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Glycosyl Composition of Polysaccharide from *Tinospora Cordifolia*

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Polysaccharide from *Tinospora cordifolia* was isolated, purified, hydrolysed, trimethylsilylated and then subjected to GC-MC studies. The polysaccharide composition was estimated as follows: glucose 98.0%, arabinose 0.5%, rhamnose 0.2%, xylose 0.8%, mannose 0.2% and galactose 0.3%.

Keywords: Tinospora cordifolia (*Menispermaceae*), polysaccharide

Tinospora cordifolia (Willd) Miers ex. Hook & Thoms (*Menispermaceae*) is a succulent climbing shrub, indigenous to and found distributed throughout most part of India. In spite of the fact that this plant is so extensively used in folk medicine and traditional medicine, the pharmacological action of the active principles involved has not been worked out to date. The aqueous extract as well as the tincture prepared from it are now officially in the Indian Pharmacopoeia (1). The present work is about the study and characterization of the polysaccharide from *T. cordifolia.*

EXPERIMENTAL

Isolation

Standard procedure was followed for the isolation of the polysaccharide (2). The shade dried stem bark of *T. cordifolia* was treated with water at room temperature for 48 hours under stirring. From the aqueous extract, the polysaccharide was precipitated by addition of 95% ethanol (3 times the volume of aqueous extract). The solution was concentrated below 60 °C with a rotary flash evaporator under reduced pressure. The precipitate was collected by centrifugation at 20000 rpm (15 min), dissolved in water and dialyzed against distilled water. The collected polysaccharide was dried over fused calcium chloride under reduced pressure in a vacuum desiccator. The desiccated polysaccharide was redissolved in water. The associated proteins were removed using the Sevag method (3). The solution was shaken with chloroform in a separating funnel till it formed

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an emulsion/gel, which remained in the water-chloroform interface and was removed. To facilitate the denaturation, a pH 4–5 buffer (0.025 mol L^{-1} phosphate buffer) was used instead of water, and a small quantity of 1-butanol was added (5 mL). Since this technique removed only small quantities of protein, it was repeated four times resalting in significant, losses of polysaccharide. The clear aqueous layer was again treated with 95% ethanol to precipitate the polysaccharide, dialyzed against distilled water for 48 hours in the cold; the solution in the dialysis bag was lyophilized to a colourless powder.

The polysaccharide was purified by gel filtration chromatography on a Sephadex G 200 (Pharmacia Fine Chemicals, Italy). Phosphate buffered saline (PBS), 0.001 mol L^{-1} was used as eluent. Lyophilized crude sample (500 mg) was suspended in the buffer and chromatographed through a column of Sephadex G-200 (2.5 cm ×75 cm) equilibrated with the buffer. 3-mL fractions were collected in test tubes and were monitored at 490 nm using the phenol-sulphuric acid method (4). Two peaks were obtained: a major peak for fractions 12 to 30 and a minor one for fractions 38 to 42, with an area ratio 5:1. The major peak fractions were pooled and lyophilized. To test the homogeneity of the purified polysaccharide, sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis was performed (Sisco Chemicals, India). A 10% gel at pH 7.2 was used. Total carbohydrate content was determined by the phenol-sulphuric acid method using arabinose as standard.

Average molecular mass was determined by gel filtration on Sephadex G 200 using a series of dextrans of different molecular sizes as reference standards. From the data obtained, the average molecular mass was calculated by linear correlation between the logarithm of the molecular mass of the standards and the ratios of their elution volumes to the void volume of the column (5).

Hydrolysis

Complete hydrolysis of the homogeneous polysaccharide was done according to Kram and Franz (6) by 1 mol L^{-1} H_2SO_4 for 6 hours in a sealed tube over a boiling water bath. Graded hydrolysis was carried out with 25 μmol L^{-1} H_2SO_4 at 100 °C. Sulphate ions and charged sugars were removed from the hydrolysate by passage through a column of Dowex 50-X4 200–400 mesh (H^+ form) (Sisco Chemicals, India) coupled to a column of Dowex 1-X8 200–400 mesh (formate form) (7).

Thin layer chromatography (TLC) and paper chromatography (PC) of the hydrolysate after 15, 30, 45, 60, 90 and 120 minutes indicated an early release of D-glucose, followed by D-mannose. These were identified by co-chromatography with authentic samples. The following solvent mixtures were used: 1-butanol/ethanol/water (5:1:4), 1-butanol/2-propanol/water (1:6:3), ethyl acetate/pyridine/water (10:4:3), ethyl acetate/pyridine/water (2:1:2) and 1-butanol/ethanol/water (31:11:8).

Detection of sugar spots was achieved by aniline-diphenylamine-phosphoric acid, anthrone and alkaline silver nitrate.

Trimethylsilylation and GC-MS

A hundred mg of freeze-dried sample was transferred to a 13 ×100 mm test tube. Freshly prepared 1 mol L^{-1} methanolic HCl (250 μL) was added and the resulting solu-

tion was heated at 80 °C for 16 hours. This converted the polysaccharide into a mixture of methyl glycosides. The methanolic HCl was removed by adding 100 μL *t*-butyl alcohol and then evaporating it with a stream of air at room temperature (8). The methyl glycosides were silylated by using 5 mL of anhydrous pyridine (reagent grade pyridine dried over KOH pellets), 1 mL hexamethyldisilazane (HMDS) and 0.5 mL trimethylchlorosilane (TMCS), purchased conveniently in these proportions as Tri-Sil (Pierce Chemical Company, USA). The sample was heated to 80 °C for 20 minutes, and the silylating agent was gently evaporated at room temperature. The solution became cloudy on addition of trimethylchlorosilane owing to precipitation, presumably of ammonium chloride. No attempt was made to remove it, which in no way interfered with the subsequent gas chromatography. The derivative was redissolved in hexane (10 mL) and insoluble salts we allowed to settle. The supernatant was transferred to a clean test tube and carefully evaporated. The residue was dissolved in 100 μL hexane and 1 μL of this solution was analyzed by GC-MS. GC-MS analysis was performed with a fused-silica, 30 m (0.25 mm i.d.) capillary column in a splitless mode (Supelco sp 2330, Quadrex, USA). The following temperature programme was used: two minutes at an initial temperature of 80 °C, increased to 170 °C at 30 ° min^{-1}, then to 240 °C at 4 ° min^{-1}, and held for 5 min at 240 °C. The MS operating pernameters were: ionization voltage 70 eV, scan sate 110 amu S^{-1}, electron multiplier energy: 1600 V, ion source temperature: 200 °C. The response factors relative to the internal standard, myo-inositol, were determined empirically by injecting the standards and determining the peak areas for each sugar derivative. The components of trimethylsilyl derivatives of sugars were identified by comparison of their mass spectral data with the reference spectra in thedata base using the probability-based matching search algorhythm supplied by the manufacturer.

RESULTS AND DISCUSSION

The relative retention times of the trimethylsilyl derivatives and their main fragments are listed in Table I. The molecular ion pair was weak, but stronger pairs appea-

Table I. Retention times on GC and main fragments in MS of trimethylsilyl derivatives of T. cordifolia

Sugar	Retention time (min)	Main fragments (m/z)
Arabinose	11.27	59, 73, 133, 147, 204, 217
	11.50	59, 73, 133, 147, 204, 217
Rhamnose	12.00	73, 89, 117, 133, 147, 204, 217
Xylose	13.78	73, 89, 101, 116, 133, 147, 191, 204, 217
	14.36	73, 89, 101, 116, 133, 147, 191, 204, 217
Mannose	18.03	73, 103, 117, 133, 147, 204, 217, 231
Galactose	19.37	73, 89, 103, 117, 133, 147, 204, 217, 242
Glucose	21.67	59, 73, 89, 103, 125, 133, 147, 204, 217
	22.62	59, 73, 89, 117, 129, 133, 147, 204, 217

red for various fragments; m/z values in Table I match with the expected fragments. Response factor and peak area of the trimethylsilyl derivatives, along with myo-inositol are given in Table II. The percent of glycosyl residues was then calculated by dividing the peak areas by the appropriate response factor and the resulting quotients were normalized to 100%. The same table shows the percent of glycosyl residues of polysaccharide from *T. cordifolia*. Accordingly, this polysaccharide seems to be mostly a glucose polymer (Table II shows mol % of glucose as 98%). In order to gain information on sequences, glycosyl linkage composition are also to be known. This can be accomplished by subjecting the polysacharides to different chemical modifications.

The total parcentage of sugars colorimaty and was found to be 76.4%.

Table II. Trimethylsilyl derivatives of T. cordifolia

Peak	Response factor	Peak area	Mass (μg)	nmol CHO mg^{-1} sample	Glyceryl residue (mol, %)
Arabinose	0.48	813 366	2.79	37.13	0.5
Rhamnose	0.44	802	2.03	24.75	0.2
Xylose	0.67	1802 784	4.35	57.99	0.8
Mannose	0.63	822	1.47	16.35	0.2
Galactose	0.75	1134	1.71	19.00	0.3
Glucose	0.86	16560 113991	369.64	4102.56	98.0

CONCLUSIONS

The present paper givas the glycosyl composition of the polysaccharide from *T. cordolifolia*. To get an idea about the structure of this polysaccharide, various types of glycosyl lincages have to be defermined as well.

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S A Ž E T A K

Glikozilni sastav polisaharida iz biljke *Tinospora cordifolia*

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Polisaharid iz biljke *Tinospora cordifolia* je izoliran, pročišćen, hidroliziran, trimetilsiliran i analiziran GC-MS metodom. Polisaharidni sastav bio je sljedeći: glukoza 98,0%, arabinoza 0,5%, ramnoza 0,2%, ksiloza 0,8%, manoza 0,2% i galaktoza 0,3%.

Ključne riječi: Tinospora cordifolia (*Menispermaceae*), polisaharid, GS-MS

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