Host-plant oligosaccharins in the honeydew of *Schizaphis graminum* (Rondani) (Insecta, Aphididae)

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Summary. The honeydew of the aphid *Schizaphis graminum* (Rondani) contains hetero-oligosaccharides (mol. wt > 2200 D) having neutral sugar and uronic acid compositions similar to the pectic substances of the host plant, *Sorghum biocolor* (L.) Moench. These oligosaccharides are pectic fragments which result from the depolymerization of pectic substances in the phloem by aphid salivary polysaccharases.

Key words. Aphids; honeydew; phloem; pectin; pectinase; polysaccharase; cell wall; translocation; phytoalexin.

Honeydew is excreted by aphids simultaneously with ingestion of phloem-sap[1]. Hence, honeydew provides a medium for the indirect analysis of plant natural products which are translocated in the phloem. Previous analyses of aphid honeydew showed the presence of numerous plant compounds including mono-, di-, and trisaccharides[2,3], cyclitols[4], sterols and other lipids[5], phenolics, plant growth substances and organic acids[6], amino acids[2] and indolizidine alkaloids[7]. Aphid honeydew also frequently contains oligomers of fructoglucans and dextrins. But, these oligomers play a role in osmoregulation and are polymerized by transglucosylases in the aphid gut[8]. Until now, components of plant matrix polysaccharides (e.g., cellulose, hemicellulose, pectic substances, etc.) have not been isolated and identified in honeydew. This communication is the first demonstration of pectic fragments in aphid honeydew.

Materials and methods. Honeydew was collected at four, separate, 8-h periods from colonies of *Schizaphis graminum* (Rondani) biotype C (the greenbug) maintained on plants of *Sorghum bicolor* (L.) Moench cv. 'BOK-8'. Honeydew droplets were collected in shallow dishes filled with ethanol (to prevent contamination of honeydew with fungal and/or bacterial metabolites) placed beneath aphid-infested plants. The ethanol was evaporated, the residue dissolved in distilled water and fractionated by gel filtration on Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA, USA) (1.5 × 25 cm glass column, 24 ml/h) into 2-ml fractions using distilled water as eluent. Estimation of mol. wts of oligosaccharides was based upon gel filtration (Bio-Gel P-2) of a standard series of malto-oligosaccharides (Sigma Chemical Co., St Louis, MO, USA). Anion exchange chromatography of oligosaccharides was performed on Dowex 3 (Dow Chemical Co., Midland, MI, USA) (same column conditions as above) by eluting the unbound oligosaccharides for 1 h with dilute ammonium hydroxide (pH 8.0) then eluting the acidic oligosaccharides with acetic acid (pH 2.0) until the effluent was negative for carbohydrates. Methods for the identification of sugar constituents by gas-liquid chromatography and gas chromatography mass spectrometry were previously outlined[4,9]. Procedures for hydrolysis of oligosaccharides, in 2 N trifluoroacetic acid, determination of total sugars, using a phenol-sulfuric acid assay, and uronic acid content, by a carbazole-sulfuric acid-borate assay, were the same as used earlier[9]. Histochemical staining of fresh cross sections (~ 20 µm thickness) of sorghum leaves for location of pectic polyuronides was performed by firstly treating sections with 10% ferric chloride, rinsing, treating with 1% potassium ferrocyanide, then with 2% hydrochloric acid and rinsing. Subcellular structures containing acidic polysaccharides having unmethylated uronic acids appeared bright blue under the light microscope due to the formation of ferric ferrocyanide (Prussian blue) – pectic polyuronide complexes[10]. Sorghum pectic substances were isolated by macerating freshly cut leaves in acetone, extracting the marc in boiling 1% acetic acid for 1 h, freeze drying the extract, and precipitating the pectic substances two times in 60% ethanol. The precipitates were then fractionated by gel filtration on Sephadex 4B (Pharmacia Fine Chemicals, Piscataway, NJ, USA) (0.9 × 50 cm glass column, 2.4 ml/h) using 0.05 M acetate buffer (pH 5.5) as eluent. Polysaccharides eluting in the void volume were hydrolyzed and their neutral sugar and uronic acid compositions analyzed as above.

Results and discussion. Initial gel filtration of honeydew from greenbugs feeding on sorghum showed that greater than 50% of the carbohydrate content consisted of oligosaccharides having a mol. wt of 900 D to ≥ 2200 D. The other lower MW carbohydrates (i.e. mono- to tetrasaccharides) were typical to aphid honeydew[2] and are listed in table 1. The oligosaccharides in the gel filtration fractions containing larger carbohydrates (mol. wt = 900 to 1800 D, ~ 48% of total honeydew carbohydrate) consisted chiefly of typical honeydew oligomeric glucans and dextrins[2,8]. However, the sugar composition of the largest oligosaccharides in aphid honeydew (MW ≥ 2200 D, ~ 2% of total honeydew carbohydrate) included significant amounts of arabi-

Table 1. Relative percent of individual lower mol. wt carbohydrates (i.e *mono-* to tetrasaccharides) and larger oligosaccharides (i.e. pentasaccharides and larger) to total carbohydrates in honeydew of aphids of the greenbug [*Schizaphis graminum* (Rondani) biotype C]

Carbohydrate	Relative percent
Monosaccharides	
Fructose	15.1
Glucose	7.4
myo-Inositol	0.8
Disaccharides	
Sucrose	8.0
Trehalose	3.1
Maltose	1.1
Trisaccharides	
Fructosylsucrose	4.6
Unknown	1.6
Fructosylmaltose	2.7
Maltotriose	1.7
Tetrasaccharides	
4 unknowns*	4.1
Pentasaccharides and larger**	49.8

*Upon hydrolysis yielded only glucose and fructose in a ratio of 8:1; **not analyzable in the unhydrolyzed form.

Table 2. Sugar composition (relative percent) of largest oligosaccharides (mol. wt ≥ 2200 D) in honeydew of aphids of the greenbug [*Schizaphis graminum* (Rondani) biotype C] in unbound and acid fractions after anion exchange chromatography (Dowex 3) and in the pectic substances of its host plant, *Sorghum bicolor* (L.) Moench cv. 'BOK-8'

Sugar	Anion exchange fractions		Host plant
	Unbound	Acid	
Arabinose	0.9	31.3	22.0
Xylose	0.6	2.6	< 0.1
Rhamnose	0.5	1.9	6.2
Fructose	2.7	0.1	0
Galactose	1.2	30.9	32.3
Glucose	93.9	18.3	14.6
Uronic acid	< 0.1	14.9	24.8

nose, xylose, rhamnose, galactose and uronic acid (table 2). These latter sugars were not detected in the monomeric form nor as components of the lower MW oligosaccharides (table 1, mono- to tetrasaccharides). Purification of the largest oligosaccharides (MW $\geqslant$ 2200 D) by anion exchange chromatography revealed that the oligosaccharides in the unbound fraction ($\sim$ 0.5% of total honeydew carbohydrate) were oligoglucans similar to those found in the honeydew of aphids fed on artificial diets containing only sucrose[8] and, hence, were polymerized by the aphid. Conversely, the oligosaccharides in the acid fraction (1.8% of total honeydew carbohydrate) were polymers consisting chiefly of arabinose, galactose, and uronic acid, with significantly less glucose than those oligosaccharides in the unbound fraction (table 2). Furthermore, the neutral sugar and uronic acid composition of these acidic oligosaccharides was qualitatively similar to pectic substances extracted from the same host plant, sorghum variety 'BOK-8' (table 2). Greenbugs cannot synthesize polyuronides or pentans, thus, the acidic oligosaccharides in their honeydew appeared to be of plant origin. To test whether the pectic fragments could have originated in the phloem, cross sections of sorghum leaves were treated with a stain specific for pectin. This staining showed that there was an especially high concentration of pectic substances in the phloem (intracellularly in companion cells and intercellularly between sieve tube elements).

Greenbugs generally cause extensive damage to vascular tissues of susceptible varieties of their host plants[11]. Furthermore, greenbugs feed chiefly from the phloem of their host plants and possess salivary enzymes capable of depolymerizing a wide array of plant matrix polysaccharides (e.g. pectin, arabino-galactan, cellulose, etc.)[9]. Hence, much of the hetero-oligosaccharides in greenbug honeydew are likely to be breakdown products of matrix polysaccharides in the phloem. It is possible, therefore, that pectic fragments which escape being ingested by aphids are translocated elsewhere in the plant.

Oligosaccharides produced from the depolymerization of plant cell walls have been found to elicit phytoalexin synthesis in a number of different plants[12]. More recently, oligouronides were shown to trigger synthesis of proteinase inhibitors[13]. Perhaps phloem translocation of specific hetero-oligosaccharides, produced by polysaccharase activity, is responsible for the variety of systemic responses commonly observed in plants infested by sap feeding insects[14] or infected with bacteria and fungi[15] (e.g. premature senescence, gall formation, hypersensitivity, etc.).

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Cannflavin A and B, prenylated flavones from *Cannabis sativa* L.

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Summary. Two novel prenylated flavones, termed Cannflavin A and B, were isolated from the cannabinoid free ethanolic extract of *Cannabis sativa* L. Both compounds inhibited prostaglandin E_2 production by human rheumatoid synovial cells in culture.
Key words. Cannabis; PGE_2 inhibition; non-cannabinoids.

The fact that the use of *Cannabis sativa* in Western medicine has undoubtedly been overshadowed by its use as a social drug[4], has inhibited research into the medicinal properties of this herb. Nevertheless, cannabis does have a tradition for the treatment of a variety of disorders in Indian and Arabian culture[5], including its use as an analgesic and anti-inflammatory agent. The anti-inflammatory action of extracts of cannabis was confirmed pharmacologically by Gill and others[6], but the mechanism of action and the chemical nature of the active principles was unknown. Further work concerned with the anti-inflammatory activity of the hallucinogenic constituent, tetrahydrocannabinol (THC) was inconclusive[7]. Fairbairn and Pickens[8] demonstrated that the cataleptic effects of THC could be inhibited both by cyclooxygenase inhibitors and a cannabinoid free extract of cannabis, thereby providing the first evidence for the presence of noncannabinoid modulators of prostaglandin levels from this plant. That these, unknown agents might be responsible for the analgesic and antiflammatory properties of cannabis was further suggested from the fact that this extract demonstrated appropriate activity in the mouse phenylbenzoquinone writhing test for nonsteroidal anti-inflammatory drugs[9]. The same extract was later shown to inhibit prostaglandin E_2 (PGE_2) release from human rheumatoid synovial cells in culture[10]. This communication describes the structures of Cannflavin A (**1**) together with a lower mol. wt analog, Cannflavin B (**3**) (fig.), isolated from the biologically active ethanolic extract of cannabis following removal of the hallucinogenic cannabinoids.

Cannflavin A (**1**) was isolated from the herb alcoholic extract,