

SUMMARY

The qualitative and quantitative monosaccharide composition and the physicochemical properties of the pectin substances from the leaves of *Eremurus regelii* Vved. have been studied. A galacturonan has been obtained by the partial acid hydrolysis of the pectin. On the basis of the results of periodate-nitric acid oxidation, enzymatic hydrolysis, and methylation, it has been shown in the galacturonan the galacturonic acid residues are linked by α -(1 \rightarrow 4)-glycosidic bonds.

LITERATURE CITED

1. M. I. Igamberdyeva, D. A. Rakhimov, and Z. F. Ismailov, *Khim. Prir. Soedin.*, 429 (1974).
2. G. V. Buzina, O. F. Ivanova, and L. B. Sosnovskii, *Khlebopek. Konditersk. Promst.*, No. 4, 15 (1965).
3. A. G. Gorin, *Khim. Prir. Soedin.*, 80 (1967).
4. G. O. Aspinall and A. Ganas-Rodriguez, *J. Chem. Soc.*, 4020 (1958).
5. S. Hakomori, *J. Biochem. (Tokyo)*, 55, 205 (1964).
6. E. L. Hirst and E. Percival, in: *Methods in Carbohydrate Chemistry*, Vol. V, R. L. Whistler, ed., Academic Press (1965), pp. 294-295.
7. Yu. S. Ovodov, *The Gas-Liquid Chromatography of Carbohydrates* [in Russian], Vladivostok (1970).
8. H. Bouveng, H. Kiessling, B. Lindberg, and J. E. McKay, *Acta Chem. Scand.*, 16, 615 (1962).

CARBOHYDRATES OF *Allium*.

IX. CARBOHYDRATE COMPONENTS OF *Allium cepa*

M. A. Khodzhaeva, Ya. V. Rashkes,
E. S. Kondratenko,* and D. A. Rakhimov

UDC 547.917

The carbohydrate components of the bulbs of *Allium cepa* L. (white variety) have been characterized. It has been established that the main carbohydrate component is the ethanol-soluble fraction. Information has been obtained on the elementary compositions and origin of a number of fragments in the mass spectrum of the peracetate of 1^F-kestose.

We have previously described the ethanol- and water-soluble components of the bulbs of *Allium cepa* L. (red variety) [1]. In the present paper we consider the carbohydrate components of the white variety of this species. In contrast to the red variety, here we observe that the ethanol-soluble fraction is the main carbohydrate component.

By successive extraction of the bulbs we obtained the ethanol-soluble fraction (80% ethanol) (ES) (75.5%, on the absolutely dry raw material), the water-soluble polysaccharides (WSPSs) (6%), the pectin substances (PSSs) (4.9%), and the hemicelluloses (HMCs) (0.17%).

The ES fraction, after purification with carbon, was analyzed by paper and thin-layer chromatography, with the detection of fructose, glucose, sucrose, and oligosaccharides with R_f 0.08 and 0.2 remaining in the ethanol.

When the ES material was fractionated on a column of Sephadex G-15, ten fractions, ES-I-ES-X, were obtained; their characteristics are given at the top of following page.

On comparing their chromatographic mobilities on PC and TLC, their molecular weights, and their specific rotations, ES-X and ES-IX were identified as fructose, glucose, and sucrose, respectively.

*Deceased.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from *Khimiya Prirodnykh Soedinenii*, No. 2, pp. 174-178, March-April, 1985. Original article submitted June 4, 1984.

	ES-I	ES-II	ES-III	ES-IV	ES-V	ES-VI	ES-VII	ES-VIII	ES-IX	ES-X
Volume of eluent (water), ml	134	145	152.6	163.6	170.6	181.6	196.6	214.6	228.6	260
Yield, % on the ES mol. wt.	7.54	6.70	6.70	11.75	7.54	13.5	18.9	19.0	2.5	5.4
	2060	1620	1476	1150	990	830	666	504	342	180

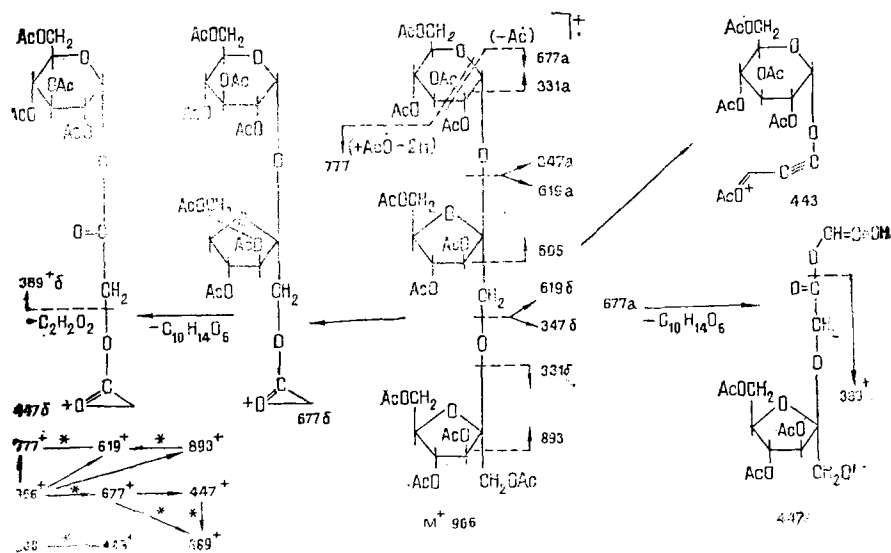
In the products of the complete acid hydrolysis of ES-I-ES-IX, fructose and glucose were detected by TLC and PC in various ratios, i.e., they were all glucofructans.

The amounts of fructose and glucose in ES-VIII determined by method [2] were 66 and 34%, respectively.

From its monosaccharide composition, molecular weight, molecular rotation $[\alpha]_D^{20} + 28^\circ$ (c 4.0; H₂O), and chromatographic mobility ES-VIII was a trisaccharide — 1^F-kestose. The acetylation of the 1^F-kestose with acetic anhydride in pyridine gave a syrupy peracetate with $[\alpha]_D^{20} + 33^\circ$ [3].

The mass spectrum of the peracetate of 1^F-kestose has been described previously [3]. Its characteristic feature, showing the presence of two successively linked fructofuranose residues, was an ion $(M - CH_2OAc)^+$ with m/z 893. The intensity of the peak of this ion in relation to the 100% peak of the ion with m/z 331 was of the same order as in [3].

At the same time, new information on the elementary composition and origin of a number of fragments in the spectrum of 1^F-kestose, and also some qualitative and quantitative differences between the spectra of the two samples, impelled us to again return to a discussion of this spectrum.



Scheme of the fragmentation of the peracetate of 1^F-kestose under EI.

The main results are reflected in the scheme, where, together with the ion formed by the cleavage of the glycosidic (C-O) and exocyclic (C-C) bonds (m/z 331, 347, 605, 619, and 893), fragments are shown which arise on the breakdown of the carbohydrate units. The sequence of acts in the formation of these ions has been confirmed in the majority of cases by the method of defocusing the ion beam in the first field-free space of the mass spectrometer and are given on the right of the scheme.

A distinguishing feature of these ions is the fact that they can arise from the fragmentation of any of the terminal units, in view of which two alternative forms can be ascribed to them. The composition of the ion with m/z 777 indicates that it is formed in the cleavage of the C₃-C₆ chain of the terminal unit with migration of the acetoxyl radical to the charged fragment and the opposite migration of two hydrogen atoms.

The subsequent breakdown of this fragment was recorded, leading to the appearance of an ion with m/z 619 ($C_{26}H_{35}O_{17}$).

When the same bonds of the terminal units were cleaved, with the additional elimination of the acetyl radical, ions with m/z 677a and 677b and the composition $C_{28}H_{37}O_{19}$ arose. As defocusing experiments showed, these ions, together with ions having m/z 447, were the precursors of the ions with m/z 389 that are the most abundant in this series. The formation of the latter is shown in the scheme as the result of the fragmentation of the central carbohydrate unit with the subsequent splitting out of a $C_2H_2O_2$ fragment.

One of the strongest peaks in the region of mass numbers above m/z 331 is the peak of an ion with m/z 443 and the composition $C_{19}H_{23}O_{12}$. It takes its origin from a fragment with m/z 605 ($C_{25}H_{38}O_{17}$) and, consequently, is formed by the elimination of the $O-C_5-C_6$ chain of the fructofuranosyl unit with the additional loss of a molecule of acetic acid.

We shall not dwell on the products of the breakdown of the ion with m/z 331, since the corresponding processes have been described repeatedly [4]. We may mention only that the precursors of this ion may be, as defocusing experiments showed, in addition to M^+ , ions with m/z 619, 605, and 389.

The WSPSs consisted of a polydisperse polymer in the product of the complete acid hydrolysis of which a predominating amount of fructose and traces of glucose were detected by PC and TLC. The IR spectrum of the WSPSs showed absorption bands at 840, 870, and 940 cm^{-1} which are characteristic for glucofructans of the mixed type.

The PSs formed a white fibrous substance with $[\alpha]_D^{20} + 130^\circ$ (c 1.0; H_2O), the monosaccharide composition of which was represented by rhamnose, arabinose, xylose, glucose, and galactose in a ratio of 3.4:1:1.1:13.6:14.5, respectively. The IR spectrum of the PSs had characteristic absorption bands — 815, 830, 870, 900, 920, 940, 960, 990, 1010, 1080, 1120, 1235, 1865, 1450, 1680, 2880, 2940, 3080, 3280, and 3400 cm^{-1} . Titrimetric results for the PSs were determined by the method of Buzina et al. [5]: K_C — free carboxy groups, 3.74%; K_e — methoxylated carboxy groups, 2.39%; λ — the degree of esterification, 39%.

An acid hydrolysate of the hemicellulose was found to contain rhamnose, arabinose, xylose, and galactose in a ratio of 2.2:1:2.0:11.34.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at $40 \pm 5^\circ\text{C}$. IR spectra were taken on a UR-20 instrument in tablets with KBr. Specific rotations were determined on a Zeiss polarimeter in a tube 1 dm long with a volume of 10 ml. Mass spectra were taken on a MKh 1310 mass spectrometer with an SVP-5 system, with a temperature of the ionization chamber and of the evaporating bulb of $130\text{--}150^\circ\text{C}$, an ionizing voltage of 50 V, and a collector current of 30 μA . The resolving power was 10,000, the reference substance perfluorokerosine, and the accuracy of the mass measurements $5 \cdot 10^{-6}$. Defocusing: H and E constant, scanning of the accelerating voltage from 2 to 4.8 kV at the rate of 0.1 kV/sec.

The conditions for GLC were similar to those described in [6]. PC was performed by the descending method on FN 11,17 paper in system 1) butan-1-ol-pyridine-water (6:4:3), and TLC on LS 5/40 silica gel in systems 2) butan-1-ol-pyridine-water (1:1:1), and 3) benzene-acetone (1:1). The revealing agent was aniline hydrogen phthalate.

The carbohydrate components were isolated in succession as described in [6].

Fractionation of the ES Material and the WSPSs. Determination of Molecular Weights. To isolate homogeneous fractions, the ES material (5.7 g) was subjected to preparative chromatography on a column of Sephadex G-15 ($56 \times 2.7\text{ cm}$). Elution was performed with distilled water, 3-ml fractions being collected and analyzed by the phenol-sulfuric acid method. The volumes of the eluates (V_e) are given below. The molecular weights were determined on a column of Sephadex G-100 ($50 \times 1.6\text{ cm}$) which was calibrated by the passage of 10 mg each of dextran-10,000, inulin, raffinose, sucrose, and fructose. The molecular weights of ES-I-ES-X ($V_e = 54.0, 55.5, 56.5, 57.5, 58.8, 59.5, 61.0, 64.0, 68.0$, and 74.0 ml) are given above. The WSPSs proved to be polydisperse and their V_e s ranged from 33 to 64 ml, which corresponds to molecular weights of from 504 to 40,000.

Acid Hydrolysis of ES-I-ES-X and the WSPSs. The hydrolysis of fractions ES-I-ES-VI was carried out with 0.1 N H₂SO₄ at 100°C, that of ES-VII-ES-X with 1% oxalic acid at 100°C for 30 min, and that of the WSPSs with 0.5 N H₂SO₄ at 100°C for 2 hours. The hydrolysates were neutralized with barium carbonate and, after filtration, evaporated to syrups. The hydrolysis products were analyzed by PC (system 1) and by TLC (system 2). Fructose and glucose in various ratios were detected in all the samples by Ivanov's method [2].

Acetylation of ES-VIII. A solution of 0.5 g of the ES-VIII fraction in 3 ml of pyridine was treated dropwise with acetic anhydride (3 ml) over 2 h. The reaction mixture was left for 12 h and was then dissolved in chloroform (20 ml), and the chloroform layer was washed repeatedly with water and evaporated. The peracetate was purified on a column of silica gel with elution by chloroform. The homogeneity of the ES-VIII peracetate was checked by TLC (system 3). After concentration, a viscous syrup was obtained with $[\alpha]_D^{20} + 33^\circ$ (c 4; chloroform).

Relative Intensities of the Peaks in the Mass Spectrum above 100 a.u.m. (%). 966 (M⁺; 0), 907 (M - OAc - 1.8), 893 (M - CH₂OAc, 15), 851 (893 - 42; 0.76), 777 (0.60), 677 (2.2), 619 (15), 605 (0.52), 577 (619 - 42; 0.55), 563 (605 - 42; 0.48), 561 (0.59), 559 (619 - 60; 0.66), 547 (619 - 72; 0.55), 545 (605 - 60; 0.86), 539 (0.85), 503 (563 - 60; 0.93), 487 (547 - 60; 0.92), 457 (577 - 120; 2.0), 447 (0.53), 443 (2.9), 389 (14), 347 (0.86), 345 (2.3), 331 (100), 317 (2.1), 289 (10), 287 (3.3), 275 (6.3), 273 (6.5), 271 (6.8), 269 (10), 229 (7.0), 227 (7.3), 211 (96), 187 (8.0), 169 (84), 127 (20), 109 (64), 101 (23).

Acid Hydrolysis of the PSs and the HMCs. Samples weighing 0.1 g were hydrolyzed with 5 ml of 2 N H₂SO₄ each in sealed tubes at 100°C for 48 h. The hydrolysates were neutralized with barium carbonate and, after filtration, were evaporated to the state of syrups. Part of each syrup was analyzed by PC (system 1), and from the other part the corresponding aldonitrile acetates were obtained for analysis by GLC. The hydrolysate of the PSs was found by GLC to contain rhamnose, arabinose, xylose, glucose, and galactose in a ratio of 3.4:1:1.1:13.6:14.5, and the hydrolysate of the HMCs rhamnose, arabinose, xylose, and galactose in a ratio of 2.2:1:2.0:11.4.

SUMMARY

The carbohydrate components of the bulbs of *Allium cepa* L. (white variety) have been characterized. It has been established that the main carbohydrate component is the ethanol-soluble fraction. Information has been obtained on the elementary compositions and origins of a number of fragments in the mass spectrum of the peracetate of 1^F-kestose.

LITERATURE CITED

1. M. A. Khodzhaeva and E. S. Kondratenko, Khim. Prir. Soedin., 383 (1984).
2. I. N. Ivanov, Methods in Plant Physiology and Biochemistry [in Russian], Leningrad (1935), p. 122.
3. W. W. Binkley, R. C. Dougherty, D. Horton, and J. D. Wander, Carbohydr. Res., 17, 127 (1971).
4. K. G. Das and B. Thayumanavan, Org. Mass. Spectrom., 10, 455 (1975).
5. G. V. Buzina, O. F. Ivanova, and L. B. Sosnovskii, Khlebopek Konditersk. Promst., No. 4, p. 15 (1965).
6. M. A. Khodzhaeva and E. S. Kondratenko, Khim. Prir. Soedin., 228 (1983).