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Note

N.m.r. characterization of trehalulose from the excrement of the sweet potato whitefly, *Bemisia tabaci*

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As with other homopterous insects such as aphids, whiteflies feed by inserting their piercing–sucking mouthparts into the phloem tissue of plants[1,2]. Plant phloem sap has a high concentration of carbohydrates, typically sucrose, but a relatively low concentration of such other nutritional components as minerals and amino acids. In order to obtain sufficient levels of these relatively scarce components, a large quantity of phloem sap is processed by homopterans. The material which is not utilized passes through the alimentary canal and exits the animal through the anus as honeydew.

Two of the authors (DNB and WBM, unpublished results) recently found that 1-*O*-α-D-glucopyranosyl-D-fructose (trehalulose) constituted a large percentage (up to 51%) of the carbohydrate found in the honeydew produced by the sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), when the insect was feeding on poinsettia plants (*Euphorbia pulcherrima* Willd.). Trehalulose (**1**) was characterized through extensive n.m.r. studies detailed here.

EXPERIMENTAL

Honeydew collection. — Approximately 100 *B. tabaci* insects were placed in petri-dish clip cages on the underside of poinsettia leaves, and allowed to feed for 48 h. Using this method, the excreta fall to the bottom of the petri dish. Honeydew from ~2000 insects was collected, solubilized in glass-distilled water, and passed through a column (5 mL) of Dowex MR-3 mixed-bed ion-exchange resin. Neutral substances were eluted with distilled water, and evaporated to a clear syrup under vacuum at 40°.

Preparative l.c. of trehalulose (**1**). — The l.c. system consisted of an LKB 2150 pump and a Rheodyne 7010 injector equipped with a 20-μL sample loop. Two Bio-Rad

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HPX-87C (calcium based) stainless-steel columns (300 x 7.8 mm i.d.) were connected in series and maintained at 85°. A guard column (Bio-Rad micro guard) was used at room temperature between the injector and analytical column. The isocratic system was operated at 0.3 mL min^{-1} using degassed water as the mobile phase. Detection was via refractive index (Knauer 198).

The syrup (from the foregoing) was diluted slightly in l.c.-grade water, and multiple injections (20 μL) made into the l.c. Fractions of 10 sec were collected with a fraction collector. Fractions enriched in trehalulose (**1**) were combined and evaporated in a N_2 stream at 40°. Subsequent l.c. analyses indicated the purity of the trehalulose (**1**) collected to be in excess of 98%. The total mass of trehalulose (**1**) collected was 8 mg. Retention times of known standards and of purified trehalulose were determined using the foregoing system with a single analytical column.

N.m.r. spectra. — ^{1}H-N.m.r. spectra were recorded at 500 MHz on a Bruker AM-500 spectrometer, and ^{13}C-n.m.r. (APT) at 100 MHz on a Varian XL-400 instrument. ^{1}H–^{1}H COSY and ^{1}H–^{13}C 1-bond HETCOR spectra were very helpful in making the assignments in Table II.

RESULTS AND DISCUSSION

L.c. characteristics. — L.c. retention behavior of trehalulose (**1**) and several mono-, di- and oligo-saccharides are given in Table I. Trehalulose was baseline-separated from all of the disaccharides tested, including sucrose, isomaltulose, trehalose, and maltose. Earlier reports[3] used amino l.c. columns and acetonitrile-based solvents for large-scale preparative l.c. of trehalulose. In our small-scale purification

TABLE I

L.c.[a] retention behavior of trehalulose and several other sugars

Carbohydrate	*Relative retention time*[b]
Stachyose	0.657
Melezitose	0.703
Raffinose	0.712
Trehalose	0.800
Sucrose	0.801
Maltose	0.811
Isomaltulose (palatinose)	0.830
Lactose	0.844
Trehalulose	0.941
Glucose	1.000
Galactose	1.128
Mannose	1.159
Fructose	1.271
Mannitol	1.692

[a] L.c. conditions as follows: Bio-Rad HPX-87C stainless-steel column (300 x 7.8 mm i.d.), temp. = 85°, degassed water at 0.6 mL per min. [b] Relative to glucose, which had a retention time of 10.48 min.

TABLE II

N.m.r. shifts (δ, D_2O) and coupling constants (Hz, in parentheses) for trehalulose tautomers **1**–**3**.

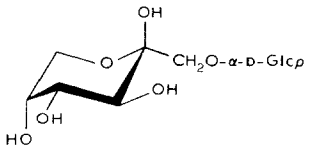
	1		**2**		**3**
Carbon number	$^{13}C^a$	$^1H^b$	^{13}C	^1H	^{13}C
Glc-1	99.8 d	4.96 d (3.7)		4.99 d (3.8)	
Glc-2	72.8 d	3.57 dd (9.7, 3.7)			
Glc-3	74.3 d	3.77 t (9.7)			
Glc-4	70.8 d	3.42 t (9.4)			
Glc-5	73.2 d	3.72 dd (9.4, 2.0)			
Glc-6	61.8 t	3.78 dd (12.3, 9.4) 3.87 dd (12.3, 2.0)			
Fru-1	70.4 t	3.45 d (10.3) — 3.92 d (10.3)	69.8 t		
Fru-2	99.1 s	—	102.2 s		107.8 s
Fru-3	69.2 d	3.83 d	77.6 d		77.9 d
Fru-4	70.9 d	3.90 dd (10.1, 3.3)	75.7 d		83.2 d
Fru-5	70.4 d	4.01 br s	82.0 d		83.6 d
Fru-6	64.9 t	3.70 br d (12.8) 4.06 br d (12.8)	63.6 t		62.7 t

[a] Relative to 1,4-dioxane at δ 67.4. [b] Relative to sodium 4,4-dimethyl-4-silapentanesulfonate at δ 0. [c] Not observed.

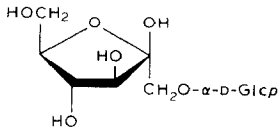
work (with a calcium based l.c. column), we used water as the l.c. mobile phase and thus eliminated exposure to toxic acetonitrile.

N.m.r. parameters. — The n.m.r. parameters obtained for trehalulose (**1**), given in Table II, are much more complete than those reported earlier[3]. The spectra are complicated by the occurrence of three tautomers in D_2O in the ratio 20:4:1. By analogy with fructose itself[4], these would be expected to be the β-pyranose, β-furanose, and α-furanose, respectively. Comparison of the ^{13}C-n.m.r. parameters with those of the methyl fructosides[5] confirms that this is the case. APT, COSY, and HETCOR spectra made it possible to correct some tentative assignments made earlier[3] for the two major tautomers and to make assignments for the α-furanose.

Trehalulose biosynthesis. — Bacteria in the genus *Erwinia* have been found to synthesize trehalulose and release it to the bathing medium when sucrose is given as a



1



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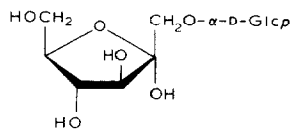

3

carbon source[6–8]. Avigad[9] and Cheetham[10] found fructose to be a non-competitive inhibitor of the hydrolytic activity of isomaltulose synthase (EC 5.4.99.10), the enzyme apparently responsible for trehalulose and isomaltulose formation from sucrose. When fructose is present in the incubation medium, the enzyme catalyzes the formation of a wide variety of di- and tri-saccharides. Trehalulose was formed as a major product (along with isomaltulose) during sucrose cleavage in the presence of high fructose concentrations[9,10].

The foregoing findings provide a speculative model for the formation of trehalulose by *B. tabaci*. Homopterans maintain bacteriod symbionts in their mycetocytes[11]. Currently, it is unknown whether isomaltulose synthase is present in the *Bemisia* symbionts. Its presence, however, based on the findings described here, could provide the mechanism for trehalulose synthesis. For most plant species, sucrose serves as the phloem-mobile, or translocated carbohydrate[12], and thus composes the majority of the carbohydrate available to *B. tabaci*. Sucrose arriving in the mycetocyte could be hydrolyzed by bacteriod invertase (EC 3.2.1.26) or isomaltulose synthase. Under these conditions, fructose could accumulate and non-competitively inhibit the hydrolytic reaction of isomaltulose synthase. The trehalulose formed would then be expelled from the insect gut into the excretory tract, and excreted from the body. Indirect evidence supporting this model comes from the fact that honeydew from *B. tabaci* feeding on pumpkin (a plant species that primarily translocates the oligosaccharides stachyose and raffinose[13]) contained lower percentages of trehalulose than honeydew produced by *B. tabaci* feeding on sucrose-translocating species (e.g. poinsettia) (Byrne and Miller, unpublished results). Additional, direct experiments are necessary to confirm this model.

It would appear that trehalulose is a carbohydrate that is unavailable to the insect for further metabolism. Trehalulose formation may be an adaptation by the symbionts to periodic shortages of carbohydrate, allowing sequestering of a carbon source in a form unavailable to competing organisms (*i.e.* the *Bemisia* host). Such a mechanism has been identified in *Pseudomonas*[14,15].

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