AN INVESTIGATION OF THE GLYCOSIDES OF POLEMONIUM COERULEUM

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Polemonium coeruleum L. (greenvalerian polemonium) is a perennial herbaceous plant growing in the European part of the USSR and in Western and Eastern Siberia [1]. The roots of greenvalerian polemonium are used as a substitute for imported senega [2]. They possess good expectorant and sedative properties.

All the organs of the plant contain saponins but the roots are particulary rich in them. N A. Burgim and S. A. Nosovitskaya [3] have isolated two groups of saponins—acidic (96%) and neutral (3.9%)—and have determined some of their properties.

We have investigated the roots of P. coeruleum L. collected in 1965 in the Altai. It was found by thin-layer chromatography on silica gel that a methanolic extract of the roots contained at least five compounds giving a characteristic coloration with antimony trichloride. In order of increasing polarity we have called them polemoniosides A, B, C, D, and E.

To isolate the individual glycosides, the methanolic extract was transferred into an aqueous solution and this was extracted with ethyl acetate and butan-1-ol. The butanolic fraction was separated chromatographically on a column of silica gel. Polemonioside B with mp 232–234° C and polemonioside C with mp 241–243° C were isolated.

Polemonioside B dissolved in aqueous ethanol and, sparingly, in water, and had a well-defined acidic nature. Thus, it passed into aqueous alkali solutions and was easily titrated, which made it possible to determine its molecular weight (1100–1150).

Polemonioside C dissolved readily in water and sparingly in butan-1-ol and ethanol. In contrast to polemonioside B it possessed neutral properties. Its IR spectrum had a strong absorption band characteristic of an ester grouping. The glycoside proved to be extremely unstable to the action of alkalies: on being heated even with 10% ammonia it was converted completely into polemonioside B. By making use of these properties, we determined its ester number and from this we calculated the molecular weight of polemonioside C (1400–1450). The polemoniosides are very unstable to the action of alkalis and acids.

We also showed that polemonioside C is cleaved with the formation of B on EDE-10P anion-exchange resin.

Polemonioside B was split by dilute mineral acids, forming D-galactose and a product which, from its elementary analysis and molecular weight (650), is a progenin. The latter, in its turn, was hydrolyzed by Kiliani's mixture, forming a uronic acid and a neutral product with mp 198–200° C (mol. wt. 480). When polemonioside C was heated with 5% hydrochloric acid, the hydrolysate was found to contain L-arabinose and D-galactose, and the above-described progenin.

Polemonioside C is the main component of the mixture of glycosides; its yield was 20% of the extract. The amount of polemonioside B in the mixture was only 2–3%. According to N. A. Burgim and S. A. Nosovitskii [3], the ratio of the glycosides is the reverse of this. This can be explained by the fact that, in order to isolate the total glycosides completely, they acidified the extract with mineral acids. Under these conditions the bulk of the neutral polemonioside C could be converted into the acid polemonioside B.

EXPERIMENTAL

Chromatography was carried out with type AKS silica gel and with "Leningrad slow" paper using the solvent systems: 1) butan-1-ol–acetic acid–water (4 : 1 : 5) and 2) benzene–butan-1-ol–pyridine–water (1 : 5 : 3: 3).

The air-dry roots of Polemonium coeruleum L. (1 kg) were exhaustively extracted by being boiled with

chloroform (yield 32.5 g), methanol to which 5–10% of water had been added (275 g), and water (31.5 g).

The methanolic extract was evaporated to dryness in vacuum at 40–50° C, dissolved in 1 *l* of water, and exhaustively extracted with ethyl acetate and butan-1-ol. The yields amounted to 41.4 and 182.2, g, respectively, while the residue weighed 52.4 g.

About 500 g of the butanolic extract was transferred to a chromatographic column containing 2600 g of silica gel and was eluted successively with butan-1-ol, butan-1-ol saturated with water, and water. The separation of the fractions was checked by thin-layer chromatography in system 1; 254 fractions were collected and of them nos. 191-197 contained 15 g of polemonioside B with mp 232–234° C, $[\alpha]_D^{20}$ +43° (c 1.4; methanol-pyridine (1 : 1).

Found, %: C 56.69, 56.72; H 7.50, 7.80.

Fractions 221–254 contained 100 g of polemonioside C with mp 241–243° C, $[\alpha]_D^{20}$ + 38° (c 1; water).

Found, %: C 53.68, 53.63; H 7.36, 7.36.

Separation of polemoniosides C and B. 35 g of the intermediate fractions 198–200 from the preceding experiment, containing mainly polemonioside C with some polemonioside B, were transferred to a column containing 1000 g of EDE-10P ion-exchanger in the OH^- form. Elution was carried out with water until the neutral glycoside had been washed out completely, and then with a 10% aqueous methanolic solution of acetic acid. Evaporation of the solutions yielded 9 g of polemonioside C and 26 g of polemonioside B.

Acetate of polemonioside B. A solution of 0.3 g of polemonioside B in a mixture of 15 ml of pyridine, 5 ml of acetic anhydride, and 3 ml of dimethylformamide was left at room temperature for a day. Then it was poured into ice water and the precipitate that had formed was filtered off, transferred to a column containing 4 g of silica gel, and eluted with 100 ml of chloroform. This gave 0.2 g of the acetate of polemonioside B with mp 145–147° C, $[\alpha]_D^{20}$ +20° (c 1; ethanol).

Found, %: C 59.96, 60.09; H 7.32, 7.10.

When a 0.029-g sample of polemonioside B was titrated to phenolphthalein 0.256 ml of 0.1 N caustic soda was consumed. Found, %: mol. wt. 1130, 1130.

Hydrolysis of polemonioside B. 1) A mixture of 0.02 g of polemonioside B and 3 ml of 5% aqueous methanolic HCl solution was heated in a sealed tube. The precipitate that deposited was filtered off and washed with water. The filtrate was found by chromatography in systems 1 and 2, by comparison with an authentic sample, to contain D-galactose. The residue was passed in choroform solution through a layer of silica gel (1 g). The yield of progenin was 0.1 g, mp 212–217° C, $[\alpha]_D^{20}$ + 20° (c 1; ethanol).

Found, %: C 66.66, 66.76; H 8.56, 8.63; mol. wt. 641, 660 (Rast).

In a similar manner to that described above, 0.8 g of hydrolysis product yielded 0.6 g of the acetate of the progenin with mp 140–142° C.

Found, %: C 64.33, 64.30; H 8.29, 8.02; mol. wt. 850 (Rast).

2) The progenin obtained from the preceding experiment (0.2 g) was heated with a mixture of 3.5 ml of glacial acetic acid, 5.5 ml of ethanol, and 1 ml of HCl at 70–80° C for 5 hr. Then a tenfold volume of water was added. The precipitate that deposited was reprecipitated from acetone with petroleum ether, giving 0.1 g of aglycone with mp 198–200° C, $[\alpha]_D^{20}$ + 50° (c 1; ethanol).

Acetate of polemonioside C. Polemonioside C (0.7 g) was treated with a mixture of 5 ml of dimethylformamide, 16 ml of pyridine, and 7 ml of acetic anhydride for a day. The precipitate that deposited when a large volume of water was added was filtered off, transferred to a column containing 10 g of silica gel, and eluted with chloroform and a mixture of chloroform and ethyl acetate (1 : 1) (200-ml portions). This yielded 0.1 g of the acetate of polemonioside C (mp 152–154° C, $[\alpha]_D^{20}$ +20° (c 1.6; ethanol; found, %: C 57.50, 57.68; H 6.68, 6.67) and 0.5 g of the acetate of polemonioside B.

A weighed sample (0.2742 g) of polemonioside C in 25 ml of ethanol was heated in the water bath with 2.3 ml of 0.1 N caustic potash for 15–20 min. The excess of alkali was back-titrated with 0.4 ml of 0.1 N HCl.

Similarly, the titration of 0.2115 and 0.2230 g of polemonioside C consumed, respectively, 1.45 and 1.55 ml of alkali. Found, mol. wt.: 1445, 1455, 1440.

Hydrolysis of polemonioside C. 1) A mixture of 0.5 g of polemonioside C and 7 ml of 5% HCl was heated for 2 hr. The precipitate that had deposited was filtered off and washed with water, and was then passed in choroform solution through silica gel (10 g). This gave the progenin with mp 211–216° C. L-Arabinose and D-galactose were identified in the filtrate by paper chromatography in systems 1 and 2.

2) A mixture of 4 g of polemonioside C and 50 ml of 2% aqueous sodium hydroxide was heated at 70–80° C for 2 hr. The reaction mixture was diluted twofold with ethanol and neutralized with KU-2 ion-exchange resin. Then the resin was filtered off and the filtrate was evaporated to small volume. Polemonioside B deposited.

In the same way, when 0.2 g of polemonioside C was heated with 10% ammonia in a sealed tube, 0.2 g of polemonioside B was obtained.

CONCLUSIONS

Two individual glycosides, polemonioside B and polemonioside C, have been isolated from the roots of Polemonium coeruleum L. for the first time and some of their properties have been studied.

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