THE GLYCOSIDES OF ELEUTHEROCOCCUS SENTICOCCUS MAX.
I. ISOLATION AND SOME PROPERTIES OF ELEUTHEROSIDES B AND E

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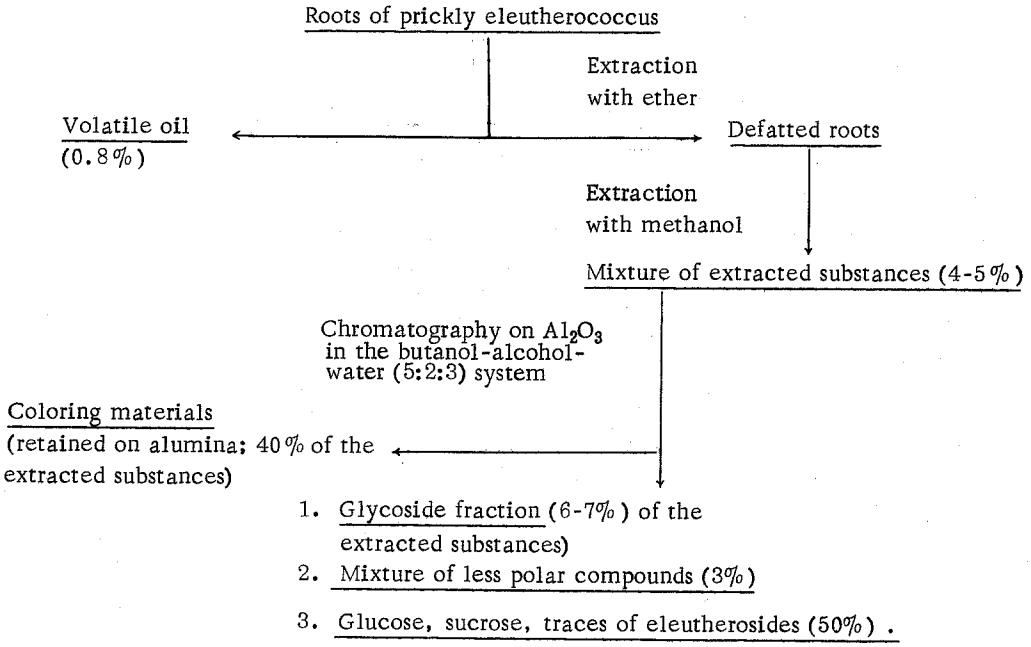
The liquid extract of the roots of Eleutherococcus senticoccus Max. (prickly eleutherococcus), one of the representatives of the family Araliaceae, which is widely distributed in the maritime region of the U.S.S.R., has an activity similar to an extract of the roots of Panax ginseng C. Mey (ginseng), frequently even surpassing it [1].

We have isolated from the roots of prickly eleutherococcus a physiologically active glycoside fraction* containing seven or more glycosides. Its chromatograms are shown in Fig. 1. Development was carried out with the chloroform-methanol system (4:1 by volume) and the spots were developed by means of concentrated sulfuric acid or $SbCl_3$ in chloroform at 120°. The name eleutherosides is proposed for the glycosides of the prickly eleutherococcus. In the present work we describe the processes for the isolation and separation of the eleutherosides and some properties of eleutherosides B and E, which can readily be isolated in the crystalline state.

A preliminary study of a defatted methanolic extract, comprising 4-5% of the weight of the air-dry roots (by chromatography on paper and a thin layer of alumina and silica gel) showed that the extract contained, in addition to the eleutherosides, glucose, sucrose, substances somewhat less polar than the eleutherosides, and also a mixture of coloring materials. The separation of the mixture and the isolation of the glycoside fraction was carried out by the following scheme:



The eleutherosides obtained were chromatographically identical with the glycosides present in the initial methanolic extract and also with the eleutherosides obtained by means of Sephadex (cf. [2]) or by chromatography on silica gel in various solvent systems. The mixture of substances less polar than the eleutherosides that was isolated had a non-glycosidic nature according to the results of acid hydrolysis.

To separate the glycoside fraction into its individual components, we used chromatography on silica gel columns with gradient elution by means of mixtures of chloroform and methanol (100:0→50:50, by volume) or of ethyl acetate and ethanol (100:0→85:15, by volume). In this way we succeeded in isolating a series of eleutherosides in the chromatographically pure state. Preliminary data show that the ratio of eleutherosides, A, B, C, D, E, F, and G in the glycoside fraction is approximately 8:30:10:12:4:2:1.

Eleutherosides B and E were obtained in the crystalline state. Paper chromatography of the products of acid hydroly-

*The pharmacological trials of the glycoside fraction were carried out in the Laboratory for Plant Materials of the Biological and Soil Institute of the Far-Eastern branch of the Siberian Division of the U. S. S. R Academy of Sciences.

sis of these eleutherosides showed that the carbohydrate part of both glycosides contained only glucose. The chromatographic behavior of eleutherosides B and E shows that eleutheroside B is a mono- or bioside and eleutheroside E is, most probably, a tri- or tetraoside. Eleutheroside B has a greater R_f value in all solvent systems than panaxoside A [3] and gyposide [4]; eleutheroside E occupies an intermediate position between panaxoside A, which is a trioside, and the more highly polar glycosides from ginseng. The marked solubility of eleutheroside B in chloroform and ethyl acetate, and its good solubility in methanol indicate that this compound is of low polarity. Eleutheroside E is practically insoluble in the solvents mentioned above and dissolves only in aqueous methanol, which indicates the presence of three or more glucose residues in the carbohydrate chain of this glycoside.

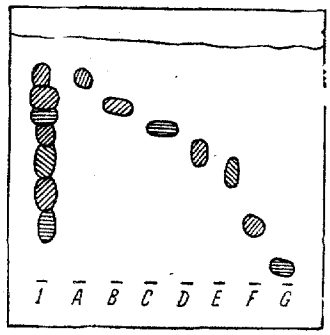


Fig. 1. Chromatograms of the glycosides of the prickly eleutherococcus in a thin fixed layer of silica gel: 1) Mixture of glycosides; A-G) Eleutherosides.

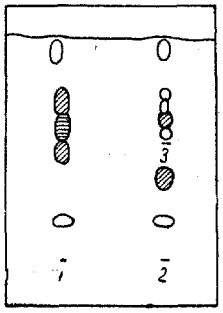


Fig. 2. Chromatograms of a mixture of the genins of the eleutherosides and the products of their conversion: 1) Hydrolyzate of eleutheroside B; 2) Hydrolyzate of eleutheroside E.

On acid hydrolysis of eleutherosides B and E, a mixture of genins and their unidentified conversion products is formed. Chromatograms of the hydrolyzates of the eleutherosides obtained in a thin fixed layer of silica gel are shown in Fig. 2. Development was carried out with ethyl acetate, and the spots were shown up by means of concentrated sulfuric acid or by $SbCl_3$ in chloroform at 120°.

The IR spectra of the two eleutherosides (Fig. 3) are similar to one another. The absorption band at 1600 cm^{-1} which shows the presence of either double bonds conjugated with a carbonyl group or an aromatic system, is noteworthy.

The analytical data obtained for eleutherosides B and E and also the direct determination of the content of glucose in the acid hydrolyzate using the method described by Somogyi [5, 6] indicate that eleutheroside B is most probably a bioside and eleutheroside E a tri- or tetraoside.

EXPERIMENTAL

Chromatography was carried out on chromatographic paper from the Leningrad Goznak mill with a weight of 110 g/m^2 and with alumina of activity grade III and silica gel of the KSK type with the following grain sizes: for column chromatography, 100-200 mesh; in a thin fixed layer, > 200 mesh; for preparative chromatography the silica gel was activated by 2 hours heating at 140°. The following systems of solvents were used as mobile phases (proportions by volume): I, n-butanol-ethanol-water (5:2:3), II, chloroform-methanol (100:0→50:50). All the solvents were evaporated in vacuum at 40-50°. The IR spectra were taken in KBr tablets on a UR-10* spectrophotometer.

Isolation of the eleutherosides. Preparation of the glucosidic fraction. 1.5 kg of air-dry pulverized roots of the prickly eleutherococcus was extracted with ether (2×5 liters), and the volatile extracts were evaporated. This gave 12 g of a mixture of lipophilic substances. The defatted roots were extracted for 5-7 days with hot methanol in an apparatus of the Soxhlet type. The methanolic solution was evaporated; the yield of the mixture of extracted substances amounted to 60-70 g. The mixture obtained was dissolved in a minimum amount of system I and was filtered through a layer of alumina (15×15 cm) with elution by the same solvent system. After evaporation of the eluates, 40-45 g of a mixture of glycosides, carbohydrates, and substances of a non-glycosidic nature was obtained. The mixture was chromatographed on an alumina column (7.5×70 cm) in system I. The fraction was analyzed chromatographically in a thin layer of alumina in the same system. This gave a glycoside fraction containing 4.5-5 g of a mixture of eleutherosides. In addition, about 2 g of a mixture of less polar substances of a non-glycosidic nature and 30-35 g of a mixture of sucrose and glucose containing a very small amount of eleutherosides were isolated.

* The analysis were carried out by two members of the staff of the Laboratory of the Chemistry of Natural Compounds of the Far-Eastern Branch of the Siberian Division of the U. S. S. R. Academy of Sciences, L. P. Glebko and Zh. I. Ul'kina.

Separation of the mixture of eleutherosides. 1) The glycosidic fraction (5.0 g) was rechromatographed on an alumina column (4 × 45 cm) with development by means of system I. This gave about 1 g of eleutheroside B, 0.1 g of eleutheroside E, and 2.5-3 g of a mixture of the other eleutherosides.

2. The glycosidic fraction (40 g) was transferred to a silica gel column and was eluted with system II, 40-ml fractions being collected. Analysis of the fractions was carried out by chromatography in a thin fixed layer of silica gel, the development of the fraction containing eleutherosides A-D being carried out with the chloroform-ethanol (85:15) system and that of the fractions containing eleutherosides E-G with the chloroform-methanol (4:1) system (table).

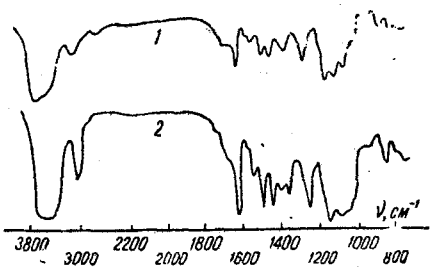


Fig. 3. IR spectra of eleutherosides B and E: 1) Eleutheroside B; 2) Eleutheroside E.

Eleutherosides B and E readily crystallize from methanol or ethanol, even from solutions contaminated with the other eleutherosides. Eleutheroside B was recrystallized from the same solvents and eleutheroside E from aqueous methanol, since in the crystalline state it is very sparingly soluble in alcohols.

Eleutheroside B has mp 182° (from ethanol), $[\alpha]_D^{20}$ 0 ± 2° (c 2.0 in methanol).

IR spectrum: 3500; 2950; 1670; 1600; 1530; 1480; 1360; 1340; 1140; 1100; 1010; 850 cm^{-1}.

Found %: C 54.64; 54.57; H 6.50; 6.53.

Eleutheroside E has mp 235° (from methanol), $[\alpha]_D^{20}$ 0 ± 2° (c 5.0 in 50% aqueous methanol).

IR spectrum: 3500; 2950; 1600; 1530; 1475; 1430; 1380; 1340; 1250; 1140; 1085; 825 cm^{-1}.

Found %: C 54.84; 54.86; H 6.28; 6.50.

Fraction number	Eleutherosides	Yield, g
1	*A*	3.15
2	*A*+*B*	3.15
3	*B*	6.00
4	*B*+*C*+*D*	11.15
5	*C*+*D*	4.60
6	*D*+*E*	1.35
7	*E*	1.15
8	*E*+*F*	0.48
9	*F*	0.60
10	*F*+*G*	0.72
11	*G*	0.15

Acid hydrolysis of eleutherosides B and E. 1. 5-10 mg of eleutheroside B was heated for 2 hours in a sealed tube at 100° with 1-2 ml of Kiliani's mixture (conc. HCl-glacial Ch_3COOH-water, 10:35:55, by volume) or for 4-5 hours at 95-100° with 1-2 ml of a mixture of 5% sulfuric acid and methanol (2:1, by volume). The hydrolyzates were neutralized with Dowex-1 (HCO_3 form. The neutralized solution was concentrated and was then used for paper chromatography. To determine the presence of uronic acids, the acid hydrolyzate was chromatographed with Kiliani's mixture. Paper chromatography in various solvent systems showed the presence in the hydrolyzates of a single monosaccharide — glucose.

2. The hydrolysis of eleutheroside E was carried out in the same way as that of eleutheroside B. Paper chromatography with various solvent systems showed only the presence of glucose in the hydrolyzates.

3. 5-10 mg of eleutheroside B or eleutheroside E was heated for 4 hours at 95° with 1-2 ml of mixture of 5% sulfuric acid and methanol (2:1, by volume). The reaction mixture was extracted with chloroform (3 × 1 ml), and the chloroform extracts were washed with water and evaporated to small volume. The resulting solution of genin and the products of its conversion were used for chromatography in a thin fixed layer of silica gel with ethyl acetate (cf. Fig. 2).

Quantitative determination of glucose in eleutherosides B and E. An accurately weighed sample (about 5 mg) of eleutheroside B or eleutheroside E was dissolved in 1-2 ml of a mixture of 5% acid and methanol (2:1) and heated for 5 hours at 100°. The reaction mixture was treated as described above. The volume of the solution obtained was made up to 8 ml with distilled water and was titrated as described by Somogyi [5, 6]. The content of glucose in eleutheroside B is 53-55% and in eleutheroside E 60-63%.

SUMMARY

1. From the roots of prickly eleutherococcus (Eleutherococcus senticoccus Max.) we have isolated a physiologically active glycosidic fraction containing seven or more different glycosides which have been named eleutherosides.

2. Eleutherosides B and E have been obtained in the crystalline state. They are glycosides of genins of undetermined nature, eleutheroside B probably being a mono- or a bioside and eleutheroside E a tri- or a tetraoside.

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