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COMPARISON OF CELL WALL COMPOSITIONS OF A DESERT XEROPHYTE AND A RELATED MESOPHYTE

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Key Word Index—*Anabasis syriaca*; *Spinacia oleracea*; Chenopodiaceae; xerophyte; mesophyte; cell wall composition; polysaccharide; lignin.

Abstract—A comparison was made of the cell wall compositions of stem internode tissues from two members of the Chenopodiaceae. Cell walls from *Anabasis syriaca* (a desert xerophyte) contained non-cellulosic polysaccharides rich in arabinose, xylose and galacturonic acid. The non-cellulosic polysaccharides from cell walls of *Spinacia oleracea* (a mesophyte) were rich in glucose. *Anabasis syriaca* cell walls contained relatively more cellulose and lignin than those of *Spinacia oleracea*.

INTRODUCTION

The genus *Anabasis* contains a number of species of xerophytic and halophytic shrubs, including six which are found in desert habitats in Syria, Jordan and Israel [1, 2]. Perennial plants growing in such habitats are adapted to resist desiccation and this is likely to involve adaptations in the cell wall structure [3]. This report describes investigations into the carbohydrate and lignin compositions of cell walls from one abundant desert growing perennial member of the genus, *Anabasis syriaca*. The results are compared with those from a mesophytic species from the same family (Chenopodiaceae), *Spinacia oleracea*, in order to see whether adaptation to the desert habitat involves major changes in cell wall composition.

RESULTS AND DISCUSSION

TFA was used to hydrolyse non-cellulosic polysaccharides in the cell wall material [4]. The results (Table 1) show that the predominant sugars found in the non-cellulosic polysaccharides of *Anabasis syriaca* were xylose, arabinose and galacturonic acid. Galactose, glucose, rhamnose, mannose and glucuronic acid were present in smaller amounts, together with traces of fucose. It is likely, therefore, that the major polymers present were arabinoxylan, perhaps including some glucuronoarabinoxylan and galacturonan. Some of the galacturonic acid may have been present as rhamnogalacturonan. However, the majority of the galacturonic acid containing polymers could be extracted from the pectin fraction (Table 2) which contained very little rhamnose. Hence, most of the galacturonic acid must have been present in polymers which did not contain significant amounts of rhamnose. In addition to galacturonic acid, the pectin fraction contained arabinose and galactose (perhaps as arabinogalactan) and glucose (perhaps as non-cellulosic glucan).

The non-cellulosic polysaccharides from *Spinacia oleracea* were very different in composition. Although xylose, arabinose and galacturonic acid were present, they were overshadowed by glucose, which constituted *ca* 75% of the total sugars. This is likely to represent a non-cellulosic glucan. The second most abundant constituent was glucuronic acid, which may have been present partly as a glucuronoarabinoxylan.

A possible artefactual source of glucose in the non-

Table 1. Sugar composition of non-cellulosic polysaccharides

	Anabasis syriaca		*Spinacia oleracea*	
Sugar	μg/mg	Relative mol %	μg/mg	Relative mol %
Rha	10.4	4	1.4	1
Fuc	1.2	1	—	—
Ara	67.6	28	6.3	3
Xyl	85.0	35	6.6	3
Man	7.6	3	2.5	1
Gal	16 2	6	4.7	2
Glc	15.2	5	168.6	75
GalA	52.6	17	13.3	6
GlcA	6.0	2	20.8	9

Table 2 Sugar composition of pectin extracts

	Anabasis syriaca		*Spinacia oleracea*	
Sugar	μg/mg	Relative mol %	μg/mg	Relative mol %
Rha	0.6	1	—	—
Ara	6.2	12	1.9	5
Xyl	—	—	1.9	5
Man	1 2	2	2.5	5
Gal	2.4	4	2.3	5
Glc	7 0	1[illegible]	37.2	80
GalA	46 4	70	—	—

cellulosic wall fraction of *Spinacia oleracea* could have been starch that had not been adequately degraded during preparation of the wall material. However, the amylase used was shown to be highly active under the conditions used and treatment of the cell wall preparation with amylase from another source did not remove the glucose. Furthermore, residual starch would have been expected to have been removed along with pectins by extraction with hot aqueous ethylenediaminetetra acetic acid (EDTA) and the glucose found in this extract was only 22% of that in the total non-cellulosic polysaccharide fraction. Hence, the glucose in the non-cellulosic polysaccharides appears to have been derived from a wall glucan. This is an unusual finding since such glucans are characteristic of monocotyledonous rather than dicotyledonous plants, but it is now recognized that they are found in considerable amounts in some tissues in the latter group of plants [5].

Both cellulose (38.6 μg/mg for *Anabasis*; 11.4 μg/mg for *Spinacia*) and lignin (Fig. 1) were present in considerably greater amounts in *Anabasis syriaca* than in *Spinacia oleracea*. This is to be expected, since these polymers are the ones which contribute most to the strength of the cell wall. In a desert plant, therefore, they would contribute to the prevention of wilting under water stress, perhaps by increasing the elastic modulus of the tissue [3].

Information concerning the chemical composition of the lignin in the two species was obtained by comparing the UV spectra of the lignin extracts under alkaline and neutral conditions. The difference spectra (Fig. 1) show characteristic peaks at 245, 304 and 350 nm. The peaks at 245 and 304 nm may be attributed to non-conjugated phenols, while those at *ca* 350 nm are due to phenols with large, conjugated side chains, such as the hydroxycinnamic acid derivatives [6]. The relative heights of the peaks in the two species indicate that *Anabasis syriaca* contains a higher proportion of phenols with conjugated side chains than *Spinacia oleracea*.

The differences between the cell wall compositions of the two species are quite considerable. While it is not clear which of these differences might be due to adaptation to different habitats, the existence of such differences between plants of the same family suggests that habitat may have an important role in influencing the molecular architecture of plant cell walls.

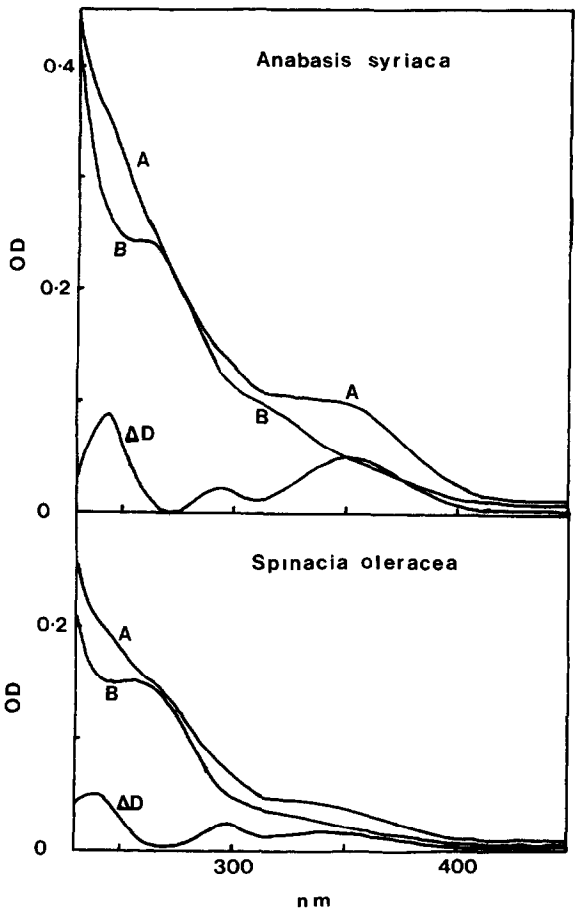

Fig. 1. UV spectra and difference spectra of lignin extracts Lignin extracts were adjusted to pH 12.5 (A) and pH 7.0 (B), and UV absorption spectra were recorded. The difference spectra (ΔD) were then calculated. The *Anabasis syriaca* extract contained lignin corresponding to 0.4 mg cell wall material per ml solution analysed; the *Spinacia oleracea* extract contained lignin corresponding to 0.5 mg cell wall material per ml

EXPERIMENTAL

Plant material. Anabasis syriaca was obtained from the desert adjacent to the new campus of Yarmouk University, outside Irbid, in NW Jordan Stem internodes were excised from plants *ca* 50 cm high. *Spinacia oleracea* was obtained from the local market in Irbid and stem internodes were excised Both species were harvested when at or near their maximum size under the relevant growth conditions They were both, therefore, mature as far as vegetative growth was concerned The *S oleracea* was at the physiological stage immediately preceding flowering.

Preparation of cell wall material. Internode samples (up to 100 g wet wt) were homogenized with twice their weight of buffer (10 mM NaOAc, pH 5, containing 3 mM $CaCl_2$) at 4° in a Polytron homogenizer (speed 8, in 10–15 10-sec bursts). The homogenate was filtered through two layers of muslin and material retained by muslin was rehomogenized and filtered in the same way The filtrates were combined and centrifuged at 1000 *g* for 15 min at 4°. The pellet was resuspended in 25 ml buffer and sonicated (6 10-sec bursts over 2 min). The sonicated suspension was recentrifuged as above and washed (× 4) with cold buffer, resuspending and centrifuging each time. The pellet was resuspended in 10 ml buffer containing 800 units salivary α-amylase (type IX-A, Sigma), and incubated at 30° for 1 hr. The material was then centrifuged at 1000 *g* for 15 min and the pellet extracted × 3 with 10 ml $CHCl_3$–MeOH (2:1) and twice with 10 ml PhOH–HOAc–H_2O (1:1:1, *w/v/v*) at 70° for 1 hr to remove proteins [7]. The pellet was washed twice with H_2O and once with Me_2CO, desiccated under red. pres over NaOH and stored at −18°.

Hydrolysis Cell wall material (5–10 mg) was hydrolysed with 2 M TFA (1 ml) at 120° and 1 kg/cm² for 1 hr [4] The hydrolysate was centrifuged and the pellet (cellulose) retained for carbohydrate estimation. The supernatant was rotary evaporated, desiccated over NaOH to remove the TFA and analysed by GC

Cellulose estimation. Cellulose was estimated as the amount of carbohydrate remaining after TFA hydrolysis. The carbohydrate was estimated by the method of ref [8], using glucose as standard.

Pectin extraction. Pectin was extracted from cell wall material

(5–10 mg) as described in ref. [9]. The extract was extensively dialysed and the material retained in the dialysis tubing rotary evaporated to dryness and hydrolysed by TFA.

Gas chromatography. Monosaccharides and uronic acids were analysed as their alditol acetates as described in ref. [10] Sugar standards were subjected to TFA hydrolysis before use. Alditol acetates were separated isothermally at 215° on packed columns (3 m × 4 mm) packed with 3% SP2330, with N_2 at 25 ml/min as carrier gas and a FID Identification and quantification of hexitol acetates derived from uronic acids was confirmed using a capillary column (WCOT with CP Sil 5 liquid phase, 0.14 μm film thickness), 25 m × 0.23 mm, temp. programmed from 190° to 230° at 5°/min; an FID was used, with an injection vol of 1 μl and a split ratio of 50:1; the carrier gas was N_2 at 1 ml/min.

Lignin analysis Lignin was analysed by the method of ref. [11] as modified in ref [6] Cell wall material (8–10 mg) was extracted for 16 hr with 1 ml 0.5 M NaOH at 70°. The material was centrifuged at 10000 *g* for 1 min and the pellet washed twice with 1 ml H_2O Supernatant and washings were combined, neutralized to pH 8 5–9.0 with 1 M HCl and made up to 4 ml with H_2O. Samples (0.4 ml) were diluted to 2 ml either with 0.05 M NaOH or with 0 05 M NaPi buffer, pH 7, and analysed by UV spectroscopy within 3 hr

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POLYSACCHARIDE OF THE RED ALGA *RISSOELLA VERRUCULOSA*

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Key Word Index—*Rissoella verruculosa*; Rhodophyceae; κ-carrageenan; neocarrabiose unit; neocarrabiose sulphate unit.

Abstract—The Mediterranean red alga, *Rissoella verruculosa*, contains a κ-like carrageenan. ^{13}C NMR studies indicate that the polysaccharide contains neocarrabiose sulphate units alternating with non-sulphated neocarrabiose residues.

INTRODUCTION

In a previous paper we reported on the sterol content of *Rissoella verruculosa* [1] which is the most abundant red alga of the Mediterranean French coast near Banyuls-sur-Mer. In view of the possible economic value of this alga, we have determined the chemical composition of its polysaccharide.

RESULTS AND DISCUSSION

Rissoella verruculosa is an annual seaweed. The yields of polysaccharide are particularly high and remain unchanged (50%/dry wt) from April to July. Acid hydrolysis of the polysaccharide gave the following monosaccharides (% total): galactose (91.8), xylose (2.7), mannose (0.6) and glucose (4.9). An $[\alpha_D]$ of +12.5° for the polysaccharide and its IR spectrum suggested a D-configuration for 4-*O*-linked, 3,6-anhydro-α-galactopyranose units (A-unit) alternating with 3-*O*-linked β-D-galactopyranose (G-unit) units as found in κ-carrageenan (Fig. 1). The IR spectra were quite similar to those of a commercial sample of κ-carrageenan with absorptions at 930 cm^{-1}, indicating 3,6-anhydrogalactose units, and 840 cm^{-1}, indicating 4-sulphate groups on galactose units (Fig. 2).

The chemical composition of the galactan of *R. verruculosa* is given in Table 1. The polysaccharides obtained after 'normal' and 'high milk reactivity' treatments yielded similar amounts of sulphate, excluding any 6-sulphate-galactose groups (μ-precursor of κ-carrageenan). The 'high milk reactivity' treatment consists of extraction of the alga with a dilute aqueous calcium hydroxide solution. This treatment changes μ-carrageenan into κ-