POLYSACCHARIDES OF Matricaria chamomilla

I. MONOSACCHARIDE COMPOSITION OF THE POLYSACCHARIDE COMPLEX

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The inflorescences of Matricaria chamomilla L. (German camomile) have long been used as a raw material for the production of essential oils [1]. The other biologically active substances, including carbohydrates, have been studied inadequately.

We have investigated the monosaccharide composition of the water-soluble polysaccharide of this plant, which, according to the results of pharmacological tests performed in the laboratory of the pharmacology of physiologically active substances of KhNIKhFI [Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry] (G. V. Obolentseva) possess pronounced anti-inflammatory, emollient, and enveloping properties.

The nondemineralized polysaccharide was hydrolyzed with 1 N sulfuric acid, and the hydrolyzate was chromatographed on paper in several solvent systems. It was found that the polysaccharide contains seven monosaccharide components (Table 1).

The polysaccharide was demineralized by treatment with KU-2 cation-exchange resin (H^+) and AV-17 anion-exchange resin (HCO_3^-). A gray-white powder was obtained which had the properties of an organic acid with pH 3.35. The amount of galacturonic anhydride in it was 45% by potentiometric titration [2]. Since the polysaccharide complex contains a considerable amount of galacturonic acid, we used extraction with a mixture of 0.25% solutions of ammonium oxalate and oxalic acid (equal volumes) as proposed by Bishop for the isolation of acids of the glycuronoglycan type of pectins [3]. A polysaccharide differing only very slightly in its content of uronic and hydride (51.3%) was obtained.

To establish the quantitative ratio of the neutral sugars present in this polysaccharide and to investigate the optimum conditions for their isolation, we studied the hydrolysis of a sample to monosaccharides by taking fractions of the substance that had remained unchanged after definite intervals of time. The fractions were purified and were then decomposed with acid. The resulting hydrolyzates were chromatographed (see Table 1). The figures in the table show that the splitting off of the neutral sugars of the polysaccharide was complete between the 8th and 9th hour.

TABLE 1

Monosaccharide	R in relation to rhamnose	Composition of the monosaccharides on stepwise hydrolysis of the polysaccharides, time, hours					
		2	4	6	8	9	10–24
Galacturonic acid	0,95	+	+	+	+	+	+
Galactose	0,42	+	+	+	—	—	—
Glucose	0,50	+	+	—	—	—	—
Arabinose	0,61	+	+	—	—	—	—
Xylose	0,72	+	+	—	—	—	—
Rhamnose	1,00	+	+	+	—	—	—
Substance VII	1,13	+	—	—	—	—	—

Note. The R values for the sugars were obtained in system 1.

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TABLE 2

Value of R in relation to galacturonic acid for the oligouronides investigated	Quantitative composition of the oligouronides in the hydrolyzates as a function of the time of hydrolysis, time, h				
	8	12	16	20	24
0,19	+	+	—	—	—
0,39	+	+	+	—	—
0,80	+	+	+	+	—
1,00	+	+	+	+	+

Note. The R values for the investigated compounds were obtained in system 3.

The products of partial hydrolysis after treatment with 1 N sulfuric acid for 8 h were found to contain galacturonic acid and oligouronides (Table 2). In the nature of the dynamics of hydrolysis (see Table 2), the polysaccharide complex of the camomile is hydrolyzed by a strong mineral acid in the same way as known pectins [4].

The amounts of neutral monosaccharides in the hydrolyzate of the polysaccharide were determined by the method of G. N. Zaitseva and T. G. Afanas'eva [5] (Table 3).

To isolate and identify the individual monosaccharides, the determined complex of the polysaccharides was hydrolyzed with sulfuric acid, the reaction mixture was neutralized with barium carbonate, and the barium salts of the galacturonides were separated off and galacturonic acid was then obtained from them. The neutral hydrolysis products were separated on a column containing a powder of degraded cellulose using water-saturated butan-1-ol as eluting solvent [6]. The physicochemical properties of the monosaccharides isolated are shown in Table 3.

Substance (VII) is present in the hydrolyzate in the form of traces (0.25%) and its R value referred to rhamnose is 1.13 (system 1).

For a strict identification of the uronic acid, the polysaccharide was partially hydrolyzed with 1 N sulfuric acid. From the hydrolyzate was isolated the product of partial hydrolysis – degraded polyuronic acid – and this was converted into the methyl ester and reduced with sodium tetrahydroborate [7]. The degraded polyuronic acid was converted into an almost neutral glycan. The latter was hydrolyzed with 1 N sulfuric acid, and galactose, a uronic acid, and an aldobiuronic acid were detected by chromatography. The action of concentrated nitric acid on the mixture of these acids [8] gave crystals of mucic acid (see Table 3). After the appropriate working up, crystals of D-galactose formed from the D-galacturonic acid were isolated from the hydrolyzate.

Thus, the results of a chemical investigation of the polysaccharides of the inflorescences of the German camomile have shown that they contain seven monosaccharides, D-galacturonic acid being the main component.

EXPERIMENTAL

The polysaccharides were extracted from a standard raw material corresponding to the requirements of the State Pharmacopeia of the USSR (X-th ed., Moscow, 1968). They were chromatographed on paper in the following solvent systems: 1) butan-1-ol–pyridine–water (6:4:3); 2) butan-1-ol–ethanol–water (4:1:5); 3) ethyl acetate–formic acid–water–acetic acid (18:1:4:3), and 4) butan-1-ol–acetic acid–water (4:1:5). Paper of type "M" ["slow"] from the Volodarskii Leningrad mill No. 2 was used. The optical activities of the carbohydrates were determined on a SPU-M spectropolarimeter.

The polysaccharides were dried in vacuum (12 h, residual pressure 0.03 mm Hg, 60-65°C). The ash content was determined on combustion as samples of the polysaccharides in a muffle furnace at 600°C.

Isolation of the Polysaccharides. The comminuted camomile flowers were extracted with ethanol containing 5% of ether (1:15) for 2 h. Purification was performed twice.

TABLE 3

Component	Amount, %	mp, °C	$[\alpha]_D$, deg	Sugar derivatives	mp's of the derivatives, °C
Galacturonic acid	45	154–156	–	D-Galactose	163–164
				Mucic acid	108–110
Galactose (II)	12,2	164–165	+83,0		110–111
Glucose (III)	2,3	144–145	+52,7	Osotriazole	194–196
Arabinose (IV)	10,2	157–159	+104,0		68–70
Xylose (V)	20,8	143–145	+93,6	Benzimidazole	221–223
Rhamnose (VI)	5,3	90–92	+8,1	Osazone	179–181
Substance (VII)	0,25	—	—	—	—

A. The polysaccharides were extracted with hot water (1:20) for 1.5 h. The extract was filtered and treated with 96% ethanol (1.5 volumes). The precipitate of polysaccharides was separated off and was washed with ethanol and acetone. Yield 5% on the weight of the raw material; ash content 25%.

B. The raw material was extracted with a mixture of equal volumes of 0.25% solutions of ammonium oxalate and oxalic acid (1:20). The remaining operations were similar to those in method A. The yield of polysaccharide was 5.5%.

Preliminary Investigation of the Products of Acid Hydrolysis of the Polysaccharide. A solution of 1 g of the nondemineralized polysaccharide in 50 ml of 1 N sulfuric acid was heated in the boiling water bath for 8 h. The hydrolyzate was neutralized with barium carbonate, filtered, and evaporated in vacuum to 2 ml. The hydrolysis products were chromatographed on paper in systems 1, 2, and 4 which led to the detection of galacturonic acid, galactose, glucose, arabinose, xylose, and rhamnose and an unidentified monosaccharide (VII) (see Table 1).

The monosaccharide compositions of the water-soluble polysaccharide and that obtained by extraction with 0.25% solutions of ammonium oxalate and oxalic acid were the same.

Preparation of the Demineralized Polysaccharide. A solution of 10 g of the nondemineralized polysaccharide in 100 ml of water was passed successively through columns (20× 180 mm) of KU-2 cation-exchange resin (H^+) and AV-17 anion-exchange resin (HCO_3^-). The material was eluted with water. The polysaccharides were precipitated with ethanol (four volumes). The precipitates were centrifuged and were washed with ethanol and acetone. Yield 4 g, ash content 0.5%, $[\alpha]_D^{20°}$ +158° (c 0.2%; water), amount of galacturonic acid 45% according to potentiometric titration and amount of methoxy groups 3.8% by the Zeisel method [9].

Stepwise Hydrolysis of the Polysaccharide. A. A solution of 4 g of the polysaccharide in 200 ml of 1 N sulfuric acid was heated in the boiling water bath for 2 h. The hydrolyzate was neutralized with barium carbonate and filtered. The filtrate was evaporated to 20 ml, the products of partial hydrolysis were precipitated with 100 ml of ethanol, and the precipitate was separated off. Then the filtrate was evaporated to small volume and chromatographed. The residue, consisting of the products of the partial hydrolysis of the polysaccharides and the barium salts of the galacturonic acid, was thrice reprecipitated with ethanol and was used for rehydrolysis. These operations were repeated each 2-4 h (see Table 1).

B. Four 0.1-g samples of polysaccharide were hydrolyzed as described above for 8, 12, 16, 20, and 24 h. The precipitates, consisting of the products of partial hydrolysis of the polysaccharide and the barium salts of the galacturonic acid, were separated off and were reprecipitated with ethanol. The purified precipitates were each dissolved in 5 ml of water and the solution was treated with KU-2 resin (H^+) and filtered. The filtrates were evaporated and were chromatographed on paper in system 3 (see Table 2).

Quantiative Determination of the Neutral Monosaccharides. A solution of 0.2 g of the polysaccharide in 10 ml of 1 N sulfuric acid was hydrolyzed for 9 h as described previously. The hydrolyzate was neutralized with barium carbonate and filtered. The filtrate was evaporated to 2 ml and precipitated with 20 ml of 96% ethanol. The precipitate of barium salts of oligouronides that had formed was filtered off, washed with 80% ethanol, and twice reprecipitated with ethanol from aqueous solution. The aqueous ethanolic filtrates were combined and evaporated in vacuum to dryness. The dry residue was transferred to a 5-ml measuring flask and water was added up to the mark. This solution was used for the quantitative determination of the neutral monosaccharides. The monosaccharides were separated in system 1 (see Table 3).

Isolation of the Monosaccharides. The demineralized polysaccharide (10 g) was hydrolyzed with 1 N sulfuric acid as described above. The barium salts of galacturonic acid and of the oligouronides were used to isolate the galacturonic acid, and the combined neutral monosaccharides (3 g) were separated on a column filled with a powder of degraded cellulose.

Isolation of Galacturonic Acid. The barium salts of the galacturonides (2.5 g) were hydrolyzed with 1 N sulfuric acid (1:50) in the boiling water bath for 20 h. The hydrolyzate was neutralized with barium carbonate and was filtered, and the filtrate was evaporated to 12 ml. The barium salt of the galacturonic acid was precipitated with ethanol and was twice reprecipitated from aqueous solution and was then dried. The residue (2 g) was dissolved in 50 ml of water and the solution was passed through a column of KU-2 cation-exchange resin (H^+). The eluates were evaporated to dryness and dissolved in 2 ml of ethanol. The galacturonic acid that separated out from the ethanolic solution was recrystallized from water (see Table 3). The oxidation of this substance with concentrated nitric acid gave mucic acid with mp 208-210°C.

Separation of the Neutral Sugars. Mixtures of the neutral sugars (3 g in each case) were each dissolved in 3 ml of water and deposited on a column containing a powder of degraded cellulose (40× 900 mm). The sugars were separated by elution with water-saturated butan-1-ol. Fractions of 100 ml were collected and were evaporated to dryness, and the qualitative composition of the monosaccharides in them was analyzed by paper chromatography in system 1. Fractions containing mixtures of sugars were used for reseparation under similar conditions (the properties of the monosaccharides isolated and their derivatives are given in Table 3).

Identification of the Uronic Acid in the Form of the Corresponding Hexose. A. Isolation of a Polysaccharidic Acid. The polysaccharide (20 g) was hydrolyzed with 1 N sulfuric acid for 9 h. The precipitate was separated from the liquid by centrifuging and it was washed with 1% sulfuric acid, water, ethanol, and acetone.

B. Esterification of the Polysaccharidic Acid. The acid (8 g) was esterified in 200 ml of a 1 M solution of sulfuric acid in methanol at +2°C for 16 days with periodic stirring. The esterified acid was filtered off and washed with ethanol until the reaction for sulfate ion was negative and was then washed with ether and dried at room temperature. Yield 7.2 g, $[\alpha]_D$ +205°; proportion of $-OCH_3$ groups 14.6%.

C. Reduction of the Esterified Acid. A suspension of 7.2 g of the acid in 180 ml of 80% aqueous ethanol was treated with 3 g of sodium tetrahydroborate with constant stirring. The mixture was left at room temperature for 12 h, and the polysaccharides were filtered off and were washed with acidified methanol and then with methanol until free from chlorides, and, finally, with ether. Yield 4 g.

D. Isolation of D-Galactose. The glycan (4 g) was hydrolyzed with 1 N sulfuric acid as described above for 4 h. The hydrolyzate was neutralized with barium carbonate. Then it was evaporated to small volume and the barium salts of the acidic sugars were precipitated with ethanol and filtered off. On oxidation with concentrated nitric acid, the filtrate gave mucic acid (see Table 3). The filtrate contained only D-galactose, which was crystallized from ethanol, $[\alpha]_D$ +83° (c 0.2%; water), mp 163-164°C.

CONCLUSIONS

1. A polysaccharide has been isolated from the inflorescences of Matricaria chamomilla L. It has been established that it is composed of D-galacturonic acid (45%), D-galactose (12.2%), D-glucose (2.3%), L-rhamnose (5.3%), L-arabinose (10.2%), and D-xylose (20.8%).

2. The information obtained permits the polysaccharide of the camomile to be assigned to the class of pectin substances.

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