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127. Masashi Tomoda and Masako Kitamura: The Polysaccharide from *Lupinus luteus* Seed. II.*1 Purification and Properties of Two Polysaccharides.

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The polysaccharides extracted from the seed of ***Lupinus luteus*** were fractionated with the chromatography on DEAE-cellulose, and main two fractions obtained were purified by the gel filtration on Sephadex G-50 and G-100. The each of two polysaccharides so obtained was homogeneous on gel filtration and thin-layer electrophoresis. The component carbohydrates of both of them were D-galactose, L-arabinose, D-galacturonic acid, D-xylose and L-rhamnose, and the molar ratios of these sugars in the two polysaccharides were 15:10:6:2:2 in one, and 6:4:3:1:1 in the other.

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The chemical properties of the polysaccharides extracted from the seeds of *Lupinus luteus* were described in the previous paper.*1 D-Galactose, L-arabinose, D-xylose, L-rhamnose and D-galacturonic acid were identified as the component sugars of the polysaccharides by means of paper chromatography of hydrolysate and syntheses of derivatives, but in point of homogenity, the problem of the composition had not been solved. The polysaccharides have now been fractionated by the chromatography on DEAE-cellulose, then treated on Sephadex columns and two pure polysaccharides were obtained, and their properties are described in the present paper.

The extraction of polysaccharides from the seeds was, on the whole, similar to that described in the previous paper. The substance obtained was absorbed on a DEAE-cellulose column. Five fractions were eluted with water and further stepwise increments in the ionic strength of alkaline solvents (Fig. 1).

Fraction 1 was obtained from the eluate with water, fract. 2 with 0.01*M* borate buffer, fract. 3 with 0.05*M* borate buffer, fract. 4 with 0.1*N* sodium hydroxide solution and fract. 5 with 0.5*N* sodium hydroxide solution, after dialysis, concentration and dryness *in vacuo*. As shown in Table I, fract. 2, 3 and 5 were small amounts in both yields and of carbohydrates.

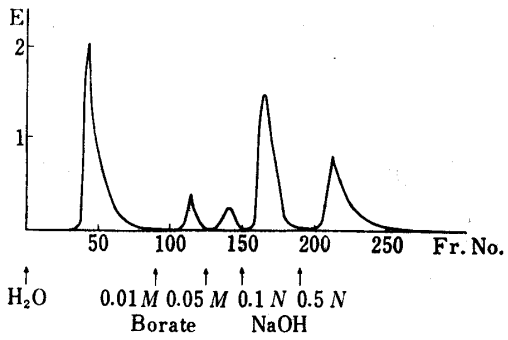

Fig. 1. Chromatogram on DEAE-cellulose

The water solution of fract. 1 was fractionated again on a Sephadex G-50 column and then, two more gel filtrations on a Sephadex G-100 column were carried out. Similarly, fract. 4 was treated with the gel filtrations on Sephadex columns. After these treatments on Sephadex G-50 and G-100, the two polysaccharides which are homogeneous on gel filtration with Sephadex G-200 separately were obtained (Fig. 2 and 3). The authors named provisionally them polysaccharide W, which is the substance obtained from fract. 1, and polysaccharide H, the substance from fract. 4.

Polysaccharides W and H have also been shown to be respectively homogeneous on thin-layer electrophoresis, and it was shown that the component sugars of the both

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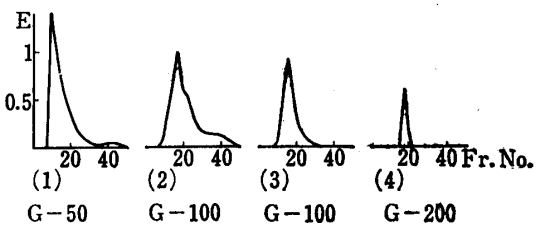

Fig. 2. Gel filtration of Frac. 1 on Sephadex

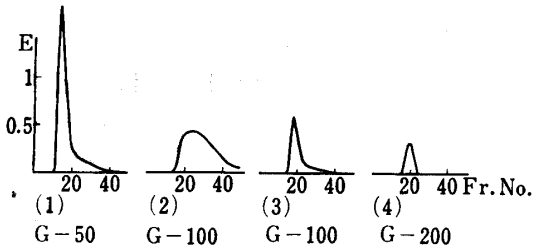

Fig. 3. Gel filtration of Frac. 4 on Sephadex

substances are D-galactose, L-arabinose, D-xylose, L-rhamnose and D-galacturonic acid, the carbohydrates which had been already identified as their derivatives*[1] by means of thin-layer chromatography of the hydrolysates.

They were obtained as white powders, and soluble in water, giving viscous solutions. It is presumed that their moieties of uronic acid are sodium salts. Specific rotations of them were as follows: "W," $[\alpha]_D^{20} = +38°$ (c=0.5, H_2O), and "H," $[\alpha]_D^{20} = +56°$ (c=0.5, H_2O). The molecular weights of the preparations gave values of 480,000 in "W" and 210,000 in "H" by the measurement of osmotic pressure. The results of quantitative determinations of sugar components of the pure polysaccharides and other fractions are given in Table I.

Table I. Quantitative Determination of Sugar Componets (%)

	Polysac. W	Frac. 2	Frac. 3	Polysac. H	Frac. 5
D-Galactose	48.0	3.1	7.5	43.8	15.0
L-Arabinose	24.7	1.3	6.3	23.0	3.7
D-Galacturonic acid	18.0			21.4	
D-Xylose	5.0			5.8	
L-Rhamnose	5.1			6.4	
(Glycerol)			(+)		

The presence of glycerol in the fract 3. was detected but the other constituents except carbohydrate in fract. 2, 3 and 5 have not been examined. The molar ratios of component carbohydrates of the two pure polysaccharides were as follows: D-galactose: L-arabinose: D-galacturonic acid: D-xylose: L-rhamnose were about 15 : 10 : 6 : 2 : 2 in "W" and 6 : 4 : 3 : 1 : 1 in "H." Owing to these results, it will be able to conclude that fundamental composition of the polysaccharide in *Lupinus luteus* seed is a chain consisting of D-galactose and L-arabinose in the ratio 3:2.

Experimental

Extraction of Polysaccharides——The seeds (500 g.) of *Lupinus luteus* were stripped of their skins and milled, then extracted with 10% aqueous sodium chloride solution (1.7 L.) by stirring and standing overnight, followed by filtration and repetition of the process three times. After suction filtration, the residue was stirred with 0.2% aqueous sodium hydroxide (1.8 L.) three times for 16 hr. at a time. And then, the residue was heated with 0.2% aqueous sodium hydroxide (1 L.) for 1 hr. in a boiling water bath, after which it was filtered and the filtrate poured into 4 volumes of methanol. The precipitate was filtered off, washed with ethanol and dried at 60° *in vacuo*. The resulting product was extracted with water (200 ml.), the solution was centrifuged to separate small insoluble material, then evaporated *in vacuo* to dryness. Yield, 2.5%. The product was a pale yellow colored powder.

Chromatography on DEAE-cellulose——DEAE-cellulose (Brown Co.) was purified by treatment with 0.5*N* sodium hydroxide until the washings showed no color by phenol-sulfuric acid method,[1] and the excess of sodium hydroxide was removed by repeated washing with distilled water. For the separation of 1 g. of the sample, a DEAE-cellulose column (5 × 30 cm.) was used, and fractions were collected (20 ml. each). The polysaccharides were eluted stepwise with water and by increasing the ionic strength of alkaline eluting solution, and determined by phenol-sulfuric acid method[1] (E at 490 mμ). Elution of the sample from a DEAE-cellulose column gave the five groups : fract. 1, tubes 38 to 74; fract. 2, tubes 110 to 122; fract. 3, tubes 132 to 148; fract. 4, tubes 158 to 180; fract. 5, tubes 206 to 236 as shown in Fig. 1. The yields were 0.52 g. in fract. 1, 0.03 g. in fract. 2, 0.04 g. in fract. 3, 0.26 g. in fract. 4 and 0.09 g. in fract. 5.

Gel Filtration on Sephadex Columns——Sephadex G-50, G-100 and G-200 (Pharmacia Co.) were stirred and decantated to remove fines and washed repeatedly with distilled water. Columns of Sephadex G-50 (4 × 42 cm.), Sephadex G-100 (4 × 52 cm.) and Sephadex G-200 (5 × 50 cm.) were used, and fractions were collected 20 ml. each for 1 hr. The polysaccharides in eluates were measured by phenol-sulfuric acid method[1] (E at 490 mμ). As the result of get filtration of fract. 1 on Sephadex G-50, the eluates obtained from tubes 8 to 24 in Fig. 2 (1) were collected and concentrated *in vacuo*, then re-separation of the polysaccharide fraction with Sephadex G-100 was carried out twice. The polysaccharide was obtained from tubes 10 to 22 in Fig. 2 (2) and then, from tubes 12 to 20 in Fig. 2 (3). The yield was 0.14 g. from 0.4 g. of starting fraction. Similarly, in the case of purification of fract. 4, the eluates obtained from tubes 12 to 23 in Fig. 3 (1), tubes 17 to 30 in Fig. 3 (2) and tubes 15 to 24 in Fig. 3 (3) were collected and concentrated *in vacuo*. The yield was 0.09 g. from 0.25 g. of starting fraction.

Thin-layer Electrophoresis——8 g. of Wakôgel B5 (Wakô-junyaku Co.) were mixed with 20 ml. of buffer solution and stirred well. For the other plates, 5 g. of Cellulose MN300 (Macherey, Nagel & Co.) were blended in a homogenizer for 2 min. with 30 ml. of buffer solution. Glass plates (10 × 20 cm.) were coated with 0.25 mm. thick layer by means of an applicator. Each thin layer plate was used after 1 hr. standing at room temperature. Electrophoresis was carried out with the following absorbent and buffers : A, Wakôgel B5 with pyridine-acetic acid buffer of pH 4.3 (pyridine : acetic acid : water, 1:2:97); B, Cellulose MN300 with pyridine-acetic acid buffer (the same as A); C, Cellulose MN300 with 0.1*M* borate buffer of pH 9.0. The reagents for detection made use of anisaldehyde-sulfuric acid[2] in case of A and periodate-permanganate[3] as to B and C. The conditions of electrophoresis were 200 volt for 2 hr. to A and B, and 1 hr. to C. The results are shown in Fig. 4.

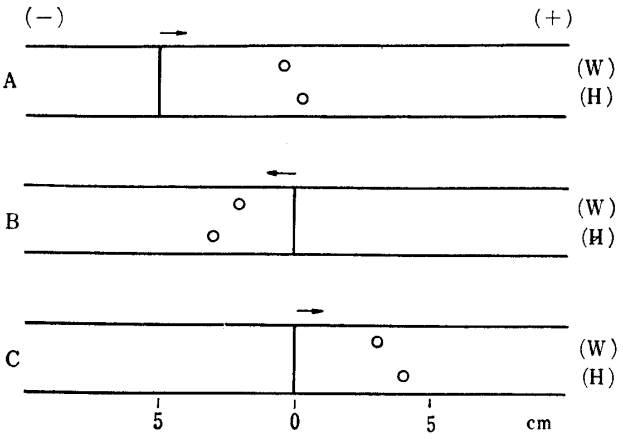

Fig. 4. Thin-layer Electrophoresis

Thin-layer Chromatography——The procedures of hydrolysis of polysaccharides with *N* sulfuric acid and neutralization with barium carbonate were similar to that described in the previous report and the hydrolysates were used as samples. 15 g. of Cellulose MN 300 were mixed with 90 ml. of water in a homogenizer and five glass plates (20 × 20 cm.) were coated with 0.25 mm. thick layer by the use of an applicator. The plates were dried for 10 min. at 105° and stored overnight at room temperature before use. The samples were spotted in line at 1.5 cm. from an edge and the solvents were allowed to ascend to a height of 10 cm. from starting point at 20°. The following solvent systems were used : A, BuOH : pyridine: HOAc : H_2O (10:6:1:3, by vol.); B, EtOAc : pyridine : H_2O (10:3:2, by vol.); C, PhOH : H_2O (5:1, by vol.). The sugars and glycerol spots were revealed with periodate-permanganate[3] and benzidine-acetic acid reagent.[4] Table II shows Rf values of sugars and glycerol in the three solvent systems.

Determination of Sugars——Galactose was determined by anthrone method.[5] Pentose was estimated by orcinol method.[6] Rhamnose was measured by thioglycolic acid method.[7] For the quantitative analysis of galacturonic acid, carbazole method[8] was employed. For the fractional determination of neutral sugar, the paper chromatography of hydrolysate was carried out with Tôyô-roshi No. 51 paper and a solvent

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Table II. Rf Values of Sugar Components

	Solvent A	Solvent B	Solvent C
(Glycerol)	0.72	0.84	0.82
L-Rhamnose	0.64	0.81	0.65
D-Xylose	0.48	0.61	0.48
L-Arabinose	0.40	0.46	0.57
D-Galactose	0.28	0.22	0.44
D-Galacturonic acid	0.07	0.03	0.22

system of BuOH : C_6H_6 : pyridine : H_2O (5:1:3:3, by vol., upper layer), and after end of development and dryness, the parts containing each sugar were cut out and extracted with water and each sugar in the extract was estimated colorimetrically.