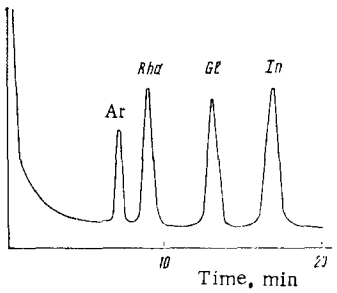

Fig. 1. Gas-liquid chromatography of a mixture of the monosaccharides obtained in the hydrolysis of eleutheroside L, in the form of the TMS ethers of the corresponding polyols: peaks: 1) arabinose; 2) rhamnose; 3) glucose; 4) inositol (standard).

anol (4 : 1); 5) butan-1-ol–ethanol–water (7 : 2 : 5); 6) chloroform–methanol–water (95 : 5 : 3 drops), 7) chloroform–benzene–ethyl acetate (3 : 2 : 1) and 8) chloroform–ethyl acetate (18 : 3).

The glycosides and their genins were revealed with a saturated solution of antimony trichloride in chloroform with additions of antimony pentachloride and thionyl chloride. The monosaccharides and their derivatives were revealed on paper with aniline phthalate and on TLC with a solution of orcin [5].

The gas-liquid chromatography (TLC) of the trimethylsilyl ethers (TMS) of the monosaccharides and the polyols derived from them was performed on a Tswett-2 chromatograph (Experimental Design Bureau for Automation, Dzerzhinsk) with a flame-ionization detector.

A stainless-steel column containing 10% of SE-30 on Chromosorb W (60-80 mesh) washed with acid, with a programmed temperature from 165 to 200°C at the rate of 2.5 deg/min, was used for separation. The carrier gas was nitrogen at the rate of 30 ml/min.

To calculate molecular weights, two accurately weighed samples of the glycosides (50-200 mg) were taken in each case, and they were hydrolyzed with 2 N sulfuric acid (4 ml) for 6 h. The precipitates of the genin were separated off, washed with water, and dried in vacuum with heating to constant weight.

All the solutions were evaporated in vacuum at 35-40°C. The melting points were determined in a Boetius apparatus (Karl Zeiss, Jena, German Democratic Republic), and the specific rotations on an S-PU-EM spectrophotopolarimeter. The IR spectra were recorded on a UR-20 spectrophotometer (Karl Zeiss, Jena, German Democratic Republic). The analyses for the eleutherosides and their derivatives corresponded to the calculated figures.

Isolation of the Glycoside Fraction. The air-dry leaves of the eleutherococcus collected in August-September (380 g) were exhaustively extracted with hot methanol. The solution was evaporated to a volume of 1.5 liter. The extract was boiled with OU-A carbon for 10 min, evaporated, and diluted with a sevenfold volume of water. The precipitate was separated by centrifuging and was crystallized from methanol. This gave crude eleutheroside H. Yield 2 g (0.6%). The aqueous extract was extracted with butanol. On cooling, the evaporated butanolic extract deposited a precipitate containing a mixture of the glycosides L and M. Yield 4.2 g (1.1%).

Isolation of the Individual Oleanolic Acid Glycosides. The mixture of eleutherosides L and M (16 g) was chromatographed on a column of silica gel (8.5 × 35 cm) in system 4. This gave 2.3 g of eleutheroside L with mp 200-202°C (isopropanol), $[\alpha]_D^{20}$ −21.0° (in ethanol). Found, %: mol. wt. 1224; 1251. $C_{59}H_{96}O_{25}$. Calculated: mol. wt. 1204. In addition, 3.8 g of eleutheroside M was isolated with mp 217°C (isopropanol), $[\alpha]_D^{20}$ −18.0° (in ethanol). Found; mol. wt. 1251, 1252. $C_{59}H_{96}O_{25}$. Calculated: mol. wt. 1204.

The acetylation of eleutheroside L with acetic anhydride in pyridine gave the corresponding acetate with mp 155°C (ethanol), $[\alpha]_D^{20}$ −0.5° (in ethanol). The acetate of eleutheroside M was obtained similarly, mp 148°C (ethanol), $[\alpha]_D^{20}$ −1.0° (in ethanol).

Identification of the Genins of Eleutherosides L and M. After recrystallization from ethanol, the genins proved to be identical with one another and with an authentic sample of oleanolic acid [by their behavior on TLC in systems 6-8, by the nature of their IR and mass spectra, by the melting points of the samples obtained – 287-288°C (ethanol), $[\alpha]_D^{20}$ + 67.0° (in ethanol)– and by a mixed melting point]. The genins were treated with an ethereal solution of diazomethane. The resulting methyl esters were identical with one another and with methyl oleanolate (by their behavior on TLC in systems 6 and 7). The acetates of the genins were also identical with the acetate of oleanolic acid (TLC in systems 6 and 7).

Alkaline Hydrolysis of Eleutherosides L and M. A mixture of 67 mg of glycoside L and 3 ml of 2% caustic soda was heated in an atmosphere of argon for 2 h. The precipitate that deposited was separated off, dissolved in methanol, and deionized with Amberlite IR-120 (H^+). This gave eleutheroside I. Yield 31 mg, mp 238-246°C, $[\alpha]_D^{20}$ +30.7° (in methanol). Found: mol. wt. 693, 749. $C_{41}H_{66}O_{11}$. Calculated: mol. wt.

734. Eleutheroside M (60 mg) was treated similarly, giving eleutheroside K. Yield 22 mg, mp 220-221°C $[\alpha]_D^{20}+32.2°$ (in methanol). Found: mol. wt. 693, 749. $C_{41}H_{66}O_{11}$. Calculated: mol. wt. 734. The filtrates were neutralized with Amberlite IR-120 (H^+), evaporated, and hydrolyzed with sulfuric acid. Rhamnose and glucose were detected in the hydrolysates by PC in systems 1 and 2 and by the GLC of the corresponding TMS ethers.

Eleutheroside L (29 mg) and eleutheroside M (35 mg) were heated with 0.5% caustic soda solution and a tenfold excess of potassium tetrahydroborate for 5 h in an atmosphere of argon. The precipitates that deposited were separated off. The filtrates were treated with Amberlite IR-120 (H^+) and hydrolyzed with sulfuric acid. In addition to the complete set of monosaccharides, sorbitol was identified in the hydrolysates by PC in systems 1 and 2 and by the GLC of the corresponding TMS ethers.

Acid Hydrolysis and Determination of the Monosaccharide Compositions of the Eleutherosides. Eleutherosides I and K (10 mg) were hydrolyzed with sulfuric acid (1 ml) at 100°C for 7 h. The hydrolysates were neutralized with $BaCO_3$, treated with Amberlite IR-120 (H^+), evaporated, and used for the determination of the monosaccharide composition. Eleutherosides L and M were hydrolyzed similarly. In the hydrolysates of I and K, rhamnose and arabinose were detected by PC in systems 1 and 2. In the hydrolysates of eleutherosides L and M, rhamnose, arabinose, and glucose were detected. The GLC of the corresponding TMS ethers confirmed the results obtained.

For additional identification, the monosaccharides were separated preparatively. On a column of cellulose powder (2 × 48 cm) 400 mg of the mixture of monosaccharides obtained on hydrolyzing the combined eleutherosides L and M was deposited. Elution was performed with butan-1-ol 2/3 saturated with water. The separation was monitored by PC in system 1. Fractions 17-34 contained L-rhamnose, yield 94.6 mg $[\alpha]_D^{20}+8.0$ (in water). Literature data: $[\alpha]_D^{20}+8.5°$ (in water) [6]. Fractions 48-64 consisted of L-arabinose, yield 28.4 mg $[\alpha]_D^{20}+91.2°$ (in water). Literature data: $[\alpha]_D^{20}+105°$ (in water)[6]. From fractions 72-92 D-glucose was isolated; yield 98.2 mg $[\alpha]_D^{20}+53.1°$ (in water). Literature $[\alpha]_D^{20}+52.7°$ (in water) [6].

Quantitative Analysis of the Monosaccharides. Eleutheroside L (46 mg) was hydrolyzed and treated by the method described above. To the aqueous solution of monosaccharides was added 6 mg of sodium tetrahydroborate and the mixture was stirred for 4 h. The solution was neutralized with Amberlite IR-120 (H^+) and evaporated with an excess of methanol, and the residue was dried. Inositol (4.64 mg) was used as internal standard. The TMS ethers of the polyols were obtained in the usual way [8]. The correction coefficients K for the detector used were as follows for the TMS ethers of the polyols mentioned: sorbitol 0.66; rhamnitol 0.80; arabitol 0.90. The ratio of arabinose to rhamnose to glucose was 1:2:2 (see Fig. 1). Similar results were obtained for eleutheroside M.

Periodate Oxidation of the Oleanolic Acid Glycosides. The oxidation mixture was prepared in the following way: 134 mg of periodic acid was dissolved in a small amount of water and neutralized with sodium bicarbonate to pH 7. The solution was diluted with 15 ml of acetate buffer (pH 4.5).

The eleutherosides (10-15 mg) were oxidized in 5 ml of the mixture in the dark at 20°C for 12 h. After the addition of ethylene glycol, the solution was reduced with sodium tetrahydroborate (with stirring) for 3 h. Then it was neutralized with Amberlite IR-120 (H^+) and evaporated with an excess of methanol. The resulting syrups were hydrolyzed as described above. The hydrolysates were studied by PC in systems 1 and 2. No monosaccharides were detected.

SUMMARY

From the leaves of Eleutherococcus senticosus four glycosides of oleanolic acid have been isolated, and they have been called eleutherosides I, K, L, and M. Eleutherosides I and K are biosides whose carbohydrate chains consist of L-arabinose and L-rhamnose residues. Eleutherosides L and M are pentaosides and differ from I and K, respectively, by the fact that they contain a trisaccharide consisting of D-glucose and L-rhamnose attached by an O-acylglycosidic bond to the carboxy group. The absence of branching and of 1,3 bonds in the carbohydrate chains of the eleutherosides has been shown.

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