CARBOHYDRATES, AMINO ACIDS, PHYTIN, AND MACROELEMENTS OF THE HERBAGE OF *Trifolium pratense*

A. L. Kazakov and I. I. Samokish

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Two monosaccharides have been found by paper chromatography in an aqueous extract of the herbage of red clover (*Trifolium pratense*). One of them has been identified as glucose. After hydrolysis of a dry water-soluble extract by paper chromatography in the presence of markers, galactose, arabinose, xylose, and mannose were detected. By paper chromatography in the presence of markers the amino acids phenylalanine, leucine (isoleucine), methionine, aspartic acid, proline, alanine, and histidine have been identified. Phytin with decomp. p 276°C has been isolated and identified, giving, after hydrolysis, inositol with mp 225-226°C, and Ca^{2+}, Mg^{2+}, and PO_4^{3-} have been detected. The amount of ash in the herbage of clover is 8.4% and the amount insoluble in HCl 1.5%. The amounts of macroelements were determined by the flame photometry of a solution of the ash (mg-%): K^+ 1620; Na^+ 310; Ca^{2+} 1240; Mg^{2+} 1090.

Red clover is used for medicinal purposes in folk medicine and scientific medicine [1-4]. Valuable nutrients have been found in clover growing in the Urals: proteins, albumins, carbohydrates in the form of starch and water-soluble polysaccharides, and mineral salts [5].

A chemical study of red clover growing in the Northern Caucasus that we have performed [6-9] has shown the presence of pharmacologically active substances in it — flavonoids and phenolic acids possessing vitaminic, hypolipidemic, and antiatherosclerotic activities.

Continuing an investigation of the biologically active substances of red clover, we have studied its carbohydrate composition, macroelements, and amino acid complex.

In an aqueous extract from clover herbage we have detected chromatographically in the free state glucose and an unidentified monosaccharide. When an aqueous extract was precipitated with an excess of ethanol, the free monosaccharides remained in the mother solution. The total polysaccharides were not detected on paper chromatograms by the aniline phthalate reagent, which shows the absence of open hemiacetal groups of water-soluble polysaccharides and oligosaccharides.

By paper chromatography of a hydrolysate of the dry water-soluble extract we established the presence of four carbohydrate residues revealed by the aniline phthalate reagent. Identification with authentic samples of monosaccharides showed a predominating amount of galactose and arabinose. Two other monosaccharides, identified as xylose and mannose, were present in small amounts and present no practical interest.

When the dry water-soluble extract was hydrolyzed with 5% sulfuric acid, a white precipitate was formed which was identified by qualitative reactions as potassium sulfate. Magnesium ions were found in the hydrolysate [10].

The ash content after combustion and calcination (red heat) of a sample of clover herbage amounted to 8.4%. The ash insoluble in hydrochloric acid amounted to 1.5%. The amounts of macroelements calculated on the air-dry crude clover herbage were (mg-%): K^+ 1620; Na^+ 310; Ca^{2+} 1240; Mg^{2+} 1090. The determinations were made by the flame photometry of a solution of the ash.

The high ash content of the clover herbage and the presence of a considerable amount of calcium and magnesium ions gave grounds for performing an additional investigation for the presence of phytin (a mixture of the calcium and magnesium salts of inositol phosphate).

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The amino acid composition was studied by paper chromatography in the presence of markers. The presence in an aqueous extract of the dry clover herbage of 10 substances of amino acid nature was shown, the following being identified: phenylalanine, leucine (isoleucine), methionine, aspartic acid, proline, alanine, and histidine.

EXPERIMENTAL

Preparation of a Dry Extract of Clover Herbage. The comminuted herbage was covered with water (1:20) and the mixture was heated for 15-20 min and was then cooled. The aqueous extract was concentrated in vacuum and was dried in a drying cabinet at 60-70°C. A dry brown powder was obtained.

The hydrolysis of the dry extract was carried out with a 5% solution of H_2SO_4 in the boiling water bath for 4 h. After the hydrolysate had cooled, a white crystalline precipitate deposited which was filtered off on a glass filter and was washed with cold water. It was shown to contain calcium sulfate. Magnesium ions and monosaccharides were found in the filtrate.

Determination of Calcium and Magnesium Ions. The precipitate obtained on hydrolysis was boiled with 10 ml of water, and then the hot filtrate (60-70°C) was treated with a hot (60-70°C) 1 N solution of oxalic acid. This led to the formation of a white crystalline precipitate of calcium oxalate. Part of the filtrate was neutralized, and 1 N solutions of NH_4CL, NH_4OH, and Na_2HPO_4 were added. A white crystalline precipitate of $MgNH_4PO_4$ was formed.

Identification of the Carbohydrates. The sulfuric acid hydrolysate was neutralized with crystalline $BaCO_3$ and filtered, and the filtrate, without evaporation, was chromatographed on paper from the Leningrad mill in the following solvent systems: 1) butan-1-ol—CH_3COOH—H_2O (4:1:2); 2) butan-1-ol–ethyl acetate—H_2O (7:2:1); 3) butan-1-ol—acetone—H_2O (4:5:1); 4) n-propanol—CH_3COOH—H_2O (7:2:1); 5) n-propanol—acetone—H_2O (4:5:1); and 6) n-propanol—ethyl acetate—water (7:2:1).

For identification we used authentic samples of monosaccharides as markers. The time of running of the chromatograms was 12 h in an ascending flow of solvent. When the separation of the mixture being analyzed was not sufficiently clear, two separations of 12 h each with intermediate drying of the chromatograms was used [13-15].

Isolation and Identification of Phytin. The air-dry comminuted herbage (0.25 kg) was extracted with 96% ethanol in an extractor with continuous reflux. The remaining meal was exhaustively treated with hot water without the preliminary elimination of the ethanol residues. The aqueous extract was evaporated in vacuum to small volume, an equal volume of ethanol was added to it, and the precipitate that formed was allowed to stand for 12 h and was then filtered off through filter paper and was washed with ethanol (yield 0.47%).

After reprecipitation from C_2H_5OH—H_2O (1:1), a white crystalline powder with mp 276°C, insoluble in chloroform, ether, and ethanol was obtained. After combustion, 30% of incombustible residue was left, theanalysis of which showed the presence of Ca^{2+}, Mg^{2+}, and PO_4^{3-} ions.

On hydrolysis with a 5% solution of H_2SO_4 for 4 h followed by cooling, the hydrolysate deposited a crystalline precipitate of $CaSO_4$. In the filtrate after neutralization with crystalline $BaCO_3$, a white crystalline precipitate of hydrolysis products with mp 225-226°C was obtained, and this was identified as mesoinositol [11]. When it was chromatographed on paper, it was revealed by the Tollens reagent and its R_f value coincided with that of an authentic sample of mesoinositol [12]. On the basis of the results obtained, the substance was identified as phytin (a mixture of the Ca and Mg salts of mesoinositol hexaphosphate).

Identification of the Amino Acids. An aqueous extract of the dry clover herbage was obtained. The herbage was wetted with water, boiled for 10-15 min, and filtered. The aqueous extract was chromatographed [13] on paper of the Leningrad mill by the ascending method in systems 1 and 2. In the majority of cases, two separations of 12 h each with intermediate drying of the chromatograms in air was used. To detect the amino acids, the chromatograms were sprayed with a 0.25% solution of ninhydrin in propanol and were heated at 110-120°C. Seven substances of amino acid nature revealed by ninhydrin were identified chromatographically in the presence of amino acid markers.

SUMMARY

The carbohydrate and amino acid compositions of red clover growing in the Northern Caucasus have been studied qualitatively by paper chromatography.

The amounts of macroelements potassium, sodium, calcium, and magnesium in the total ash content have been determined quantitatively.

Phytin, a mixture of Ca and Mg salts of inositol phosphate, has been isolated and identified.

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A STUDY OF THE PHOSPHOLIPIDS OF THE COTTON PLANT OF VARIETY 159-F IN THE PROCESS OF ITS DEVELOPMENT

F. Yu. Gazizov, A. Sh. Isamukhamedov, and S. T. Akramov

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The distribution of phosphorus with the secretion of phospholipids (PLs) in six stages of development of the cotton plant has been studied. It has been shown that additional extraction with methanol leads to the isolation of further PLs. Both the seeds and cotton-plant bushes during growth contain phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, X_1, and X_2, and also unidentified PLs, one of which, Y_2, is phosphatidic acid according to qualitative reactions, chromatographic mobility, and literature information.

The completing stage of the development of the cotton plant is represented by the ripe seeds, the phospholipids (PLs) of which have been relatively well studied [1, 2].

Ganieva and Rakhmanova [3], who investigated the lipids of cotton-plant leaves, showed that the phases of development and the positions of the leaves on the plant are not reflected

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