Chemistry of Natural Compounds, Vol. 32, No. 4, 1996

POLYSACCHARIDES OF SAPONIN-BEARING PLANTS. IX. STRUCTURES OF OLIGOSACCHARIDES FROM THE GLUCOGALACTAN OF *Allochrusa gypsophiloides*

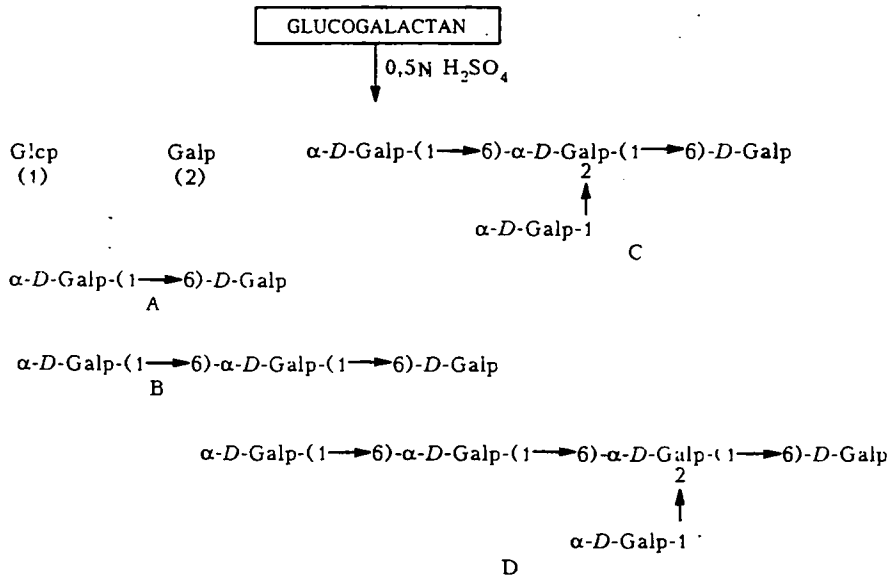
A. O. Arifkhodzhaev

UDC 547.917

The partial acid hydrolysis of the glucogalactan from A. gypsophiloides *has yielded four oligosaccharides, two of which have been obtained for the first time and are branched tetra- and pentasaccharides. Their structures have been established by periodate oxidation, methylation, and reduction and also from the results of* ^{13}C *NMR.*

In the preceding paper we reported an investigation of a glucogalactan from the roots of *A. gypsophiloides* [1]. For a complete determination of the structure of the polysaccharide we have carried out partial acid hydrolysis and in the products have detected, by PC, glucose (**1**), galactose (**2**), and four oligosaccharides (see Scheme 1). Preparative separation of the oligosaccharides yielded chromatographically pure compounds. The individuality of each of them was checked by acetylation, followed by deacetylation, which in the final account led to the initial oligosaccharides (A-D) the monosaccharide compositions of which were represented by *D*-galactopyranose alone (PC and GLC). The structures of A-D have been studied by chemical and spectral methods.

Scheme 1



Partial hydrolysis of the glucogalactan

Periodate oxidation and Smith degradation formed only glycerol. Consequently, in the oligosaccharides the monosaccharides were linked to one another either by α-(1→2) or α-(1→6) bonds or by both types of bonds.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 534-536, July-August, 1996. Original article submitted February 20, 1995.

0009-3130/96/3204-0526$15.00

TABLE 1. Characteristics of Oligosaccharides A-D from the *A. gypsophiloides* Glucogalactan

Oligosaccharide	$[\alpha]_D^{21}$, deg (*c* 1.0; water)	Reduction product (hydrolysate)		Methylation product (hydrolysate)			DP	Type of bond
		dulcitol	galactose	3	4	5		
A	+141.0	1	1	1	1	–	2	α-1→6
B	+159.7	1	2	1	2	–	3	α-1→6
C	+172.3	1	3	2	1	1	4	α-1→6,α-1→2
D	+181.4	1	4	2	2	1	5	α-1→6,α-1→2

Hakomori methylation [2] yielded fully methylated products (the completeness of the reaction was checked by IR spectroscopy). After an appropriate work-up and complete acid hydrolysis, it was established by TLC and GLC that the permethylates of A and B consisted of 2,3,4,6-tetra-O-Me-*D*-galactose (**3**) and 2,3,4-tri-O-Me-*D*-galactose (**4**) in ratios of 1:1 and 1:2, respectively, while C and D were characterized by the presence of (**3**), (**4**), and 3,4-di-O-Me-*D*-galactose (**5**) in ratios of 2:1:1 and 2:2:1, respectively (Table 1). The results of the methylation of A-D completely confirmed those of periodate oxidation, showing the presence of α-(1→6) and α-(1→2) glycosidic bonds.

In the products of the reduction of the oligosaccharides with $NaHB_4$, after appropriate working up, we found dulcitol and galactose. The results of methylation and reduction made it possible to determine the degrees of polymerization of A-D (see Table 1).

The chemical results obtained were confirmed by a study of the ^{13}C NMR spectra. The spectrum of A differed little from that of B, and that of C from that of D: below we give the chemical shifts (ppm) of the main intense signals of the carbon atoms:

Oligosaccharide	C-1	C-2	C-3	C-4	C-5	C-6
A	99.6	69.8	70.6	70.9	72.3	62.5
B	99.6	69.8	70.6	70.9	72.3	62.5
C	100.1	69.7	70.6	70.8	72.4	62.6
D	100.2	69.7	70.7	70.7	72.3	62.6

The spectra of A and B also contained signals with a low intensity belonging to the reducing ends of galactopyranose. It is possible that they relate to the carbon atoms of free hexopyranoses: (ppm) 93.8 and 97.5 (C-1), 75.8 and 72.8 (C-2), 74.3 (C-3), 71.5 (C-4), and 75.5 and 77.3 (C-5), while a peak at 67.3 ppm is characteristic for substituted C-6 galactopyranose atoms.

Signals with chemical shifts in the 68.3 ppm region in the spectra of C and D corresponded to the resonance of substituted C-6 atom, while the signal of an anomeric atom present at a point of branching appeared at 103.1 ppm; the region resonance of C-1 atoms at 101.1 ppm is characteristic for (1→2)-bound hexopyranoses, and a signal at 82.1 ppm belonged to a substituted C-2 atom of galactopyranose. In addition, the spectrum contained intense resonance lines at (ppm): 93.2 (C-1), 73.3 (C-2), and 65.5 and 64.7 (C-6).

Structures have been proposed for A-D on the basis of the facts given above, and, for the first time, structures have been obtained and demonstrated for a galactotetrose and a galactopentose in which C-2 *D*-galactopyranose atoms are points of branching (see Scheme 1). The study of the oligosaccharides by various methods has confirmed the structure of the glucogalactan proposed by us previously [1].

EXPERIMENTAL

TLC and PC were conducted under the conditions given in [4], which also gives the materials used, solvent systems, and revealing agents.

GLC was performed on a Chrom-5 chromatograph with a flame-ionization detector under the conditions given in [5].

^{13}C NMR spectra were taken on a Bruker WR-60 instrument with a working frequency for carbon of 15.08 MHz under the conditions of [4].

The specific rotations of the substances were measured on a Zeiss polarimeter in a tube 0.5 dm long with a volume of 1 ml at 21°C.

The oligosaccharides were reduced in the way described in [4].

Partial Acid Hydrolysis of the Glucogalactan. A solution of 4.0 g of the glucogalactan in 10 ml of 1 N H_2SO_4 was heated at 100°C for 10 min, neutralized with $BaCO_3$, deionized with KU-2 cation-exchange resin (H^+), and evaporated to a syrup, in which PC showed the presence of glucose, galactose, and the oligosaccharides A, B, C, and D with R_f values of 0.65, 0.53, 0.43, and 0.30, respectively.

The glucogalactan hydrolysate was separated by PC, the zones corresponding to individual oligosaccharides were cut out and extracted with water, and the extracts were evaporated to dryness. This gave the four chromatographically individual oligosaccharides, A-D, with yields of 0.16, 0.64, 0.24, and 0.54 g, respectively. Their specific rotations are given in Table 1, the literature figure for A being $[\alpha]_D$ +142.5° (water) [6].

Hydrolysis of the Oligosaccharides. Compounds A, B, C, and D (0.1 g each) were hydrolyzed as in [4], and only galactose was detected in the hydrolysates; i.e., the oligosaccharides consisted of *D*-galactose residues.

Acetylation of the Galactooligosaccharides. Compounds A, B, C, and D (0.02 g) were each dissolved in 1 ml of pyridine and treated with 1.5 ml of acetic anhydride. Each mixture was stirred for five days, poured into ice water and extracted with chloroform, and the chloroform extract was evaporated to dryness, after which a single spot was identified by TLC. This confirmed the purity of the oligosaccharides, and the products were then deacetylated with metallic sodium in methanol.

REFERENCES

1. A. O. Arifkhodzhaev, Khim. Prir. Soedin., 430 (1996) [preceding paper in this issue].
2. S. Hakomori, J. Biochem. (Tokyo), **55**, 205 (1964).
3. J. H. Bradbury and G. A. Jenkins, Carbohyd. Res., **126**, 125 (1984).
4. A. O. Arifkhodzhaev and D. A. Rakhimov, Khim. Prir. Soedin., 709 (1994).
5. A. O. Arifkhodzhaev and D. A. Rakhimov, Khim. Prir. Soedin., 188 (1993).
6. J. Stanék, M. Černý, J. Kocourek, and J. Pacák, The Oligosaccharides, Academic Press, New York (1965), p. 234.