Carbohydrate Components of the Holoparasite *Cistanche deserticola* General Characteristics of the Underground Part

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The underground part of the holoparasite *Cistanche deserticola* Y. C. Ma. was subjected to composition analysis. Lipophilic substances and proteins comprise each nearly 15 % of the dry mass of the drug. The plant is rich in proteins and exhibits in comparison to wood a lower lignin content. A sirupy material which is mostly extractable with hot aqueous methanol represents 60 % of the dry drug. Only a part of this extract (83.8 %) comprises free sugars and sugar alcohols with glucose, mannose, and mannitol as the main components. The dialyzable carbohydrate fraction (DS) extracted from the methanol-insoluble drug residue (MIR) by cold water contains (*w*/%) mannose (14.9), mannitol (30.8), glucose (25.3), glucitol (8.2), and saccharose (6.8). Arabinose, galactose, xylose, and rhamnose are present in minor amounts. The sugar composition of MIR suggests the presence of a variety of carbohydrate polymers. An amylose-rich starch fraction was extracted from MIR with DMSO and characterized by ^{13}C NMR spectroscopy.

Cistanche deserticola Y. C. Ma. belongs to the family *Orobanchaceae.* The plant parasitizes on the roots of the tree *Haloxylon ammodendron* which is widely distributed in the Gobi desert. The underground part of the plant has been used as a tonic in traditional medicine of several Asian countries. Its chemical interest is due to the production of low-molecular mass compounds based on neolignan, phenylethanoid, and phenylpropanoid glycosides which have been isolated from related *Cistanche* species grown in Pakistan and China, and claimed as antistress drugs [1—3]. In the recent years, many carbohydrates of plant origin emerged as an important class of bioactive natural products [4, 5]. There is only one report [6] concerning carbohydrates of a *Cistanche* plant. It deals with the reducing sugar level in *C. tubulosa* (WIGHT.) and in the roots of infested and noninfested host plants. In the framework of a detailed investigation of the carbohydrate components of *Cistanche deserticola*, we have at first undertaken the general chemical characterization of the underground part of the parasite.

EXPERIMENTAL

Materials and Methods

The underground part of *C. deserticola* was collected in the Mongolian Gobi, Province Baian-Chongor, in August 1990. The voucher of the plant is deposited at the Herbarium of the Institute of Botany, Mongolian Academy of Sciences, Ulan-Bator. The air-dried plant was freezed in dry CO_2 and ground to yield a brownish powder. For qualitative sugar analysis, paper chromatography (p.c.) was performed by the descending method on Whatman No. 1 paper in the systems S_1 ethyl acetate—pyridine—water (φ_r = 8 2 1) and S_2 ethyl acetate—acetic acid—formic acid—water (φ_r = 18 : 3 : 1 : 4) used for neutral and acidic sugar analysis, respectively. Reducing sugars were detected with anilinium hydrogen phthalate, alditols with ammoniacal $AgNO_3$. The uronic acid content was determined by the 2-methoxydiphenyl method [7]. Moisture content was determined by drying at 105 °C to constant mass. All yield and composition calculations were made on a moisture-free basis. Protein was calculated from the nitrogen content (*w*(N)/%: 6.25) assayed on the Perkin—Elmer Elemental Analyzer, Model 240. Amino acid composition was determined, after hydrolysis by 6 M-HCl (105 °C, 20 h), under nitrogen, with an automatic amino acid analyzer T339 (Mikrotechna, Prague, Czechoslovakia). Lignin was determined by the conventional Klason method. The polysaccharide fractions were hydrolyzed with 2 M trifluoroacetic acid at 100 °C for 2 h. The hydrolyzates were separated into neutral and acidic saccharide fractions using an ion-exchange technique [8]. Lipids were estimated by extraction with benzene—ethanol (φ_r = 2 1) under reflux. Ash was determined as sulfates.

Gas chromatography (GC) was carried out on a Hewlett—Packard instrument, Model HP 5890. Gas chromatography—mass spectrometry (GC—MS) was carried out on the instrument SSQ (Finnigan). For the quantitative determination of sugars in the form of their alditol trifluoroacetates [9], a capillary column (25 m × 0.32 mm i.d.) with

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PAS 1701, and temperature range 110 °C (1 min) to 125 °C (2 °C min^{-1}) was used. The trimethylsilylated alditols and oximes [10] were analyzed on a SP-2330 silica capillary column (25 m x 0.32 mm i.d.) in the temperature range from 130 °C (0.5 min) to 240 °C (4 °C min^{-1}). Inositol was used as the internal standard in the quantitative sugar analysis. The ^{13}C NMR spectra of polysaccharide solutions ($w = 2$ % in $(CD_3)_2SO$) were recorded at 50 °C on a Bruker AM-300 spectrometer. Chemical shifts are referred to DMSO ($\delta_{TMS} = 38.5$).

Extraction Methods

A. The air-dry ground drug (1286 g) was suspended in 95 % aqueous methanol (5 dm^3) and extracted five times under reflux. The methanol extracts were combined, concentrated under reduced pressure and freeze-dried. The residue after methanol extraction (MIR) was washed with acetone and dried at 40 °C (393 g).

B. MIR (300 g) was macerated with cold distilled water (2 dm^3) under stirring for 2 h and filtered. The procedure was repeated four times. The extraction residue was dried at 105 °C for 6 h (229 g). The combined filtrates were concentrated under reduced pressure to about 0.5 dm^3 and dialyzed against distilled water for 3 d. The dialyzable fractions were collected and concentrated to a sirup-yielding fraction DS (43 g).

C. MIR (20 g) was once extracted with 90 % aqueous DMSO (0.2 dm^3) at 50 °C for 1 h. After filtration, the residue was washed with 50 % ethanol and dried at 105 °C for 6 h (15.8 g). The extract was dialyzed against distilled water, concentrated, and poured into quadruple volume of 96 % ethanol. The precipitate was filtered, dispersed in water, dialyzed, and freeze-dried yielding fraction SP (0.31 g).

RESULTS AND DISCUSSION

The results of the compositional analysis are given in Table 1. The air-dry drug retained an appreciable amount of water bound mainly in the sirupy extracellular brownish substance. In accordance, a high yield of hydrophilic extractives was obtained by treatment of the drug with hot 95 % methanol, representing about 60 % of the dry mass of the drug. The methanol extract contained 83.8 % of carbohydrates estimated by the trimethylsilylation method [10]. Mannitol, glucitol, and galactitol were the main components comprising 55.5 % of the extract, whereas glucose and mannose ($x_r = 10 : 9$) were present in minor amounts (≈ 20 %). After acid hydrolysis of the methanol extract and following conversion of the liberated sugars into alditol trifluoroacetates, rhamnose appeared and x_r of glucose, mannose, and rhamnose was 100 9 2. This indicates that glucose and rhamnose may be bound to glycosides of similar types as they have been isolated from related *Cistanche* species [1—3].

The drug contains besides of lipophilic extractives the polyaromatic substances determined as Klason lignin. Its content (7.7 %) is in comparison to that of wood tissues rather low. However, the lignin content of the methanol-insoluble residue (MIR) is higher (15.5 %) and thus closer to the values reported for wood species [11].

The sugar composition of the native *Cistanche* drug and MIR was derived from analysis of the corresponding Klason lignin hydrolyzates (Table 2). In the case of the native drug, glucose is predominating, followed by mannose and minor amounts of arabinose, rhamnose, xylose, galactose, and fucose in the decreasing order. The mannose content of the MIR hydrolyzate is diminished due to losses of carbohydrates during methanol extraction.

Table 1. Characterization of the Underground Part of the *Cistanche deserticola* Drug[a]

w(Moisture)/%	22.2[b]
w(Klason lignin)/%	7.7
w(Protein)/%	14.8
w(Ash)/%	8.3
w(Benzene—ethanol extract)/%	14.7
w(Methanol extract)/%	60.7
w(Cold water extract)/%	9.3
w(DMSO extract)/%	7.2

a) Relative to dry mass of the drug; *b*) relative to air-dry drug.

Table 2. Klason Lignin and Sugar Composition of the *Cistanche* Drug, Its Methanol-Insoluble Residue (MIR) and Fraction SP

Composition	Native drug	MIR	SP
w(Klason lignin)/%	7.7	5.9	16.2[a]
Sugar, x_r[b]			
Rhamnose	7	10	3
Fucose	1	1	0
Arabinose	14	56	10
Xylose	5	27	3
Mannose	50	21	12
Glucose	100	100	100
Galactose	4	18	18
Uronic acid[c]	8	9	nd

a) Acid-resistant portion in 2 M trifluoroacetic acid; *b*) determined as alditol trifluoroacetates; *c*) expressed as a unit of glucuronic acid; nd — not determined.

Since neutral sugars of the Klason lignin hydrolyzates were estimated as alditols, they do not reflect the actual composition and distribution of the carbohydrate components in the native drug. By this kind of determination, those carbohydrates are also analyzed which are present as mono- and oligosaccharides as

well as the alditols in the methanol extract. Therefore, a cold water extraction of MIR was performed (Table 1). After exhaustive dialysis of the extract against distilled water, the recovered dialyzable portion (DS) represents low-molecular mass compounds accounting for about 60 % of the water extract. P.c. of DS indicated the presence of glucose, mannose, arabinose, rhamnose, and saccharose. GLC (Table 3) and GC-MS [12, 13] analyses were used to establish the sugar composition of this fraction. Conversion of DS into trimethylsilylated oximes revealed the presence of glucose, mannose, glucitol, mannitol, and saccharose comprising 92.6 % of DS. After reduction of DS with deuterated sodium borohydride and following trifluoroacetylation, the obtained product was shown by GC-MS to consist mainly of glucitol and mannitol, containing different proportions of deuterium-labelled and nonlabelled fragment ions (m/z = 153, 208, 253, 265, 303, and 379). Thus, the presence of glucose and mannose in DS was confirmed. Their proportion was calculated from the content of the corresponding hexitols estimated in both experiments. DS contains also minor amounts of rhamnose, arabinose, xylose, and galactose. These sugars which are constituents of the carbohydrate polymers of the drug (see Table 2) are probably released by activities of the present enzymes during the growth as well as storage of the plant before the investigations have been started. The uronic acids of the Klason lignin hydrolyzate were identified by p.c., using authentic standards [8], as galacturonic acid, glucuronic acid and its 4-*O*-methyl derivative suggesting the presence of pectic polymers and glucuronoxylan.

The plant is relatively rich in protein calculated from the nitrogen content. However, it must be mentioned

Table 3. Sugar Composition of the Dialyzable Portion of the Cold Water Extract (DS)

	w(Original DS)/%[a]		w_r($NaBD_4$-reduced DS)/%[b]
	A	B	
Glucose	46.8[c]	25.3	0
Mannose		14.9	0
Glucitol	8.2	8.2	39.1
Mannitol	30.8	30.8	53.2
Saccharose	6.8	6.8	nd
Rhamnose	nd	3.3	3.8
Arabinose	nd	1.8	2.1
Xylose	nd	0.7	0.8
Galactose	nd	0.8	0.9

a) Determined as trimethylsilylated oximes on SP 2330; *b*) determined as alditol trifluoroacetates on PAS 1701; *c*) sum of glucose and mannose;
A — GLC analysis; B — calculated from the results of *a*) and *b*).

that a part of the nitrogen may be bound in inorganic substances. The amino acid composition of the protein moiety of the original drug and MIR, listed in Table 4, shows no substantial differences. The content of essential amino acids resembles that of flours [14], showing nutritional value.

Table 4. Amino Acid Composition of the *Cistanche* Drug and Its Methanol-Insoluble Residue (MIR)

Amino acid	x_r/mole %	
	Native drug	MIR
Asparagine	12.9	12.5
Threonine	5.8	5.7
Serine	8.9	7.0
Glutamic acid	15.9	11.7
Proline	7.1	9.3
Glycine	8.1	11.1
Alanine	9.4	8.9
Valine	4.1	6.0
Methionine	1.5	0.7
Isoleucine	4.4	5.9
Leucine	7.9	7.2
Tyrosine	4.1	3.8
Phenylalanine	3.4	4.1
Histidine	1.3	2.2
Lysine	2.0	1.9
Arginine	2.6	1.6

Extraction of the drug with DMSO (Table 1), used to isolate starch from higher plants [15], afforded a glucose-rich polymeric fraction SP (Table 2) which generated iodine blue colour typical for amylose. Of course, the ^{13}C NMR spectrum of SP in $(CD_3)_2SO$ exhibits a group of main resonances which resembles that of high-amylose starch [16]. The signals of the carbons of the α-(1,4)-linked D-glucopyranosyl units assigned on the basis of literature data [16—18] appeared at δ = 99.90 (C-1), 71.48 (C-2), 71.88 (C-3), 78.71 (C-4), 73.09 (C-5), and 60.43 (C-6).

On the basis of these results, it can be concluded that the *Cistanche* parasite growing on the carbon source of the host, *e.g.* hardwood roots, contains in comparison to wood an unusually high amount of low-molecular mass extractives. Only a part of them comprises aldoses and alditols with glucose, mannose, and mannitol as the main components. The sugar composition of the extractive-free drug suggests the presence of starch and a variety of other polysaccharides, which is in contrast to hardwood tissues [11]. Such complex of polysaccharides is usually isolated from roots, stems, and leaves of various herbs and other plants [5, 19, 20]. Detailed studies on the soluble and insoluble carbohydrate polymers of the parasite are in progress and will be published elsewhere.

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