POLYSACCHARIDES OF IRIDACEAE

V. CHARACTERIZATION OF THE NEUTRAL FRACTION OF THE WATER SOLUBLE POLYSACCHARIDE FROM Juno drepanophylla

Kh. A. Arifkhodzhaev

UDC 547.917

From the WSPS-I of the bulbs with roots of Juno drepanophylla, 15 fractions have been isolated by fractionation on DEAE-cellulose. Fractions 1 and 2 together made up 53% of the total weight of the fractions. Fraction 1 proved to be homogeneous on ultracentrifugation, on passage through Sepharose-4B, and on high-pressure LC. The results of the analysis of the methylated product of fraction 1 differed little from those for the initial polysaccharide.

We have established the polydispersity of the WSPS-I from the bulbs with roots of Juno drepanophylla. In this paper we give the results of an investigation of the fractionation of the polysaccharides on DEAE-cellulose (phosphate form), the physicochemical properties of the fractions, and the rate of monosaccharides.

The fractionation of the WSPS-I with the aid of fractional precipitation by ethanol and via the copper complex proved unsuccessful. When it was fractionated on DEAE-cellulose (OH^- form) with alkali (gradient from 0.05 to 0.5 N), the polysaccharide issued as a single peak. Fractionation on a column in the phosphate form led to the isolate of 15 fractions. Elution was performed first with water and then with phosphate buffer (0.1 M solution, pH 5.8-8.0), a 6 M solution of urea, and a 0.1 N solution of caustic soda. The salt fractions, after treatment with KU-2 resin (H^+), were dialyzed against distilled water and the products were precipitated from the concentrated solutions with methanol (1:4). The yields of the fractions, their specific rotations, and the ratios of the monosaccharides are given in Table 1 and in Fig. 1

Fractions 1 and 2 together made up 53% of the total weight of the fractions. The losses of polysaccharide on the column and in precipitation of the fractions amounted to 18%.

The different specific rotations and ratios of the monosaccharides in the fractions once more confirmed the polydispersity of the initial WSPS-I. The additional sugars rhamnose, fructose, mannose, and a uronic acid were detected in the fractions, as in the case of the amyloids of the shoots of white mustard seeds [1].

According to the results of ultracentrifugation (Fig. 2) and gel chromatography on Sephadex 4B, fraction 1 was homogeneous.

The relative viscosity of fraction 1 had the following values (23°, poise):

Concentration, %	Water	1% NaOH
0.5	11.4	2.6
0.4	8.8	2.1
0.3	5.3	1.8
0.2	3.4	1.6
0.1	2.0	1.3
0.05	1.5	1.1

The high-pressure liquid chromatography (LC) of fractions 1-15 was performed in accordance with the conditions given in the previous paper [4]. It was established that on columns 1 and 2 fractions 1 and 2 issued with retention times close to that of the WSPS-I, of dextran $2 \cdot 10^5$, and of amylopectin $2\text{-}4 \cdot 10^5$. This shows the high molecular weights of the fractions, in the interval of $2\text{-}5 \cdot 10^5$.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 415-418, July-August, 1986. Original article submitted February 28, 1986.

 0009-3130/86/2204-0386$12.50

TABLE 1. Characteristics and Monosaccharide Compositions of the Fractions of the WSPS from DEAE-cellulose (phosphate form)

Fraction	Yield, % (mg)	$[\alpha]_D^{23}$	$[\alpha]_{546}$	Monosaccharides in the form of polyol acetates, moles						
		degrees (c 0.5; water)		Rha	Fuc	Xyl	Man	Glc	Gal	UA
1	37,8 (0,620)	+76	+90			1,0		1,14	2,5	
2	15,2 (0 250)	+66	+76			1,0		1,0	1,8	
3	4,2 (0,070)	+46	+56			1,0		2,7	4,2	
4	1,8 (0,030)	+47[a]	+55			1,0		3 0	4,5	
5	4,8 (0,080)	+13	+16	Tr.	Tr.	1,0	Tr.	1,5	1,0	
6	6,1 (0.100)	+16	+20	(1,0)	(1,0)	1,0 (21,7)[b]		1,3 (23,0)	2,5 (55,0)	
7	3,1 (0,050)	+33	+42	Tr.	Tr.	1,0		1,0	2,0	
8	0,61 (0,010)	—	—	(1,4)	(1,0)	1,0 (18,0)	(1,6)	1,2 (23,3)	2,4 (43,7)	
9	6,10 (0,100)	—	—	(1,0)	(1,0)	1,0 (9,8)	(2,0)	1,2 (12,3)	2,1 (20,6)	
10	3,66 (0,060)	+27[b]	+30	(1,0)	Tr.	1,0 (27,0)	(2,3)	1,0 (26,3)	2,2 (60,0)	+
11	3 66 (0,060)	+21[c]	+25	Tr.	Tr.	1,0	Tr	2,5	6,3	+
12	6,10 (0,100)	+27[d]	+30	—	—	1,1 (9,2)	(1,0)	1,0 (8,0)	3,6 (29,0)	+
13	1,22 (0,020)	—	—	(1,0)	Tr.	1,4 (9,0)	(4,3)	1 0 (6,0)	5,5 (33,3)	+
14	2,44 (0.040)	+65[e]	+90	(4,4)	(1,0)	1,0 (28,0)	(4,5)	1,1 (31,3)	3 2 (91,0)	+
15	3,05 (0,050)	—	—	(2,4)	(1,0)	1,0 (6,2)	(3,6)	2,0 (12,0)	5,9 (37,0)	+

The ratios in parentheses are given with all the monosaccharides taken into account. a) c = 0.40%; b) c = 0.33%; c) c = 0.46%; d) c = 0.33%; e) c = 0.20%.

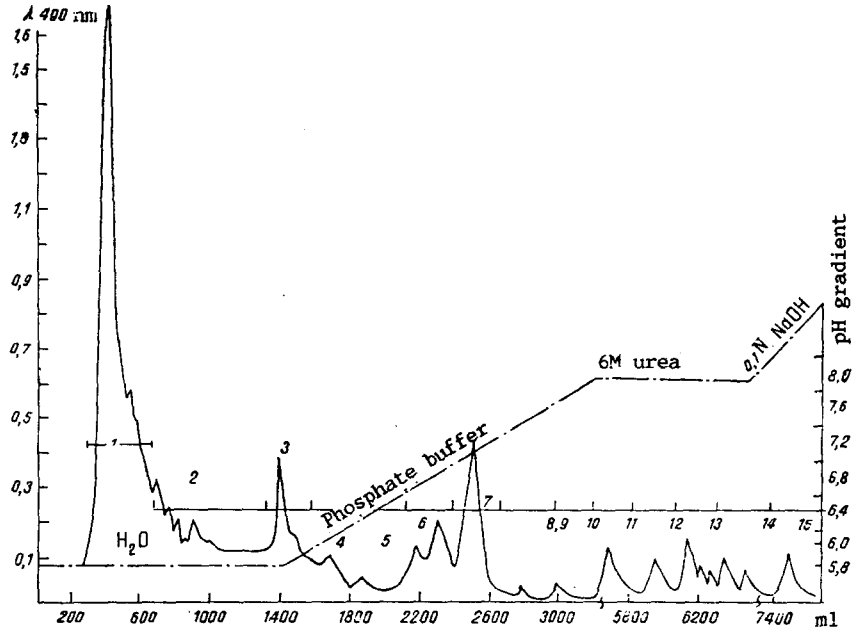

Fig. 1. Chromatography of WSPS-I on DEAE-cellulose (phosphate form, 5 × 60 cm, 20-ml fractions collected).

Sample	Column 1	Sample	Column 2
WSPS-I	59	WSPS-I	62
Fraction 1	56	Fraction 1	55
2	58	2	57
3–7	62	6	58
8	68	9	65
9–12	72	12	66
13–15	73		
T-500	73	T-500	67
T-250	77	T-2000	70
T-2000	61		
Amylopectin 2-4 million	59	Amylopectin 2-4 million	59

Analysis of the methylated fraction 1 showed approximately the same ratio of the monosaccharides as in the initial polysaccharide:

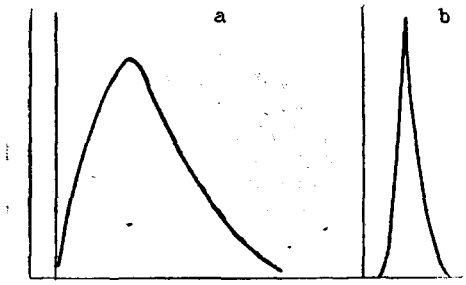

Fig. 2. Sedimentogram of WSPS-I (a) and of fraction 1 (b): a) $S = 10.3 \cdot 10^{-13}$, $D = 4.6 \cdot 10^{-7}$; b) $S = 7.6 \cdot 10^{-13}$, $D = 8.2 \cdot 10^{-7}$.

Xylose -1→	1,5—1,7
Galactose(1→	2,4
→4)-Galactose-(1→	2,7
→4)-Glucose -(1→	1,0
→4,6)-Glucose (1→	3,4—3,9

Thus, the polydispersity of WSPS-I has been established by fractionation on a column of DEAE-cellulose. According to the results of ultracentrifugation, fraction 1 was homogeneous and had a molecular weight greater than $2 \cdot 10^5$. The other fractions, with similar molecular weights, contained additional monosaccharides, according to the results of high-pressure LC. WSPS-I consists of several polysaccharide homologues differing little in composition and properties and having similar molecular weight.

EXPERIMENTAL

For conditions of chromatographic analysis and relative viscosity determinations, see [4].

Fractionation on DEAE-Cellulose. DEAE-Cellulose in the phosphate form was prepared as described in the literature [2, 3]. An aqueous solution of 2.0 g of WSPS-I was passed through a column (5 × 60 cm) filled with DEAE-cellulose and equilibrated with 0.1 M phosphate buffer (pH 5.8). Elution was carried out with water (2 × 400 ml; fractions 1 and 2); with 0.1 M phosphate buffer (pH gradient 5.8-8.0, 400 ml each, fractions 3-10); with 6 M urea solution (2 × 400 ml, fractions 11-14); and with 0.1 N caustic soda (2 × 400 ml, fraction 15). Fractions with a colume of 20 ml were collected and were analyzed by the phenol/sulfuric acid method on a FEK-56 photoelectric at λ 490 nm. Corresponding fractions were combined. All the solutions apart from the aqueous solutions were dialyzed against mains water and then against distilled water and were treated with KU-2 cation-exchange resin (H^+) and were precipitated from methanol (1:4). The precipitates were washed with ethanol, acetone, and ether, and were dried over P_2O_5. The specific rotations of the fractions and their qualitative and quantitative compositions and yields are given in Table 1. The acetates of the polyols obtained from hydrolysates of the fractions, and also the products of the hydrolysis of methylated fraction 1 were analyzed under the conditions given by Gould et al. [1].

SUMMARY

1. Fractionation of WSPS-I on DEAE-cellulose (phosphate form) has yielded 15 fractions.

2. Neutral fraction 1 was homogeneous according to the results of ultracentrifugation, high-pressure LC, and gel filtration of Sepharose-4B. Fractions 1 and 2 made up 53% of the weight of the initial WSPS-I.

LITERATURE CITED

1. S. E. Gould, D. A. Rees, and N. J. Wight, Biochem. J., 124, 47 (1971).
2. V. I. Sharkov and N. I. Kuibina, The Chemistry of the Hemicelluloses [in Russian], Moscow (1972), p. 47.
3. H. Naukom and W. Kuendig, in: Methods in Carbohydrate Chemsitry, Vol. V, R. L. Whistler, ed., Academic Press, New York (1965), pp. 14-17.
4. Kh. A. Arifkhodzhaev, Khim. Prir. Soedin., 410 (1986).