Chemistry of Natural Compounds, Vol. 33, No. 6, 1997

PLANT POLYSACCHARIDES*
VII. POLYSACCHARIDES OF *Morus* AND THEIR HYPOGLYCEMIC ACTIVITY

M. Kh. Sanavova[a] and D. A. Rakhimov[b]

UDC 547.917:615.015.31:514.64

Water-soluble polysaccharides, pectin substances, and hemicelluloses have been isolated from Morus *leaves, and their qualitative and quantitative compositions have been determined. The polysaccharides obtained by extraction with hot water have been investigated for hypoglycemic activity. A polysaccharide from* Morus alba *proved to have the highest sugar-lowering activity.*

In the Republic of Uzbekistan the genus *Morus* (fam. Moraceae) is represented by five cultivated species [1, 2]. There are reports of a study of the water-soluble polysaccharides of *Morus rubra* [3]. It has been shown that under the conditions of experimental hyperglycemia, tinctures of the leaves of some mulberry species possess a sugar-lowering effect [4-6].

With the aim of revealing the active principle, we have studied the carbohydrates of the leaves of the white mulberry *Morus alba* gathered in August in the Botanical Garden of the Uzbekistan Academy of Sciences.

The air-dry raw material was comminuted and treated with methanol to eliminate low-molecular-mass compounds. To extract the polysaccharides, the plant residue was treated successively with water, oxalate buffer, and caustic soda. The polysaccharides were precipitated from the extract with ethanol. As a result, from the aqueous extract we isolated the water-soluble polysaccharides (WSPSs), from the buffer solution the pectin substances (PcSs), and from the alkaline extract the hemicelluloses (HMCs-A and -B). The qualitative and quantitative compositions of the polysaccharides isolated were established after complete acid hydrolysis with the aid of paper chromatography (PC) and the gas-liquid chromatography (GLC) of aldononitrile acetate derivatives. The monosaccharide compositions of the polysaccharides are given in Table 1.

The quantitatively predominating polysaccharides in the leaves were the PcSs. Samples of the polysaccharides gave no color reaction with iodine, which showed the absence of a glucan of the starch type. As can be seen from Table 1, all the polysaccharides isolated had practically the same qualitative monosaccharide composition and differed by the ratios of the component monosaccharides. In the products of the hydrolysis of the WSPSs we detected rhamnose, arabinose, mannose, xylose, galactose and (predominantly) glucose. The pectin isolated was a cream-colored water-soluble powder possessing a high positive rotation. Its composition included neutral sugars and galacturonic acid, identified by electrophoresis. It proportion was 53.6%.

The alkali-soluble polysaccharides (HMCs-A and -B, total yield 4.5%) differed by their monosaccharide components: the HMCs-A contained mainly galactose and xylose, and the HMCs-B glucose and rhamnose.

Preliminary tests for hypoglycemic activity of the PcSs, the HMCs, and the WSPSs obtained from *Morus alba* by cold and hot extraction showed that the greatest sugar-lowering effect was possessed by the hot-water-extracted polysaccharide. In view of this, we made a detailed study of the above-mentioned activity of the polysaccharides obtained from three *Morus* species by extraction with water at 95°C, the general characteristics of which are given in Table 2.

*Materials presented at the IInd International Symposium of the Chemistry of Natural Compounds.

a) Institute of Endocrinology, Academy of Sciences of the Republic of Uzbekistan, Tashkent. b) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 789-791, November-December, 1997. Original article submitted January 20, 1997.

0009-3130/97/3306-0617$18.00

TABLE 1. Amounts and Monosaccharide Composition of the Polysaccharides Isolated from the Leaves of *Morus alba*

Type of carbohydrate	Yield, %	Ratio of the sugars					
		Gal	Glc	Man	Xyl	Ara	Rham
WSPSs	4.6	6.0	9.7	1.3	1.0	4.4	5.3
PcSs	7.8	12.6	17.4	1.6	1.0	5.9	4.2
HMCs-A	1.7	13.9	4.9	2.0	5.7	1.1	1.0
HMCs-B	2.8	16.5	23.6	1.0	16.1	13.3	20.6

TABLE 2. Amounts and Compositions of Hydrolysates of the Polysaccharides Obtained by Hot Extraction from *Morus*

Plant	Yield, %	Ratio of the monosaccharides					
		Gal	Glc	Man	Xyl	Ara	Rham
M.alba L.	6.1	7.1	7.1	1.0	1.3	6.2	10.7
M.nigra L.	3.6	5.0	5.0	1.0	–	3.1	10.9
M.rubra	2.4	4.3	4.6	1.0	1.7	3.3	13.8

TABLE 3. Action of Polysaccharides Isolated from Three *Morus* Species on Blood Glucose (100 mg/kg) in Alimentary Hyperglycemia

Variant	Glucose level in the blood, mmoles/liter		
	M.alba L.	M.nigra L.	M.rubra
Control	7.18±0.70 (12)	8.49±0.78 (9)	7.87±0.56 (9)
Experiment	5.22±0.65 (12)	7.00±0.40 (16)	7.68±0.32 (12)
Hypoglycemic effect, %	27.30	17.55	0

*The numbers of animals (rats) are given in parentheses.

The hydrolysis products of the polysaccharides included galactose, glucose, mannose, xylose, arabinose, and rhamnose. The last-mentioned was the main component.

It was established that the polysaccharide from *M. alba* was more effective than a control (Table 3) and was only slightly inferior in its effect to the widely known synthetic peroral drugs adebit and maninil.

Thus, a study of the carbohydrates of plants used in folk medicine is revealing promising sources of antidiabetic drugs.

EXPERIMENTAL

Descending paper chromatography of the monosaccharides was conducted on Filtrak 11; 3 paper in the butan-1-ol–pyridine–water (6:4:3 by volume) system. Acid aniline phthalate was used to indicate the spots. The GLC of the samples was conducted on a Chrom-5 instrument with a glass column (0.4 × 120 cm) filled with Chromaton (0.16-0.20 mm) bearing 5% of XE-60 at 200°C and a rate of flow of nitrogen of 60 ml/min. Flame-ionization detector. The monosaccharides were analyzed in the form of aldononitriles [7]. The amounts of sugars were determined from chromatographic peak areas. Optical rotations were determined on a Coers polarimeter, l = 1 dm at 20°C.

Isolation of the Polysaccharides. The WSPSs, PcSs, and HCSs-A and -B were extracted successively from 100 g of air-dry raw material as described in [8]. For the hot-extraction method of isolating polysaccharides, the raw material was covered with water (1:10, by weight) and heated on the water bath at 95°C for 2.5 h. The extract was separated by filtration and was concentrated by evaporation in a rotary evaporator to 0.5 volume, and the polysaccharides were precipitated with ethanol (1:2, by volume). The precipitate was filtered off and, for dehydration, it was washed with alcohol, 80 → 96°.

The acid hydrolysis of the polysaccharides (100 mg each) was performed with 2 N H_2SO_4 at 100°C: WSPSs for 8 h, and PcSs and HMCs for 48 h, followed by neutralization with $BaCO_3$. The hydrolysates were analyzed by PC and GLC.

Alimentary hyperglycemia was evoked by the administration of glucose in a dose of 9 g/kg 1 h before glucose analysis after the action of the preparation and was compared with a control. The preparation was administered *per os* in a dose of 100 mg/kg body weight of the animals. Glucose was determined by the *o*-toluidine method [9].

Electrophoresis of the uronic acids was conducted on FN-7 paper in 1% acetic acid (110 V, 7 mA, 4 h).

REFERENCES

1. Flora of Uzbekistan [in Russian], Tashkent, Vol 11 (1953), p. 82.
2. Kh. Kh. Kholmatov, Medicinal Wild Plants of Uzbekistan [in Russian], Meditsina, Tashkent (1964), p. 39.
3. A. O. Arifkhodzhaev, Khim. Prir. Soedin., 62 (1955).
4. S. M. Kit and I. S. Turchin, Medicinal Plants in Endocrinology [in Russian] (1986), p. 80
5. B. R. Khozhdaev, Kh. Kh. Kholmatov, and I. A. Kharlamov, An Experimental Study of Individual Plants Used in Folk Medicine, in: Abstracts of Lectures at an All-Union Conference [in Russian], Tomsk (1989) No. 2, p. 186.
6. V. A. Blinov, Medicinal Plants in Sugar Diabetes [in Russian], Ibn Sina, Tashkent (1933), p. 18.
7. Yu. S. Ovodov, The Gas-liquid Chromatography of Carbohydrates [in Russian], Vladivostok (1970).
8. M. Kh. Malikova and D. A. Rakhimov, Khim. Prir. Soedin., 21 (1986).
9. A. Hyvarinen and E. A. Nikkila, Clin. Chim. Acta, 7, No. 1, 140 (1962).