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POLYSACCHARIDES OF Polygonatum

VIII. STRUCTURE OF A GLUCOFRUCTAN FROM Polygonatum sewerzowii

MASS-SPECTROMETRIC PROPERTIES OF PERACETATES OF FRUCTOOLIGOSACCHARIDES

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UDC 547.917

By partial hydrolysis, tetra- and pentafructooligosaccharides have been obtained from Polygonatum sewerzowii Regel. Structures of the individual tetra- and pentasaccharides have been proposed on the basis of the results of ^{13}C NMR and mass spectrometry. In the ^{13}C NMR spectrum a signal with a chemical shift of 76.5 ppm has been detected which is characteristic for the C-4 atom of a nonterminal unit. Consequently, the pentasaccharide has mixed 2 → 1 and 2 → 6 bonds in the chain. A comparative mass-spectrometric study of isomeric trisaccharide peracetates has been performed.

We have previously reported on the study by ^{13}C NMR spectroscopy of the glucofructan pseverin from Polygonatum sewerzowii consisting of β-2 → 6- and β-2 → 1-bound fructofuranose units, i.e., having a mixed type of bonds — of inulin and levan natures [1]. A determination of the amounts of monosaccharides is the polysaccharide by the methods of Bertrand and Kolthoff [2] has shown that pseverin contains 94% of fructose residues and 6% of glucose residues.

In order to study the sequence of monosaccharide residues, pseverin was subjected to partial hydrolysis. Glucose, fructose, sucrose, and tri-, tetra- (I), and penta-(II)-fructooligosaccharides were detected in the hydrolysate. The trifructooligosaccharides were identified with markers in PC. The fructooligosaccharides (I) and (II) were obtained in the individual form by preparative PC, and glucose and fructose were revealed in hydrolysates of them.

To determine the nature of the types of bonds of the monosaccharide units, fructooligosaccharides (I) and (II) were methylated by Hakomori's method [3]. The permethylates were subjected to methanolysis. In a hydrolysate of the permethylate of oligosaccharide (I) TLC revealed the presence of 2,3,4,6-tetra-O-Me-D-glucose, 1,2,4,6-tetra-O-Me-D-fructose, and 3,4,6-tri-O-Me-D-fructose. The isolation of 3,4,6-tri-O-Me-D-fructose showed the presence of a 2 → 1 bond between fructofuranose units.

Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 146-151, March-April, 1986. Original article submitted July 3, 1985.

0009-3130/86/2202-0135 $12.50

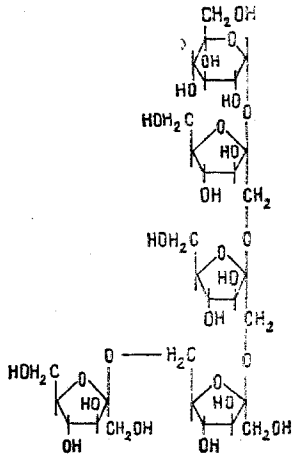
In addition to the sugars mentioned above, 1,3,4-tri-O-Me-D-fructose was detected in the hydrolysate of the permethylate of the fructooligosaccharide (II), obviously showing that oligosaccharide (II) contained both 2 → 1- and 2 → 6-bound fructofuranose residues.

The chemical results obtained were confirmed by those of ^{13}C NMR spectroscopy. In the ^{13}C NMR spectrum of fructooligosaccharide (I) strong peaks were detected with chemical shifts (ppm) of 104.3 (C-2), 82.3 (C), 78.2 (C-3), 75.7 (C-4), and 63.8 (C-6), which are characteristic for residues with the inulin type of bond. A signal at 93.2 ppm was assigned to the C-1 atom of an α-D-glucopyranose residue attached by a 2 → 1 bond to a fructofuranose residue. It follows from this that the fructooligosaccharide (I) has 2 → 1-bound fructofuranose units with a terminal glucose residue and is the tetrasaccharide nystose [4].

We studied the ^{13}C NMR spectrum of the fructooligosaccharide (I) similarly and determined the ratio between the levan and inulin units. For this oligosaccharide, moreover, it is possible to determine the length of the chain from the ratio of the integral intensities of the signals of the glucopyranose and fructopyranose residues. The chemical shifts of the carbon atoms are given below:

Residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
→(2β-*D*-Fruf-6-)-	61.9	104.8	78.5	76.1	81.3	64.3
→(2β-*D*-Fruf-1)→	61.6	104.3	78.2	75.7	82.3	63.8
α-*D*-Glcp	93.35	72.8	73.7	70.05	72.2	61.6

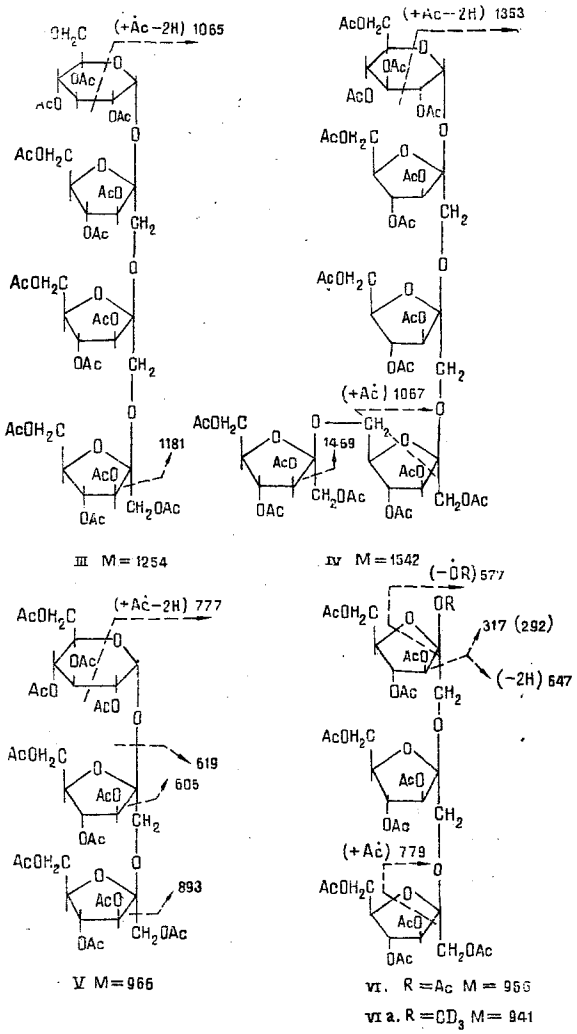
As we see, the fructooligosaccharide (II) gives signals at 81.3 and 64.3 ppm corresponding to a levan unit. The spectrum also contains signals characteristic for the C-4 atom of a nonterminal unit. The numerical ratio of the 2 → 6 and 2 → 1 bonds calculated from the integral intensities of the signals of the carbon atoms in the spectrum is 1:3. The retention of a signal at 93.35 ppm for α-D-glucose indicates that one glucose residue is attached at C-2 of a fructofuranose unit in the polymer chain. Thus, the pentasaccharide under consideration has the following structure:



III

Let us now consider the mass-spectrometric properties of the peracetates of the oligosaccharides (I) and (II) (III) and (IV) in comparison with those of the peracetates of model trisaccharides – I^F-kestose (V) and inulotriose (VI). A comparison of the spectra of (V) and (VI), in its turn, in addition to revealing common features of the breakdown of isomeric compounds, should lead to the achievement of a practical aim – the confirmation of the attachment of a glucose residue to the reducing unit in each of the fructooligosaccharides (I) and (II), since on hydrolysis of the initial glucofructan, in principle, purely fructose fragments could be formed.

We have previously [5] described features of the fragmentation of the peracetate of I^F-kestose (V) revealed with the aid of high-resolution mass spectrometry and metastable defocussing (MD) spectra. In the spectrum of the peracetate of the inulin fragment (VI) the ion with m/z 777 that is characteristic of the spectrum of (V) disappears and the peak of a fragment with m/z 647 appears, while the intensities of the peaks of the ions with m/z 605, 779, and 935 rise appreciably.



A still more substantial difference between the isomers (V) and (VI) is revealed when the MD spectra of some daughter ions are compared. The precursors of some daughter ions in the spectra of the peracetates of I^F-kestose (V) and of inulotriose (VI) are:

Daughter ion	Compound	Parental ions
317	V	577, 375
	VI	605
331	V	619, 605, 577, 517, 389
	VI	677, 619, 605, 591, 559, 503, 389
389	V	777, 677, 487, 447
	VI	677
605	V	—
	VI	893, 935

The results given above show the lower selectivity of the processes of fragmentation of the peracetate of I^F-kestose (V).

The disappearance of the peak of the ion with m/z 777 in the spectrum of (VI) may indicate that this fragment is characteristic only for a peracetate of an oligosaccharide containing a glucopyranose residue attached to the reducing end of a fructofuranose chain by a

TABLE 1. Mass Numbers and Relative Intensities (in % of the height of the m/z 331 ion) of the Main Fragments in the Mass Spectra of (III)-(VI)

n	m/z	Compound				Origin of the fragments
		III	IV	V	VI	
1	317	3,0	2,4	2,1	4,5	$(M-\dot{C}H_2OAc)^+$
2	605	3,9	0,60	0,52	14,0	
3	893	4,0	10,0	15,0	4,2	$(M-CH_2OAcFru_n)^+$
4	1181	0,10	3,6	—	—	
5	1469	—	0,002	—	—	
1	331	100	100	100	100	
2	619	7,5	13,0	15 0	17,0	$(\dot{M}-AcO)^+$
3	907	0,10	2,3	1 8	0,22	$(M-AcFru\dot{O})^+$
4	1195	0,005	0,10	—	—	
1	389	15,0	11,0	14,0	19,0	
2	677	2 0	2,2	2,2	6,0	
3	965	0,10	0,30	—	—	
4	1253	—	0,005	—	—	
1	503	1,6	2,0	0,93	2,4	$(C05-AcOH-C_2H_2O)^+$
2	791	0,60	1,1	0,30	0,60	
3	1079	0,63	0,20	—	—	
4	1367	—	0,10	—	—	
2	647	—	—	—	0,25	$C_1' - \gtrless - C_2'$ (−2H)
3	935	0,72	0,55	0,005	2,3	
4	1223	—	0,25	—	—	
2	777	0,04	—	0,60	—	$C_5' - \gtrless - O$, $C_2' - \gtrless - C_3 \times$ $\times(+Ac-2H)$
3	1065	0,13	—	—	—	
4	1353	—	0,003	—	—	
2	779	—	—	0,10	0,52	$C_5''' - \gtrless - O$, $C_2''' - C_3'''$; $(+Ac)$
3	1067	—	0,22	—	—	

1 → 2 bond. In actual fact, the spectra of the peracetates (III) and (IV) show the presence of peaks of ions with m/z 1065 and 1353, respectively, in place of the ion with m/z 777.

In the analysis of the spectrum of (VI), the conclusion suggests itself that the increase in the height of the peak of the ion with m/z 779 is due here to a process of elimination of a neutral fragment differing by 2 H from that which is eliminated in the formation of the ions with m/z 777 in the spectrum of (V), and that in this process it is the reducing unit that breaks down. The elementary composition of the ion with m/z 777 confirms this hypothesis. On this basis, in the spectra of the paracetates (III) and (IV) the homologous ions with m/z 1067 and 1365, respectively, should be absent. In actual fact, the first of these ions is absent from the spectrum of (III) and the second from the spectrum of (IV), but in the latter there is the peak of an ion with m/z 1067. This fact unambiguously indicates that the ion with m/z 779 from the peracetate of the inulin fragment (VI) is formed by the breakdown of the nonreducing unit at the $C_5''' - O$ and the $C_2''' - C_3'''$ bonds with the migration of Ac· to the charged fragment, which does not contradict the elementary composition of this ion. Correspondingly, the formation of the ion with m/z 1067 from the peracetate of the pentasaccharide (IV) takes place through the cleavage of the same bonds in the unit adjacent to the terminal unit of the levan type. We may add that, as was to be expected, in the spectrum of the peracetate of the tetrasaccharide fragment of inulin that we obtained there was an ion of low intensity with m/z 1067.

The only fact contradicting the assignment of the ion with m/z 779 given above is the absence of the peak of an ion with m/z 1067 in the spectrum of the peracetate of nystose (III) (scheme).

A fragment with m/z 647 and the composition $C_{27}H_{35}O_{18}$ in the spectrum of (VI) may serve as a qualitative indication of the presence of a fructofuranose ring at the reducing end of the molecule. It is apparently formed by the cleavage of the $C_1 - C_2$ bond with the migration of 2H to the charged fragment. Although an ion with m/z 935 in the same spectrum is

homologous in composition to the ion with m/z 647, it arises as the result of a different rearrangement process due to the elimination of the fragment CH_2OH from M^+. The spectra of the acetates (III) and (IV) also contain ions with m/z 935 and 1223, respectively, analogous in composition but arising by a different mechanism (Table 1).

Table 1 gives the mass numbers and relative intensities (the height of the peak of the ion with m/z 331 being taken as 100%) of the main characteristic fragments under discussion formed as a consequence of the cleavage of the glycosidic bonds and also by the fragmentation of the C−C bonds of the carbohydrate units. Each group includes ions homologous in composition containing increasing numbers, n, of unfragmented carbohydrate units.

As can be seen from Table 1, the presence of a glucopyranose unit attached by a 1−2 bond to an oligofructan chain does not lower the intensity of the peak of the ion with m/z 605 to the same degree in all cases. In the spectrum of the peracetate of nystose (III) it amounts to 3.9%. The same difference in the intensities of this peak for the spectrum of compounds (III) and (V) has been reported by Binkley et al. [6], although these authors consider that breakdown of this type when a terminal glucose residue is present must make a small contribution because of the electron-accepting influence of the anomeric atom of this unit.

Since a considerable proportion of the fragments under consideration may have alternate structures through the breakdown of different units, we decided to check this by introducing as isotopic label into the molecule of the peracetate (VI). When a sample of (VI) was treated with deuteromethanol at 100-110°C for 20 h, the acetyl radical at C_2' was replaced by OCD_3 in approximately 2/3 of the molecules. However, the labeled compound (VIa) cannot be considered as a complete analog of compound (VI) because of the donor influence of the OCD_3 group. This led to a marked increase in the height of the peak with m/z 292 − an analog of the ion with m/z 317 in the spectrum of (VI). The predominant localization of the charge on the reducing unit led, on the cleavage of the $C_1'-C_2'$ bond to the practically complete disappearance of the peak of the ion with m/z 647.

The figures given below show the percentage contributions of the unshifted (U) and shifted (S) fragments in the spectrum of (VI). Judging from these figures, the ions with m/z 677 and 619 are formed mainly on the breakdown or ejection of the reducing unit, and the ions with m/z 389 and 331 likewise mainly retain a nonreducing unit in their composition. Conversely, the ions with m/z 605 arise on the ejection of the nonreducing unit in the form of $AcFruOCH_2$:

U	%	S	%	U	%	S	%
893	35	868	65	389	84	364	16
677	75	652	25	331	78	306	22
619	78	594	22	317	2	292	98
605	1	580	99				

Thus, on the basis of the results obtained we propose the following structure for the glucofructan of pseverin:

Glcp 1→2 Fruf 1→[2 Fruf 1]$_2$→2 Fruf 6→[2 Fruf 1]$_3$→ 2 Fruf 6→[2 Fruf 1]$_2$→2 Fruf 6→2 Fruf

EXPERIMENTAL

The ^{13}C NMR spectra were taken on a Bruker WR-60 instrument with a working frequency for carbon of 15.08 MHz using complete proton suppression. Solutions in D_2O with a concentration of 3% were prepared and methanol was used as the internal standard, its chemical shift relative to TMS being taken as 50.15 ppm.

The review spectra of the peracetates (III-VI) were taken on a MKh 1310 mass spectrometer with a SVP5 system for the direct introduction of the sample at a temperature of the ionization chamber of 200-220°C and of the evaporator bulb of 180-250°C, using a collector current of 60 μA. For the determination of the elementary compositions of the ions and the acquisition of the MD spectra, see [5].

Methylation of the Fructooligosaccharides. A 0.01 g sample of each oligosaccharide was methylated by Hakomori's method [3]. The methanolysis of the permethylates was carried out in 2% HCl in CH_3OH, the mixture being kept at 60°C for 2 h.

Acetylation of the Fructooligosaccharides. The syrup of tetra- and pentafructooligosaccharides was evaporated to dryness, and the residue was dissolved in 0.5 ml of pyridine and was treated with 1.0 ml of $(CH_3CO)_2O$. The resulting mixture was kept for seven days. The peracetates were extracted with chloroform and the extract was evaporated to a syrup. The properties of the peracetates were studied by the mass-spectrometric method.

SUMMARY

By means of the partial hydrolysis of pseverin, tetra-, and pentafructooligosaccharides have been isolated in the individual state. A new pentasaccharide with a mixed type of bonds has been isolated for the first time and has been studied by chemical and physical methods.

On the basis of the results of ^{13}C NMR spectroscopy of fructooligosaccharides and the mass spectra of their peracetates, structures have been proposed for the tetra- and pentasaccharides from the flucofructan of Polygomatum sewerzowii. A comparative mass-spectrometric study of isomeric trisaccharide peracetates has been performed.

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FEATURES OF THE SYNTHESIS OF CARBAMOYLETHYL ETHERS OF AN ARABINOGALACTAN

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UDC 668.411

The synthesis of carbamoylethyl ethers of an arabinogalactan by the O-alkylation reaction of alcohols with acrylamide in the presence of caustic soda has been investigated. It has been shown that pronounced hydrolysis of the amide groups to carboxy groups takes place when the reaction is performed in solvents. If the reaction is performed in the solid phase with brief heating at 125°C, no appreciable hydrolysis of the amide groups is observed but the formation of the ether is accompanied by degradation of the polysaccharide itself.

Carbamoylethyl ethers of arabinogalactans (AGs) have not been described in the literature, and the aim of the present work was to study their synthesis, since they may be of interest as intermediates in the production of polymeric binders increasing the strength of paper and cardboard.

It is known that acrylonitrile is one of the most active monomers in the O-alkylation of cellulose in the presence of alkalis, reacting practically completely with cellulose under conditions [1]. To compare the reactivities of an AG and cellulose in such reactions, the first experiments on the modification of the AG were performed with acrylonitrile. When the AG was treated under the recommended conditions acrylonitrile likewise took part in a reaction with high conversion.

Siberian Scientific-Research Institute of Pulp and Board, Bratsk. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 151-154, March-April, 1986. Original article submitted January 4, 1985.

 0009-3130/86/2202-0140 $12.50