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BIOLOGICALLY ACTIVE SUBSTANCES FROM *Cacalia hastata* LEAVES. 1. CARBOHYDRATES FROM LEAVES AND THEIR HYPOGLYCEMIC ACTIVITY

D. N. Olennikov, L. M. Tankhaeva, G. G. Nikolaeva,
A. V. Tsyrenzhapov, S. M. Nikolaev, and G. V. Chekhirova

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Leaves of Cacalia hastata *L. (Asteraceae) were composed of free sugars, water-soluble polysaccharides (arabinogalactan type), pectinic substances, hemicelluloses, and cellulose. The hypoglycemic activity was determined for the water-soluble polysaccharides and pectinic substances.*

Key words: *Cacalia hastata* L., Asteraceae, water-soluble polysaccharides, cacalans A, B, and C, pectinic substances, hemicelluloses, hypoglycemic effect.

Adding cellulose or gel-forming nonabsorbing plant carbohydrates such as gums and pectinic substances (PS) to food decreases the absorption of glucose and increases less drastically the blood sugar after meals in patients with sugar diabetes. This decreases the demand for insulin and other hypoglycemic agents [1-3].

Cacalia decomposita A. Gray (Asteraceae), so-called matarik or maturin, is used to cure diabetes [4]. The species *C. hastata* L. is widely distributed to the east of Lake Baikal and is used as a wound-healing substance [5]. Extracts of its leaves were verified to have hypoglycemic activity. The chemical composition of *C. hastata* L. leaves is variable and includes various classes of biologically active substances: organic acids, tanning agents, carotinoids, ascorbic acid, phenolic acids, triterpenes, alkaloids, coumarins, etc. [6, 7].

Polysaccharides in *C. hastata* leaves are present at 29.89% of the raw mass. This made a more detailed chemical investigation of the carbohydrate components necessary.

Polysaccharides were isolated by the Kiesel method [8]. Fractionation produced alcohol-soluble carbohydrates (ASC), water-soluble polysaccharides that were soluble in cold (WSPSC) and hot (WSPSH) water, pectinic substances (PS) that were isolated by an oxalate mixture, and hemicelluloses A and B (HCA and HCB) from alkaline extraction. The tissue (pulp) remaining after isolation of all substances extracted by one method or another was arbitrarily called cellulose (C). However, it was obvious that it contained also a small quantity of difficultly extracted hemicellulose.

The following physicochemical properties of the polysaccharides were determined: optical rotation, pH of a 0.1% aqueous solution (except HCA, which is insoluble in water), and ash before and after demineralization (Table 1).

It should be noted that the isolated fractions did not give a color with iodine solution, which is consistent with the lack of glucan-type starch in *C. hastata* leaves. Nitrogen (Lassen sample) also was not observed. The monosaccharide components of the polysaccharides were investigated and the purity of the polysaccharides was checked using acidic and enzymic hydrolysis with subsequent chromatography of the hydrolysates on paper and over a thin layer of silica gel (Table 2).

Three free carbohydrates, fructose, glucose, and galactose, were shown to be present in *C. hastata* leaves using chromatography. The total content of free sugars was 2.5% of the raw mass.

The carbohydrate components isolated by hot and cold water composed about 20% of the total leaf polysaccharides.

Institute of General and Experimental Biology, Siberian Division, Russian Academy of Sciences, Ulan-Ude, fax (3012) 43 30 34. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 3-6, January-February, 2004. Original article submitted September 22, 2003.

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TABLE 1. Physicochemical Properties of Polysaccharides from *C. hastata* Leaves

Fraction	Content, % of raw mass	$[\alpha]_D^{20}$, deg (c 0.1, H_2O)	Ash		pH (c 0.1, H_2O)
			before demineralization	after demineralization	
WSPSC	3.93	+80	20.4	3.6	5.9
WSPSH	2.16	+110	18.4	4.8	5.6
PS	10.10	+190	10.7	2.1	3.8
HCA	9.00	-43 (c 0.1, NaOH)	5.9	1.3	-
HCB	4.70	-31	4.0	1.2	6.0

TABLE 2. Quantitative Monosaccharide Content (mol %) of Polysaccharides in *C. hastata* Leaves

Fraction	Ara	Fru	Gal	GalUA	Glc	Rha	Xyl
ASC	-	+	+	-	+	-	-
WSPSC (cacalan A)	37.80	3.61	43.47	-	15.12	-	-
WSPSH 1 (cacalan B)	56.00	-	44.00	-	-	-	-
WSPSH 2 (cacalan C)	62.23	-	37.77	-	-	-	-
PS	12.54	-	3.42	79.70	3.15	1.20	-
HCA	-	43.75	-	-	37.50	-	18.75
HCB	-	24.40	-	-	12.16	-	63.44
C	-	-	-	-	100.00	-	-

WSPSC are a pale brown powder with 3.93% yield of raw mass (13.15% of total polysaccharide content). Gel chromatography over a Sephadex G-75 column showed that the WSPSC are homogeneous. The homogeneity of the WSPSC was confirmed by fractional precipitation with ethanol from aqueous solution, which produced a single fraction, like fractionation with sodium acetate. The precipitate formed all at once. The precipitates were chromatographed over Sephadex G-75. They gave a single peak with identical retention times, indicative of the homogeneity and authenticity. This was confirmed after determining the qualitative and quantitative compositions of the substances, the molecular weight, the optical rotation, and the pH. We called the isolated polysaccharide cacalan A. The molecular weight was about 5000; the Gal:Ara:Glc:Frc content, 43.47:37.80:15.12:3.61.

The WSPSH are a pale gray powder with 2.16% yield of raw mass (7.23% of total polysaccharides). The homogeneity of polysaccharides isolated by hot water was determined after fractionation, as described above. Alcohol fractionation produced two precipitates, work up of which with sodium acetate caused the precipitate to form all at once. Analysis of the elution profiles showed that the total WSPSH fraction was a mixture of two components, the retention times of which coincided with that of the precipitates. The isolated substances were called cacalan B and cacalan C. Their molecular weights were about 2500 and 3200, respectively. The hydrolysates of both substances contained only galactose and arabinose in 1:2.7 and 1:1.65 ratios, respectively.

Thus, WSPS of *C. hastata* leaves are arabinogalactans.

The content of polysaccharides isolated by an oxalate mixture was 33.79% of the total polysaccharides (10.10% of the raw mass). They gave a positive Erlich reaction with galacturonic acid. The fraction behaved as a homogeneous product upon reprecipitation with calcium polyuronate. Gel chromatography also showed that the PS were a pure substance. According to quantitative analysis of the hydrolysate, neutral sugars made up 20.3% and consisted of arabinose, galactose, glucose, and rhamnose in a molar ratio 10:3:2.5:1. The content of galacturonic acid in the PS sample reached 79.7%. Partial acidic hydrolysis of PS isolated polygalacturonan in 54.79% yield with $[\alpha]_D^{20}$ +200° (c 0.1, H_2O). The products of enzymatic hydrolysis of polygalacturonan contained only galacturonic acid. Galacturonic acid was produced after enzymatic hydrolysis by the Melnik—Arasimovich method. Potentiotitrimetry determined certain PS properties such as free carboxylic groups (K_C) of 12.89%; esterfied carboxylic groups (K_E), 14.73%; acetylated carboxylic groups (K_A), 4.21%; and methoxylated carboxylic groups (K_M), 10.52%. The total content of carboxylic groups (K_T) was 27.62%; of methoxyls (K_m), 7.25%; degree of pectin esterification (λ), 53.33%; galacturonic acid (K_{GalUA}), 79.7%; molar mass of equivalent (E), 218 g/mol; characteristic viscosity of PS (η), 3.603; molecular weight of PS, 13900.

TABLE 3. Activities of WSPS and PS on Blood Glucose Content with Alimentary Hyperglycemia

Expt. conditions		Glucose, mmol/L	Hypoglycemic effect, %
Control		0.16±0.02	
Arfazetin, 100 mg/kg		0.14±0.03	+23
WSPSC, mg/kg	50	0.11±0.05	+31
	100	0.12±0.02	+30
	150	0.13±0.02	+29
WSPSH, mg/kg	50	0.09±0.04	+37
	100	0.11±0.01	+33
	150	0.10±0.03	+30
PS, mg/kg	50	0.075±0.050	+33
	100	0.100±0.020	+37
	150	0.065±0.002	+39

The products of enzymatic hydrolysis contained Ara, Gal, Glc, and Rha in a molar ratio 10:3:2.5:1. The total degree of PS esterfication was greater than 50%, which classifies them as highly esterified pectins.

HC in *C. hastata* leaves makes up 45.83% of the total polysaccharides (13.70% of the raw mass). HCA is a brown powder that is soluble in alkalis and insoluble in water, acids, and organic solvents. The HCA content reached 66% of the total amount of alkali-soluble polysaccharides. Analysis of the acidic hydrolysate found Fru, Glc, and Xyl in a molar ratio 7:6:3. HCA belongs to xyloglucofructans.

HCB is a grayish-brown powder that is soluble in water and insoluble in organic solvents. The products of acidic hydrolysis include Fru, Glc, and Xyl in a molar ratio 10:5:26. HCB of *C. hastata* leaves is a xylan.

The remainder after extraction of all polysaccharide fractions, as mentioned above, was considered to be cellulose. The cellulose was hydrolyzed in HNO_3 (2 N). Chromatography showed that the hydrolysate contained only glucose, which is consistent with the absence of HC in the sample.

WSPS and PS were studied to determine the hypoglycemic activity. Table 3 contains the comparative properties and pharmacological activities of these.

It was found that WSPSH are more effective and exceed the analogous activity of a preparation compared with the control by 14% at a dose of 50 mg/kg. The WSPSC also have hypoglycemic activity at a dose of 50 mg/kg that is 31% greater than the control. The blood glucose level decreased to 39% during the investigation of *C. hastata* PS for hypoglycemic activity.

EXPERIMENTAL

One-dimensional paper chromatography was performed in descending mode on FN-12 paper in 80% isopropanol; two-dimensional TLC, on Silufol UV_{254} plates using ethylacetate:acetic acid:water (2:2:1) and butanol:acetic acid:diethylether:water (9:6:3:1). Anilinium oxalate was used as developer. Fructose was developed using resorcinol phosphate. Optical rotations were measured on a Coers polarimeter of 10-cm length at 20°C.

Leaves of *C. hastata* L. were collected in July 2002 in Mukhorshibirsk region (Buryatiya).

Isolation of Polysaccharides. Polysaccharides were fractionated by the Kiesel scheme. Ground leaves were defatted with hexane, benzene, and chloroform in a Soxhlet extractor. Free sugars (ASC) were removed by treatment with 80% ethanol and heating. The defatted raw material was thoroughly dried and treated successively with cold (modulus 30, WSPSC) and hot water (80°C, modulus 30, WSPSH), a mixture of aqueous oxalic acid (0.5%) and ammonium oxalate (1:1) (80°C, modulus 40, PS), and aqueous KOH (10%, 40°C, modulus 50). The extracts obtained in each step were combined separately (five extracts) and evaporated to 50-100 mL. Polysaccharides after alkaline extraction were precipitated by 96% ethanol (1:5) and left to form the precipitate for 12-16 h. The precipitates were filtered, washed with ethanol and acetone, and purified by reprecipitation three times from water. The extract obtained by treatment of the raw material with KOH was acidified with acetic acid (80%) until the pH was ~4.0. The resulting precipitate was separated by centrifugation at 2000 rpm (HCA). The solution was treated with ethanol (96%, HCB); the precipitates, as above. The completeness of extraction at each step was monitored using anthrone reagent.

WSPS Fractionation. WSPSC were fractionally precipitated with ethanol from aqueous solution by the literature method [9] to confirm that they were homogeneous. For this, WSPSC (1 g) were dissolved in water (200 mL), vigorously stirred, and treated dropwise with ethanol. A single fraction PSI was obtained after adding 250 mL of ethanol. Fractionation with sodium acetate was also performed as before [10]. WSPSC (1 g) were dissolved in water (100 mL) with addition of saturated NaOH solution (2 mL). The homogeneous solution was treated dropwise with sodium acetate solution (2 N). The precipitate (PSII) formed all at once after adding 155 mL of salt solution. It was separated by centrifugation and dried by changing solvents. The yield was 87%. The mother liquor was concentrated and treated with a six-fold excess of ethanol. A precipitate did not form. Precipitates PSI and PSII were chromatographed over Sephadex G-75 gel using formic-acid solutions (0-30%).

The homogeneity of the WSPSH was found after fractionation by the above method. Purified WSPSH (1 g) were dissolved in water (60 mL). Fractionation by alcohol produced two precipitates, after adding 150 and 280 mL of ethanol, in yields of 480 and 320 mg (60 and 40%), respectively. These were denoted PSE1 and PSE2. Fractionation of PSE1 and PSE2 by sodium acetate produced precipitates all at once. Gel chromatography over Sephadex G-75 was performed for the total WSPSH fraction and separately for PSE1 and PSE2 using formic-acid solutions (0-30%).

WSPS were monitored using anthrone—H_2SO_4 [11].

PS Fractionation. PS (1 g) were dissolved in water (100 mL). The solution was placed on a column of Sephadex G-75 and eluted by formic-acid solutions (0-40%) with quantitative monitoring of the eluates gravimetrically as calcium pectate.

Gel chromatography of polysaccharide fractions was performed according to the literature [12] over Sephadex G-75 and G-100, which were calibrated using dextrans of various molecular weight (Dextran Standart 1000, 5000, 12000, 25000, 50000; Bio Chemica for GPC, Fluka).

Acidic hydrolysis was carried out with sulfuric and nitric acids [9]. The hydrolysates were analyzed by paper chromatography and TLC.

Enzymatic hydrolysis of PS was performed using the Melnik—Arasimovich pectinase method [13]. Ground and sieved PS (1.5 g) were dissolved with gentle heating in water (300 mL), mixed with enzyme preparation (100 mg), layered above with toluene (10 mL), and thermostatted at 30°C for 10-12 d.

Enzymatic hydrolysis of polygalacturonan used polygalacturonase.

Samples of galacturonic acid (according to Erlich) were taken as before [14].

The quantitative content of galacturonic acid in PS was found by the literature method [13].

Galacturonic acid from PS was isolated after enzymatic hydrolysis by pectinase by the literature method [13].

Properties of PS were determined by potentiotitrometry [13, 15]; the **quantitative composition**, by the Zaitseva—Afanas′eva method [16].

Hypoglycemic activity was determined using 40 Wistar white mice of both sexes of mass 180-200 g. Statistical treatment of the results used the Student t-criterion [17].

Alimentary hyperglycemia was induced by injecting an aqueous glucose solution at a dose of 6 g/kg one hour before glucose analysis with polysaccharide fractions of *C. hastata* as background [18, 19]. WSPSC, WSPSH, and PS were administered i.p. at doses of 50, 100, and 150 mg/kg mass one hour before administering glucose. The control preparation was the leaf collection Arfazetin, which was administered to the animals as a solution at 10 mL/kg mass by an analogous procedure. The blood glucose content was found by an enzymatic method using the LaChema reagent set.

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