SCIENTIFIC NOTE

Oligosaccharides in the Honeydew of *Coccoidea* Scale Insects: *Coccus hesperidum* L. and a New *Stigmacoccus* sp. in Brazil

AMAURI BOGO AND PETER MANTLE

Biochemistry Department, Imperial College of Science, Technology and Medicine, London, SW7 2AY, UK.

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Oligossacarídeos em "Honeydew" das Cochonilhas *Coccus hesperidum* L. e *Stigmacoccus* sp. no Brasil

RESUMO - "Honeydew" produzido por uma nova espécie de cochonilha brasileira, ainda prsentes não descrita, do gênero *Stigmacoccus* sp. (near *S. asper* Hempel) (Homoptera: Margarodidae) foi analisado por diferentes técnicas de cro- matografia, incluindo cromatografia de papel, bombardeamento de átomo - espectrometria de massa (FAB-MS) e cromatografia gasosa - espectrometria de massa (GC-MS). Foram identificados frutose e glucose como monossacarídeos e sucrose, maltose, trealulose, trealose e hexose-hexitol como dissacarídeos. O trissacarídeo erlose e o tetrassacarídeo glucosil-erlose foram identificados pela primeira vez por modernas ténicas de análise de ligação. Erlose foi também identificado em "honeydew" produzido pela cochonilha *Coccus hesperidum* L. (Homptera: Coccidae), juntamente com o pentâmero maltosil-erlose. Portanto, pode-se notar que este metabolismo específico de transformação nos açúcares desta série oligomérica ocorre largamente dentro da superfamília Coccoidea.

PALAVRAS-CHAVE: Insecta, FAB-MS, GC-MS, erlose, maltose, transformações metabólicas.

ABSTRACT-Analysis of the honeydew from an as yet undescribed, though distinctive, Brazilian *Stigmacoccus* sp. (near *S. asper* Hempel) by paper chromatography, Fast atom bombardment (FAB-MS) and Gas chromatography-mass spectrometry (GC-MS) identified fructose and glucose as monosaccharides and sucrose, maltose, trehalulose, trehalose and a hexose-hexitol as disaccharides. Erlose and glucosyl erlose have been identified as the tri- and tetra-saccharides in *Stigmacoccus* sp. and characterised for the first time in scale insects by modern techniques of linkage analysis. The same erlose oligosaccharides were recognised in honeydew of the common scale insect *Coccus hesperidum* L., together with the pentamer of this series, maltosyl erlose, therefore recognising that specific metabolic transformations of sugars into this oligomeric series occur rather widely in Coccoidea scale insects.

KEY WORDS: Insecta, FAB-MS, GC-MS, erlose,maltose, metabolic transformation.

Scale insects feeding on plant phloem sugars excrete a syrup termed honeydew which contains several oligosaccharides. The creation of these oligosaccharides from sucrose by transglucosylation reactions has been proposed as an osmoregulatory mechanism in these insects (Fisher *et al*. 1984). Understanding this osmoregulation process requires a knowledge of the carbohydrates involved.

Little is known about the composition of the honeydew secreted by Coccoidea, and particularly nothing appears to have been published, based on modern analytical techniques, on the honeydew produced by insects of the genus *Stigmacoccus* or even of the common *Coccus hesperidum* L. (Homoptera: Coccidae). Concerning the latter, White & Maher (1953) described a maltosyl-fructofuranoside arising by glucosyl transfer to sucrose. Gray & Fraenkel (1953) implied that this "fructomaltose" was widely associated with scale insects, aphids and bee honey. Wolf & Ewart (1955), using an enzyme reaction, deduced that the same oligosaccharide occurred in *C. hesperidum* together with melezitose and a gluco-sucrose which are other unusual trisaccharides present in the honeydew. Stephen (1959) named the gluco-sucrose "erlose" as a member of a family of oligosaccharide. However, even this smallest member is surprisingly omitted from the otherwise comprehensive treatise on the oligosaccharides (Liptak *et al*. 1991).

Most of the studies on scale insect honeydew oligosaccharides have concentrated on (Homoptera: Aleyrodidae) such as *Bemisia* spp. (whiteflies), in which the unusual disaccharide trehalulose was first identified in *B. tabaci* (Byrne & Miller, 1990). Later, Hendrix (1994) identified bemisiose as another unusual trisaccharide. Isaacs *et al*. (1998), studying the composition of cucurbit phloem sap and *B. tabaci* honeydew, suggested that glucose made up to 60% of the honeydew sugars. The plant sap contained low levels of sucrose and raffinose. However, no sucrose or melezitose were detected in any honeydew samples. Davidson *et al*. (1994) suggested that the unusual disaccharide trehalulose, an important constituent in honeydew of *B. argentifolii*, is produced by obligate intracellular microorganisms residing in this insect's mycetomes. Some larger oligosaccharides in this honeydew may be produced by certain *Bacillus* spp. residing in or on the insects, and may contribute artefacts to the composition of honeydew collected from rather heterogeneous surfaces. Such bacteria are not involved in an obligate relationship with the insect. Consequently, the observation of the uncontaminated excretion of honeydew on long (ca. 5 cm) hyaline anal extensions of an as yet undescribed species of *Stigmacoccus*, though near *S. asper* Hempel (Bogo *et al*. 1998), prompted application of modern linkage analysis methodology to define the composition of the oligosaccharides which arise entirely from enteric transformation of the plant sugars, sucrose and glucose, ingested by the scale insect in phloem sap.

Honeydew from scale insects (*Stigmacoccus* sp. and *C. hesperidum*) infesting respectively two different plants - *Schizolobium excelsum* Vogel, a leguminous native tree from Amazonia established in the Botanic Garden of the Federal University of Santa Catarina, Florianopolis, Brazil; and an ornamental *Hedera* sp., used as a house-plant in England - were collected by capillarity into glass tubes.

Oligosaccharide isolation: Descending paper chromatography [Whatman 3MM paper; solvent, propan-1-ol:ethyl acetate:water (7:1:2)] for 48-55 h resolved standards (fructose, glucose, sucrose and raffinose) and analytically and/or preparatively separated sugars in honeydew. Sugars were located analytically by dipping chromatograms in aniline hydrogen phthalate reagent and heating at 120° for 20 min. Oligosaccharides were eluted preparatively in warm water, repurified by paper chromatography when necessary, and freeze-dried prior to saccharide analysis.

Linkage analysis**:** Oligosaccharide composition was determined by a combination of fast atom bombardment-mass spectrometry (FAB-MS) analysis of permethylated derivatives (Dell *et al*. 1994) and gas chromatogra-

phy-mass spectrometry (GC-MS) analysis after the standard procedure of hydrolysis, reduction and peracetylation had been applied to the permethylated saccharides (Carpita *et al*. 1989). Helium was the carrier gas for GC-MS and samples were injected in 1 microlitre of hexane solvent which used a DB-5 capillary column in a temperature gradient 90°C - 190°C (20°C min^{-1}), 190°C - 210°C (1°C min^{-1}), 210°C-300°C (25°C min^{-1}) in A a Fisons MD 800 system (ThermoQuest-Masslab, Manchester, UK) in electron impact mode which revels fragment ions but not the molecular ion, and B a Micromass autospek Q system (Micromass, Withenshaw, Manchester, UK) in chemical ionisation mode to show molecular ions of derivatised monosaccharides.

Paper chromatography of the *Stigmacoccus* honeydew showed that the principal sugar was sucrose. Fructose was clearly evident and there were lesser amounts of glucose and sugars with chromatographic mobilities indicative of di-, tri- and tetrasaccharides. The composition of the disaccharide group was complex. FAB-MS data after permethylation showed an approximately 2:1 ratio of di-hexoses (m/z 477) to hexose-hexitol (m/z 493). The composition of the latter was not defined further. However, rechromatography, and subdivision of the disaccharide region to three parts according to mobility, enabled interpretation of complex GC-MS data for sugar linkage analysis, changing markedly across the three parts of the region, to indicate the presence of trehalose, trehalulose and maltose. The trisaccharide and tetrasaccharide in *Stigmacoccus* sp. honeydew appeared to be identical to the analogous components of *C. hesperidum* honeydew, in which there was an additional compound assumed to be a pentasaccharide.

The molecular masses of permethylated tri-, tetra- and penta-saccharides confirmed that they were all hexose polymers with molecular-ions in FAB-MS of m/z 658, 862 and 1067 (M+Na= 681, 885 and 1090, respectively). Linkage analysis by GC-MS showed (Table 1 and Fig. 1), for both *Stigmacoccus* sp. and *C. hesperidum* oligosaccharides, glucopyranose linked either in the 1 position or in both 1 and 4 positions, and fructofuranose linked in the 2 position, as it is in sucrose, indicating the presence of an "erlose" series of compounds.

From the ion-current intensities of tri-, tetra-, and penta-saccharides (Fig. 1) there was a systematic increase in the proportion of (1→4) Glc to 1- Glc with each unitary increase in the number of monosaccharides. The proportion of 2- fructofuranose to 1-Glc varied somewhat, but this is a normal experience in the rather harsh hydrolysis conditions which were used efficiently to cleave linkage between methylated glucose units. Fructose is variably labile under these conditions. It is therefore concluded that the oligosaccharides in *Stigmacoccus* sp. honeydew were erlose and glucosyl-erlose, and that in addition the *C. hesperidum* honeydew contained the next polymer in the series, maltosyl-erlose. Demonstration by modern linkage analysis should therefore justify full recognition of the "erlose" series of oligosaccharides and the use of this trivial name. Also between the two quite different scale insects, a common pattern of oligosaccharides seems to exist.

The analysis of the phloem sap from the host plant of the *Stigmacoccus* sp., obtained by excising the scale insect *in situ* to leave embedded mouth parts and using the same honeydew's oligosaccharide identification thecnique, showed that sucrose and glucose were the only sugars. There was no evidence of any of the oligosaccharides which have been recognised in the excreted honeydew.

The recognition of maltose in *Stigmacoccus* sp. honeydew for the first time, and which was readily collected in the field from the tip of the long anal wax filament without environmental contamination, is also consistent with the proposed structure of the triose erlose, from which maltose could arise by hydrolysis of the sucrose component.

Trehalose and trehalulose have already been described as components of *Myzus persicae* and *B. tabaci* honeydew (Fisher *et al*. 1984 and Bates *et al*. 1990, respectively).

Table 1. Gas chromatography retention time of derivatives of monosaccharides, which were released during linkage analysis of oligosaccharides from the honeydew of scale insects (*Stigmacoccus* sp. and *C. hesperidum*), together with the relative intensities of electron mass spectral fragment ions.

Derivatised monosaccharides	Important fragment ions (GC-EIMS)													
	Rt (min)	87	99	101	102	118	129	145	161	162	163	205	233	LINKAGE
2,5-di-acetyl (2D)-1,3,4,6-tetra-*O*- methyl hexitol	17.95	+					+++		+	++	+	+		2 - fructose
1,5-di-*O*-acetyl(1D) 2,3,4,6 tetra-*O*-methyl hexitol	18.64	+		+	+++	++	++	++	+	+		+		1 - glucose
1,4,5-tri-O-acetyl(1D)-2,3,6-tri-O-methyl hexitol	20.03		+		+	+++							+	1,4 - glucose
1,5,6-tri-O-acetyl(1D)-2,3,4-tri-O-methyl hexitol		+			+++	++	+							1,6 - glucose*

*Data from Dell (1994).

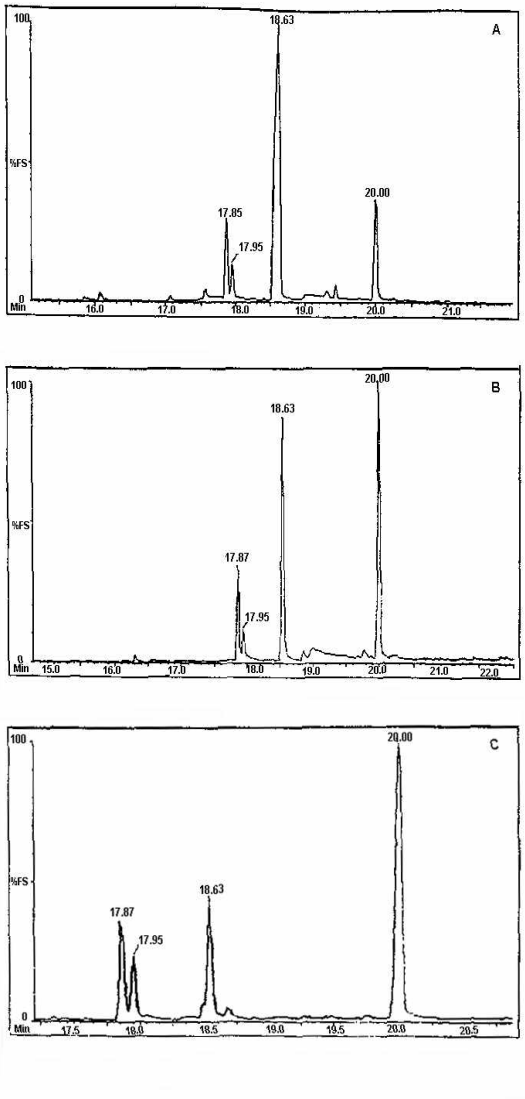

Fig. 1 Gas-chromatographic separation of derivatised monosaccharides (Table 1) from erlose (A), glucosyl-erlose (B) and maltosyl-erlose (C), showing relative ion current yield of 2-fructofuranose (17.9 min), 1-glucopyranose (18.6 min) and 1,4-glucopyranose (20 min).

However, the occurrence of maltose, trehalose, trehalulose and a hexose-hexitol appear to be unique findings for scale insects, extending the range of natural occurrence of these sugars.

The significant difference between the composition of excreted honeydew and the plant sap is attributed to metabolism within the scale insect. At present it is not possible to differentiate between activity by the insect's enzymes and those of any microbial symbionts such as have been described for other Coccoidae (Davidson *et al*. 1994; Bates *et al*. 1990). However, the present application of modern analytical techniques to even very small amounts of natural material demonstrates the potential for recognising and discovering minor components in very small volumes of scale insect excreta. It also emphasises the complex biotransformations within scale insects, which in the present example of *Stigmacoccus* sp. form an integral part of a food web involving a wide range of flying insects. Such insects are seen to feed on the clear colourless honeydew droplets at the end of the long wax anal filaments of insects attached to the bark of large forest trees in Brazil.

All the necessary stages of the *Stigmacoccus* sp. have been obtained for a full description of the new species.

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