Carbohydrate Research, 253 (1994) 329–334
Elsevier Science B.V., Amsterdam

Note

Bemisiose: an unusual trisaccharide in *Bemisia* honeydew †

Donald L. Hendrix [a,*] and Yuan-an Wei [b,1]

[a] *Western Cotton Research Laboratory, United States Department of Agriculture, Agricultural Research Service, 4135 East Broadway Road, Phoenix, Arizona 85040 (USA)*
[b] *Department of Chemistry, Guangxi University, Nanning, Guangxi 530004 (People's Republic of China)*

(Received July 17th, 1992; accepted in revised form, August 7th, 1993)

Homopteran insects feeding upon on plant phloem sugars excrete a syrup termed honeydew which contains high concentrations of oligosaccharides. The creation of these oligosaccharides from sucrose in the phloem sap by transglycosylation reactions[1] has been proposed as an osmoregulatatory mechanism in these insects[2]. Understanding this osmoregulation process would require a knowledge of the carbohydrates involved, but very few detailed analyses of the oligosaccharides in these excretions exist. A recent analysis of the smaller sugars in honeydew from the silverleaf whitefly (*Bemisia argentifolia*) honeydew showed that its most abundant saccharide component was the unusual disaccharide trehalulose[3].

In order to analyze this sugar mixture more completely, a gradient high-performance anion-exchange chromatography (HPAEC) analysis of *Bemisia* honeydew was carried out which showed that this syrup was composed of ~30 components (Fig. 1) including an unknown sugar that constituted about 3% (peak area) of the total sugars in the mixture. Chemical-ionization and electron-ionization gas chromatography, enzyme digestion, and ^{1}H and ^{13}C NMR experiments showed this saccharide to be a trisaccharide consisting entirely of α-D-glucose residues. From these results, a structure was proposed for this oligosaccharide (Fig. 2) which is identical to a trisaccharide that has been recently synthesized[4,5] but which has not been previously reported to occur in higher organisms. The common name "bemisiose" is suggested for this sugar because it was found in *Bemisia* honeydew.

An HPLC analysis of a trifluoroacetic acid hydrolyzate of bemisiose, using an anion HPLC column[6,7], showed that it consisted entirely of glucose. Methylated

† Reference to a brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

[1] Part of a thesis submitted by Y.-A. W. to the Department of Chemistry, Institute of Materia Medica, Academia Sinica, Shanghai, P.R.C.

* Corresponding author.

SSDI 0008-6215(93)E0285-9

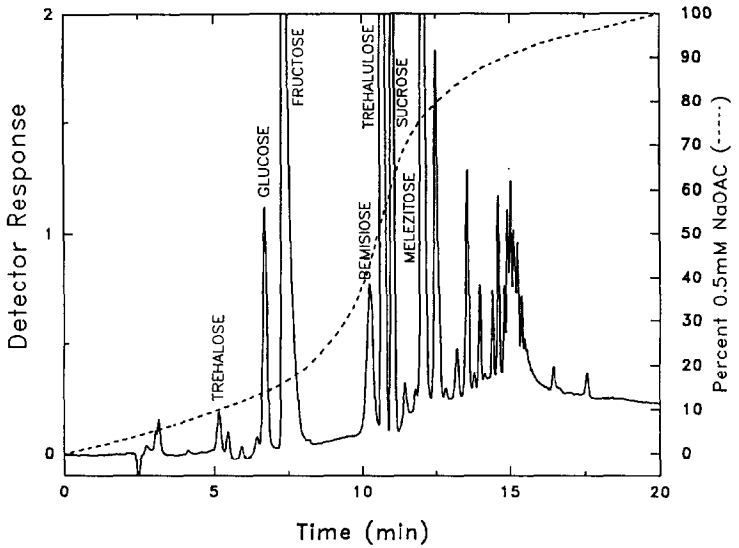

Fig. 1. Sodium acetate-gradient HPAEC analysis of *Bemisia argentifolia* honeydew.

bemisiose was analyzed directly by both chemical-ionization, using ammonia as the ionizing reactant (NH_3-CI), and electron-ionization (EI) gas chromatography–mass spectrometry. Permethylated bemisiose produced only a single peak in gas chromatography which resulted in a molecular-ion peak in CI analysis of 658 ($M + NH_4 = 676$), indicating that the molecular weight of this compound was 504. Mass/charge peaks typical of trisaccharide degradation products (155, 187, 219, 359, 391, 423, and 483) were also found in the EI spectra of permethylated bemisiose. These data are all consistent with bemisiose being a trisaccharide consisting entirely of glucose subunits.

Further investigation of the permethylated alditol acetates of bemisiose by GC–MS analysis provided additional information about the linkage between the glucose subunits in this saccharide. The data in Table I show two major peaks in

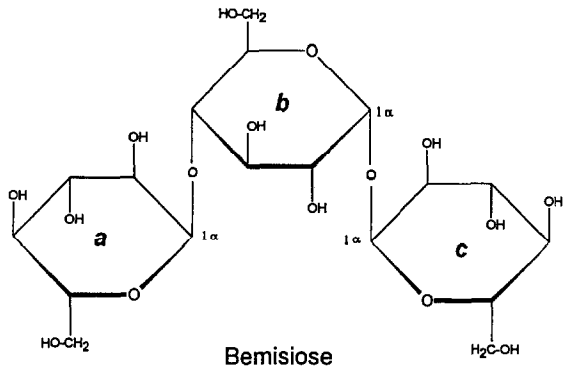

Fig. 2. Proposed structure of bemisiose.

TABLE I

GC–MS analysis of permethylated bemisiose

Peak number [a]	Main mass fragments (m/z)	Note
1	102, 129, 145, 162, 205, 217, 246	T-Glc [b], main peak
2	101, 118, 161, 174, 209, 234, 277	3-Glc, minor peak
3	99, 110, 162, 173, 234, 277	4-Glc, large peak
4	87, 118, 185, 203, 231, 305	3,4-Glc, minor peak

[a] Relative retention on a Supelco SP 2330 column (30 m). [b] Terminal glucose.

the spectra which correspond to terminal glucose and 4-linked glucose, with minor peaks corresponding to 3-linked glucose and to 3,4-linked glucose. The small amounts of 3-linked and 3,4-linked glucose detected in these assays are probably due to undermethylation of the sample. Hence, from these MS data it is concluded that bemisiose is a trisaccharide containing Glc-(1 ↔ 1)-Glc and Glc-(1 → 4)-Glc linkages.

Data from ^{1}H and ^{13}C NMR experiments confirmed the structure just proposed. In addition, the NMR spectra also revealed the conformation of the anomeric carbon atoms in bemisiose. From the ^{1}H NMR data (not shown), the coupling constants of the anomeric proton ($J_{1,2}$), referred to each glucose residue in bemisiose, could be determined (δ 5.43, d, 1 H, $J_{1,2}$ 4.00 Hz; 5.19, d, 1 H, $J_{1,2}$ 3.50 Hz; and 5.20, d, 1 H, $J_{1,2}$ 3.50 Hz). These data indicated that the anomeric configuration of all three glucose units in bemisiose was α. This conclusion was confirmed by enzymic analysis. Porcine trehalase released glucose from this molecule, and this enzyme is highly specific for the α-D-Glc-(1 ↔ 1)-α-D-Glc glycosyl linkage with respect to both the identity of the sugars involved in the bond and their anomeric configuration[8].

The NMR spectra shown in Table II agree closely with the spectra of synthesized compounds that were reported to have the same structure as that proposed for bemisiose[4,5] except for being shifted downfield by 2 ppm. Except for this shift, the peak assignments in all three papers are very close except for the assignment for atoms C-3a and C-3b. Both Koto et al.[5] and Ajisaka and Fujimoto[4] based their peak assignments only on ^{13}C NMR data. However, in 2D NMR, especially in ^{1}H-COSY spectra, it was possible to clearly identify the peaks due to the H-3a ($\delta = 3.702$ ppm) and H-3b ($\delta = 4.118$ ppm) protons. These could be correlated with the ^{13}C peaks at $\delta = 75.98$ and $\delta = 76.14$ ppm, respectively, in the HMQC spectra of this compound. The correct ^{13}C peak assignments for these two carbons are therefore the reverse of those suggested in the two earlier publications.

EXPERIMENTAL

Material.—Honeydew, collected from *Bemisia argentifolia* feeding upon upland cotton (*Gossypium hirsutum* L.) was fractionated according to size using gel-per-

TABLE II

Comparison of ^{13}C chemical-shift assignments (ppm) for bemisiose

Atom	Koto et al.[5] (Me_4Si, 0.00 ppm)	Ajisaka and Fujimoto[4] (CH_3CN, 1.3 ppm)	Hendrix and Wei (TSP-d_4, 0.00 ppm)
C-1a	101.0	100.6	102.76
C-1b	94.7	94.4	96.53
C-1c	94.5	94.2	96.29
C-2a	72.3	72.7	74.84
C-2b	72.0	71.8	73.97
C-2c	72.1	72.1	74.19
C-3a	74.3	74.0	75.98
C-3b	74.1	73.8	76.14
C-3c	73.9	73.6	75.68
C-4a	70.6	70.3	72.46
C-4b	78.2	78.0	80.05
C-4c	70.9	70.7	72.80
C-5a	73.0	73.7	75.81
C-5b	73.8	71.7	73.83
C-5c	73.4	73.2	75.31
C-6a,b,c	61.7	61.5	63.64

meation chromatography with a large (2.5 × 1500 cm) column of Bio-Gel P-2. Honeydew sugars were eluted from this column with degassed, deionized water and were detected in the eluate with a refractive-index detector. A fraction from the P-2 column that contained primarily di- and tri-saccharides was concentrated by lyophilization and fractionated further by normal-phase HPLC using two columns in series which contained amino functional groups (Alltech Econosphere NH2) and an elution solvent[9] consisting of 71% CH_3CN. Bemisiose exhibited retention times similar to that of other trisaccharides on both the gel permeation and amino columns. The amino columns were utilized to purify ~10 mg of bemisiose from the P-2 eluate for further analysis by NMR and GC–MS.

High-performance anion-exchange chromatography.—The HPAEC system consisted of a Dionex gradient pump and a pulsed electrochemical detector (PED) operated in the integrated amperometric mode, a Spectra Physics model AS3500 autosampler programmed to inject 25 μL samples onto the columns, and two Dionex Carbopac PA1 (4 × 250 mm i.d.) columns connected in series. Elution of the injected sample was carried out at 1.0 mL/min, using 200 mM NaOH as the mobile phase and a sigmoidal gradient of 0 to 0.5 M NaOAc (Fig. 1).

Partial hydrolysis of bemisiose.—Activated Dowex 50 W (H^+ form, 200–400 mesh) resin was prepared by Keleti's method[10]. A 100-μg sample of bemisiose was dissolved in 200 μL of water in a small screw-top glass test tube and 0.5 mL of activated Dowex resin was added. The tube was then sealed and the mixture was placed in a boiling-water bath and shaken periodically for 3 h to effect the hydrolysis. The hydrolyzed mixture was washed into an empty 11-mL plastic column connected to a small C_{18} column (Waters Sep-Pak). The hydrolyzate was

washed through the columns with 8 mL of distilled water. The eluant was collected, concentrated by evaporation under N_2, and the residue was subjected to HPAEC analysis.

Methylation and GC–MS analysis of bemisiose.—Bemisiose was methylated by the Hakomori procedure[11]. After permethylation, one part of the sample was analyzed directly by both CI and EI GC–MS. Samples were injected onto a 15-meter DB-1 capillary column (J&W Scientific) and a Hewlett–Packard 5985 mass spectrometer was used to analyze the column effluent. The remaining portion of the sample was converted into permethylated alditol acetate derivatives, which were analyzed by GC and GC–MS using a 30-m SP2330 column (Supelco) and the same mass spectrometer for analysis of the resulting ions.

Enzymic method to test for the α-D-glucose-(1 ↔ 1)-α-D-glucose structure in bemisiose.—A 50-μL sample of bemisiose (1 μg/μL) and 125 μL of the citrate buffer (50 mM, pH 5.7) was added to a 25-μL suspension of trehalase (Sigma Chemical Co., T-8778) in 1.5-mL microcentrifuge tubes. After vortexing the tubes they were then incubated for 30 min at 37°C. Following this incubation, the reaction was quenched by placing the tubes in a 100°C water bath for 2 min. The digested bemisiose solution was then analyzed for glucose released during the digestion, using a method based upon D-glucose 6-phosphate dehydrogenase (Sigma 115-A).

NMR spectra of bemisiose.—All NMR experiments were conducted with bemisiose that had been purified using the amino-HPLC columns[9]. Following removal of the HPLC solvent from the material collected from the HPLC column by evaporation under N_2, the collected sugar material was dissolved in water and lyophilized. A 5.9-mg sample of the resulting powder was then dissolved in D_2O and analyzed on a Varian Unity 500 spectrometer. Chemical shifts were referred to an internal standard, 4,4-dimethyl-4-silapentanoate-2,2,3,3-d_4 [TSP-d_4] (0.00 ppm), which was dissolved in the same solution as the carbohydrate.

ACKNOWLEDGMENTS

The mass-spectral analyses in this paper were carried out at and were supported in part by the USDA/DOE/NSF Plant Science Center in Athens, GA; this particular center has been funded by the Department of Energy grant DE-FG09-87-ER13810. The authors thank Dr. Russell W. Carlson, Dr. Otto Kandler, Dr. Yoshikazu Nakajima, Dr. Dwayne Fink, Mr. Terry Steele, and Dr. Ronald A. Nieman for their valuable assistance.

REFERENCES

1 E.J. Hehre, G. Okada, and D.S. Genghof, *Adv. Chem. Ser.*, 117 (1971) 309–333.
2 D.B. Fisher, J.P. Wright, and T.E. Mittler, *J. Insect Physiol.*, 30 (1984) 387–393.
3 R.B. Bates, D.N. Byrne, V.V. Kane, W.B. Miller, and S.R. Taylor, *Carbohydr. Res.*, 201 (1990) 342–345.

4 K. Ajisaka and H. Fujimoto, *Carbohydr. Res.*, 199 (1990) 227–234.
5 S. Koto, K. Yago, S. Zen, F. Tomonga, and S. Shimada, *Bull. Chem. Soc. Jpn.*, 59 (1986) 411–414.
6 D.C. Johnson, *Nature*, 321 (1986) 451–452.
7 R.D. Rocklin and C.A. Pohl, *J. Liquid Chromatogr.*, 6 (1983) 1577–1590.
8 A. Dahlqvist, *Acta Chem. Scand.*, 14 (1960) 9–16.
9 D.L. Hendrix, Y.-A. Wei, and J.E. Leggett, *Comp. Biochem. Physiol. B*, 101 (1992) 23–27.
10 G. Keleti and W.H. Lederer, *Handbook of Micromethods for the Biological Sciences*, Van Nostrand Reinhold, NY, pp. 1–3.
11 W.S. York, A.G. Darvil, M. McNeil, T.T. Stevenson, and P. Albersheim, *Methods Enzymol.*, 118 (1985) 3–40.