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CHARACTERIZATION OF THE 'RESERVE CELLULOSE' OF THE ENDOSPERM OF *CARUM CARVI* AS A β(1-4)-MANNAN

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Key Word Index—*Carum carvi*; Umbelliferae; *Phoenix dactylifera*; Palmae; endosperm; reserve polysaccharide; β(1-4)-mannan.

Abstract—The reserve polysaccharide of the endosperm of *Carum carvi* consists of more than 90% mannose and was characterized as a β(1-4)-mannan. Total or partial acid hydrolysis, enzymatic breakdown or acetolysis of either palm or *Carum carvi* mannan yielded the same mono- and oligosaccharides, indicating a similar chemical structure of the two reserve polysaccharides. However, *Carum carvi* contains only traces of the alkali-soluble mannan A dominant in the palm endosperm polysaccharide.

INTRODUCTION

In addition to large amounts of reserve protein and fatty oils, the starch free endosperm of the Umbelliferae contains the so-called 'reserve cellulose' [cf. 2], a reserve polysaccharide deposited in the cell walls. Thus far its chemical composition is unknown [1, 2]. This paper reports the isolation of the reserve polysaccharide of *Carum carvi* L. endosperm and its chemical characterization as a β(1-4)-mannan.

RESULTS

Preparation of crude polysaccharide fractions from fruits of Carum carvi

Dry ripe fruits of *Carum carvi* are about 5 mm long. The brown fruit and seed coat surround the white endosperm in which the embryo is embedded. Because of the small size of the fruits and the tight binding of the fruit and seed coat to the endosperm, pure endosperm material could not be isolated by any mechanical method. However, by boiling whole fruits in 1% NaOH [3], the fruit and seed coat as well as the embryo were nearly completely released and could be removed from the endosperm. Each fraction—fruit and seed coats, embryos, endosperm—were ground to powder and extracted to obtain the crude polysaccharide fractions. Table 1 shows the balance for the fractionation of the fruits to isolate the reserve polysaccharide from the endosperm.

Table 1. Balance of the fractionation of *Carum carvi* fruits for the isolation of the reserve polysaccharide from the endosperm

	g/100 g Dry fruits
Material removed by boiling 1% NaOH (i.g. fruit and seed coat; embryo)	67
Material extracted with boiling H_2O, 70% EtOH, petrol	17
Crude endosperm polysaccharide	16
Material removed from the endosperm polysaccharide by 7 + 14% NaOH	1.5
Material removed from the endosperm polysaccharide by cellulysin and macerase	0.5
Endosperm polysaccharide not removed by alkali and enzymes (mannan)	14

Monosaccharide composition of the polysaccharides from the fruit of Carum carvi

The monosaccharides released by total acid hydrolysis of the crude polysaccharide preparations from various parts of *Carum carvi* fruits were separated by PC in solvent C and the main constituents were determined quantitatively.

As shown in Table 2, the crude polysaccharide from the fruit and seed coat consists mainly of glucose and pentoses, whereas mannose and galactose are present in smaller amounts. Pentoses dominate in the crude polysaccharide from the embryo. This latter also contains traces of apiose (not incuded in Table 2), which has previously been described to occur in the polysaccharides of umbellifer fruits [4]. Rhamnose and galacturonic acid were also found in small amounts in the hydrolysates of the polysaccharides from the embryo and from the fruit and seed coat (not included in Table 2). The crude polysaccharide from the endosperm consists of 90% mannose, whereas glucose, galactose and pentoses are only minor components. It resembles the crude mannan isolated from the endosperm of *Phoenix dactylifera* L. (Table 2), thus indicating that the polysaccharide is a mannan.

The endosperm polysaccharide was separated into a fraction soluble in and a fraction insoluble in 14% NaOH. The alkali-soluble polysaccharide—representing about 2% of the original crude polysaccharide—contains

Table 2. Monosaccharide composition of polysaccharide preparations from *Carum carvi* and *Phoenix dactylifera* (% of total monosaccharides determined)

Species	Source	Polysaccharide preparation	Man	Gal	Glc	Ara	Xyl
Carum carvi	Embryo	crude	9.7	16.8	22.4	34.7	16.4
	Fruit and seed coat	crude	10.5	5.8	41.9	16.1	25.7
	Endosperm	crude	90.4	1.2	5.4	1.5	1.5
		soluble in 14% NaOH	61.8	5.9	7.9	1.8	22.7
		insoluble in 14% NaOH	93.8	0.9	4.2	1.1	0.0
		insoluble in 14% NaOH* treated with cellulysin and macrease	97.1	1.2	1.3	0.4	0.0
Phoenix dactylifera	Endosperm	crude	90.2	2.6	6.0	1.2	trace

* The monosaccharides determined in the hydrolysate amounted to 90% of the dry weight of the hydrolysed polysaccharide.

only about 60% manose (Table 2) and a relatively high portion of xylose (22%), thus indicating that it does not contain exclusively mannan similar to palm mannan [5, 6], but also polysaccharides from the fruit and seed coat which are rich in xylose. Even three successive precipitations with Fehling's solution could not remove xylose completely.

The alkali-insoluble polysaccharide consists almost exclusively of mannose, with small amounts of glucose, galactose and arabinose present. After incubation with a mixture of cellulysin and macerase, the percentage of mannose was further increased up to a value equalling that of mannan B from the endosperm of *Phytelephas macrocarpa* and *Phoenix dactylifera* [5, 6].

Characterization of the reserve polysaccharide from the endosperm

To characterize the chemical structure of the mannose-rich polysaccharide of *Carum carvi*, the products of partial acid hydrolysis or acetolysis were compared with those of the mannan of the endosperm of *Phoenix dactylifera*.

Since the crude and alkali-soluble endosperm polysaccharide from *Carum carvi* were contaminated with polysaccharides from the fruit and seed coat, the alkali-insoluble fraction treated with cellulysin and macerase was used for purposes of comparison. The crude mannan of the endosperm of *Phoenix dactylifera* was used without further purification, as it did not contain such contaminations and as the two alkali fractions, mannan A and B, are chemically similar [5, 6] and differ only in chain length. After partial acid hydrolysis or acetolysis, the resulting oligosaccharides were separated by one-dimensional PC in solvent D and by two-dimensional PC in the solvent A + B qnd A + D. Both preparations yielded a homologous series of manno-oligosaccharides showing the same $R_{mannose}$-values in solvent D as the homologous series of manno-oligosaccharides ranging from mannobiose to the mannopentaose found in the acetolysates of mannan A + B from *Phytelephas macrocarpa* [7]. The chromatographic characterization of the manno-oligosaccharides was confirmed by partial acid hydrolysis of the eluted oligosaccharides followed by chromatographic separation of the products in solvent D. These preparations yielded mannose and the smaller members of the homologous series of manno oligosaccharides as was to be expected according to the results of Aspinall [7]. The manno-oligosaccharides were isolated and incubated with α-mannosidase. Each of the manno-oligosaccharides remained unchanged, thus indicating β-linkages as in the palm mannans.

In addition to the homologous series of manno-oligosaccharides, two non-homologous disaccharides were found in the partial acid hydrolysate of the alkali-insoluble polysaccharide. According to their $R_{mannose}$-values in solvent D, their sensitivity to α-mannosidase and their monosaccharide content, they are most probably 4-*O*-α-D-mannopyranosyl-D-mannose and 4-*O*-β-D-mannopyranosyl-D-glucose, which have been identified by Aspinall [7] in the hydrolysate of mannan A + B from *Phytelephas macrocarpa*.

The partial acid hydrolysate of the alkali-soluble polysaccharide from *Carum* endosperm also contained the above two disaccharides and the same series of manno-oligosaccharides as found in the insoluble fraction. In addition, several pentose-containing oligosaccharides were present. The latter originated most probably from residues of the fruit and seed coat, which could not be completely removed in the course of the preparation of the endosperm.

The incubation of the polysaccharides from *Carum carvi* and *Phoenix dactylifera* with a crude enzyme prepared from germinating seeds of either *Carum carvi* or *Phoenix dactylifera* resulted in a mixture of manno-oligosaccharides having the same $R_{mannose}$-values in solvent D as those of the partial acid hydrolysates.

All results support the suggestion that the mannans of the two taxonomically very different plants are chemically similar. However, the mannan of *Carum carvi* differs from that of the Palmae in its low content (about 2%) of the alkali-soluble mannan A which is dominant in the mannan of Palmae [5–9].

EXPERIMENTAL

Plant material. Fruits of *Carum carvi* were obtained from commercial sources. Seeds of *Phoenix dactylifera* were obtained from dates bought at the local market.

Preparation of crude polysaccharide fractions from fruits of Carum carvi *and seeds of* Phoenix dactylifera. To prepare the endosperm, whole fruits of *Carum carvi* were soaked in H_2O for 18 hr and then refluxed in 1% NaOH for 24 hr under N_2, whereupon the fruit and seed coats and the embryos were almost completely released. Both could be removed from the endosperm by washing The endosperm was further stirred

in H_2O for 24 hr to remove residues of the fruit and seed coats, washed, dried, and ground to a powder which was successively extracted with boiling H_2O, 70% EtOH and petrol to remove soluble compounds and fatty oils. As by this alkali treatment of the fruits no separation of the embryos from the fruit and seed coats was possible, the following method was used to get pure preparations: fruit and seed coats were prepared by treating dry fruits in a Starmix for 2 min, whereby most of the fruit and seed coats were sheared off. Upon suspension in H_2O, the fruit and seed coats could be collected in the supernatant fractions, whereas the seeds sedimented rapidly. The seeds remained in H_2O for 1 hr whereby the rapid swelling embryos were squeezed out of the endosperm. The seeds and embryos could be separated due to the smaller sedimentation velocity of the embryo in H_2O. To obtain the crude polysaccharide preparations, the fruit and seed coats and the embryos were dried, ground to a powder and extracted in the same way as the powdered endosperm. Crude mannan from seeds of *Pheonix dactylifera* was prepared according to Keusch [8].

Purification of the polysaccharide from the endosperm. The crude polysaccharide from the endosperm was extracted with 7% NaOH followed by 14% NaOH, which resulted in a loss of 10% of the dry wt. The alkali-soluble polysaccharide was ppd with Fehling's soln [7]. It amounted to only 2% of the dry wt of the crude polysaccharide. Incubation of the alkali-insoluble polysaccharide with cellulysin and macerase (Calbiochem USA) was carried out in 0.1 M acetate buffer pH 5 at 32° for 4 hr.

Acid and enzymatic hydrolysis of the polysaccharide. Total acid hydrolysis was carried out according to ref. [8]. Partial acid hydrolysis was performed with N H_2SO_4 at 100° for 30 min. After neutralisation of the hydrolysates with $Ba(OH)_2$, the $BaSO_4$ was centrifuged off and the supernatant was tested for monosaccharides by PC and for uronic acids by paper electrophoresis. Acetolysis was carried out as follows: the alkali-insoluble was added to a mixture of Ac_2O–HOAc–H_2SO_4 (10:10:1). The mixture was kept at room temp. for 96 hr, during which the polysaccharide had nearly completely dissolved. The mixture was filtered, poured into ice-H_2O and $NaHCO_3$ was added (to pH 3–4). The pptd sugar acetates were centrifuged off and the filtrate was extracted ×3 with $CHCl_3$. The solid sugars acetates were dissolved in $CHCl_3$, combined with the $CHCl_3$ extracts, concd and dried. The sugar acetates were dissolved in $CHCl_3$–MeOH (1:2) and deacetylated by 0.1 M NaOMe for 12 hr at 0°. After neutralisation with dil. H_2SO_4, the soln was concd and applied to charcoal. Salt was removed by washing the charcoal with H_2O and sugars were recovered by washing with 50% EtOH. The conc sugar mixture was used for chromatographic analysis. The enzyme used for the enzymatic hydrolysis were prepared from germs of *Carum carvi* (2 weeks old, radicle 1 cm in length) and from the haustorium of germinating *Phoenix* seeds (4 weeks old) as in ref. [11] but 0.1 M phosphate–citrate buffer pH 5 [12] was used. The mixture contained 1 ml enzyme soln and 100 mg of polysaccharide suspended in buffer. The reaction was stopped after 2 hr by the addition of 96% EtOH. Protein and unhydrolysed polysaccharide were centrifuged off and the supernatant was analysed by PC and paper electrophoresis. Oligosaccharides isolated from partial acid hydrolysates of mannan were incubated with α-mannosidase (Boehringer, Mannheim) [13] to test for the presence of α-glucosidic linkages.

General methods. PC was carried out on Whatman 1 paper using the following solvents: (A) 88% PhOH–HOAc–M EDTA–H_2O (840:10:1:160); (B) Soln 1: *n*-BuOH–H_2O (15:1), Soln 2: $PrCO_2H$–H_2O (11:14), Solns 1 and 2 were mixed 1:1. (C) *n*-BuOH–Py–H_2O–HOAc (60:40:30:3), (D) EtOAc–Py–H_2O (10:4:3). Paper electrophoresis was carried out either with 0.05 M Na tetraborate pH 9.8 (monosaccharides) or with 0.1 M ammonium formate pH 3.7 (uronic acids). Alkaline $AgNO_3$ [14] and diphenylamine–urea [15] were used to detect sugars on the chromatograms. Quantitative determinations of hexoses and arabinose were carried out enzymatically (16) and that of xylose by the anthrone method [17].

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