determined intervals of time, 0.1 ml samples were taken, and they were diluted with methanol, filtered, and evaporated. The resulting mixtures of methylated compounds were acetylated with acetic anhydride in pyridine, the acetates were isolated, and they were used in the form of chloroform solutions of GLC analysis.

GLC Analysis. The GLC of the products of the partial methylation of methyl β-xyloside was performed on a "Tsvet 2-65" instrument, the products of the partial methylation of methyl β-arabinoside were chromatographed on a "Tsvet-4-67" instrument, and the products of the partial methylation of the other glycosides were subjected to GLC on a Pye Unicam series 104 instrument (England).

Chromosorb W (60-80 mesh) was used as the support. The retention times of the methyl ethers and the working conditions are given in Table 7.

The methyl ethers of the methyl glycosides were identified by comparison with authentic samples by means of GLC and mass spectrometry [4]. The areas of the peaks were found by the usual method. The relative error was 3-10%.

SUMMARY

The partial methylation of methyl xylo-, arabino-, lyxo- and rhamnopyranosides by Purdie's method has been studied; it is possible to use the results obtained for the isolation of individual methyl ethers by micropreparative GLC.

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THE XYLAN OF THE STEMS OF THE HERB Phleum pratense

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Phleum pratense (timothy) is a fodder cereal grass which is distinguished by a high yield, for which reason it is widely used in agriculture [1]. The structure of the polysaccharides of this raw material, like that of a number of other grasses, has been studied little [2-4].

We have investigated the chemical composition and structure of the xylan of Phleum pratense, this forming the main part of its hemicellulose.

The carbohydrates of the stem of this grass include 28.20% of readily hydrolyzable polysaccharides (RHPs) and 39.57% of difficultly hydrolyable polysaccharides (DHPs), together with 17.18% of lignin, 0.76% of total nitrogen, and 6% of ash.

A hydrolyzate of the RHP contained 59.90% of xylose, 13.36% of arabinose and mannose, which are difficult to separate, 13.05% of glucose, 2.31% of lactose, and 11.48% of uronic acids, which shows the predominance of xylan among the polysaccharides of the hemicelluloses.

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This polysaccharide was isolated by alkaline extraction. Its homogeneity was determined electrophoretically. The molecular weight of the xylan was 18,400 carbon units, $[\alpha]_D^{20} - 69.0°$. A hydrolyzate contained 87.50% of D-xylose, 12.52% of L-arabinose, and 3.65% of D-glucuronic and 4-O-methyl-D-glucuronic acids.

The IR spectrum of this xylan contained the following adsorption bands: 895 cm^{-1}, corresponding to the β form of the pyranose ring; 1720-1735 cm^{-1}, corresponding to the stretching vibrations of the carbonyl group (glucuronic acid residues); 3400-3430 cm^{-1}, corresponding to hydroxy groups included in weak hydrogen bonds; and others.

The structure of the xylan was established by periodate oxidation, Smith degradation, and methylation.

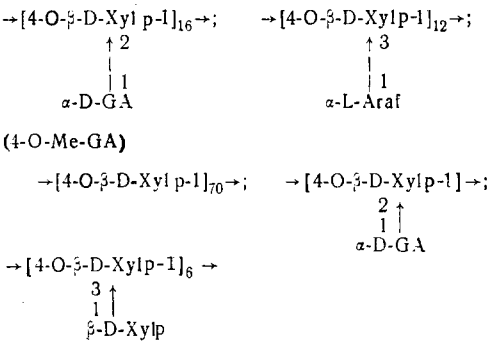
The periodate oxidation of the xylan, with the consumption of about 1 mole of $NaIO_4$ per pentose unit, led to the isolation of 21 moles of formic acid. The polyaldehyde obtained was produced and hydrolyzed, and glycerol and xylosylglycerol were found in the hydrolyzate. These products were detected by paper chromatography and were identified with the aid of markers.

Methylation of the xylan was performed by Hakomori's mathod. Its completeness was checked by thin-layer chromatography on alumina and from the IR spectra in the 2920 and 3400 cm^{-1} regions. The methylated polysaccharide obtained was hydrolyzed, and the hydrolyzates were investigated by paper chromatography in the presence of markers. We found mono-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-D-xylose in a ratio of 17 :35 :9.

It was established by the demethylation of the 2,3,4-tri-O-methyl-D-xylose and analysis of the products obtained that the tri-O-methyl derivatives of xylose and arabinose were present in a ratio of 2 :3.

The results given enable us to consider that the main chain of the xylan of timothy consists of β-D-xylopyranose residues connected by β-1,4-glycosidic bonds. Some of the xylose residues have branches. They are formed from L-arabinose residues attached to the main chain by 1 → 3 bonds, glucuronic and 4-O-methyl-glucuronic acids attached to the same chain by 1 → 2 bonds, and a small amount of xylose residues.

The structure of timothy xylan can be represented in the following way:



EXPERIMENTAL

Analysis of the Raw Material. The comminuted grass (Phleum pratense) gathered in 1972 in the variety section of the Odessa Agricultural Institute was defatted with ether, and the ethanol-soluble carbohydrates were eliminated. The raw material was dried in the air and analyzed.

To determine the amount of RHPs the polysaccharides were hydrolyzed with 2% hydrochloric acid and then the reducing substances were determined by Bertrand's method. The carbohydrates in the hydrolyzate were analyzed by paper chromatography using solvent system 1.

To determine the amount of DHPs, the residue after the analysis of the RHPs was hydrolyzed with 72% hydrochloric acid, and the reaction mixture was diluted and was analyzed by Bertrand's method.

Quantitative Determination of the Carbohydrates. The compositions and amounts of carbohydrates in the hydrolyzates were determined by partition chromatography on type S ["medium"] paper of the Volodarskii Leningrad Mill, using the following systems of mobile solvents: 1) butan-1-ol–benzene–pyridine–water (5 :1 :3 :3); 2) butan-1-ol–ethanol–water (4 :1 :2); 3) 3% solution of ammonia–butan-1-ol (40 :90); and 4) ethyl acetate–acetic acid–formic acid–water (18 :3 :1 :4). The spots were revealed with an ethanolic solution of aniline phthalate.

Simultaneously, we used thin-layer chromatography on alumina in system 1): toluene–ethanol (9 : 1). To separate 2-O- and 3-O-monomethyl-D-xyloses we used chromatographic paper impregnated with a 2% solution of sodium tetraborate.

Electrophoresis was performed by a method describe previously [5]. The xylan was isolated from the grass by alkaline extraction in an inert gas medium [6].

Characteristics of the Structure of the Xylan. To determine its monosaccharide composition, the xylan was hydrolyzed with a 2% solution of HCl for 4 h. The neutral monosaccharides were determined by paper chromatography in system 1, and the acid monosaccharides in system 4.

The xylan was oxidized with a 0.3 M solution of $NaIO_4$ for 72 h. The consumption of periodate was determined iodometrically, and the amount of formic acid by titration with a 0.02 M solution of NaOH. The completely oxidized xylan was reduced with $NaBH_4$ for 12 h, treated with KU-2 and AV-17 ion-exchange resins, and hydrolyzed at pH 1 [6].

The products of the Smith degradation – xylosylglycerol and glycerol – were detected chromatographically on paper in systems 1 and 3 in the presence of markers.

The xylan was methylated by Hakomori's method [7], the end of the reaction being found by chromatography in a thin layer of alumina in system 1 and, in parallel, by IR spectroscopy.

The completely methylated polysaccharide was hydrolyzed, and the methylated sugars were determined by paper chromatography and thin-layer chromatography using solvent system 2 for the paper and 1 for the thin-layer chromatography.

For the chromatographic identification of 2,3,4-tri-O-methyl-D-xylopyranose and 2,3,5-tri-O-methyl-L-arabinofuranose, which are difficult to separate, they were extracted preparatively from chromatograms using solvent 2. The dried mixture was demethylated in methylene chloride at −80°C. The resulting free sugars (xylose and arabinose) were identified and were determined quantitatively by paper chromatography in system 1.

SUMMARY

1. The stems of the fodder grass Phleum pratense contain a considerable amount of polysaccharides, lignin, and ash substances. They are characterized by a comparatively small amount of nitrogenous substances.

2. The hemicelluloses of the material consist mainly of an arabinoglucuronoxylan the main chain of which consists of residues of β-D-xylose connected in the 1 → 4 position. Side chains contain residues of L-arabofuranose, D-xylopyranose, and D-glucuronic and 4-O-methylglucuronic acids.

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