Characterization of Insecticidal Sugar Esters of *Petunia*

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The recent finding that leaf surface glycolipids of *Petunia* × *hybrida* Hort. plants possess insecticidal activity against sweetpotato whiteflies prompted a search for the specific compounds responsible for this biological activity. Successive liquid chromatographic fractionations of an extract of cuticular chemicals of *Petunia* plants yielded distinct fractions of two glucose esters and four sucrose esters. These different structural types of sugar esters were identified to be 2,3,4-tri-*O*-acyl-α- + *β*-D-glucopyranose, 2,3,4,6-tetra-*O*-acyl-α- + *β*-D-glucopyranose, 2,3,4-tri-*O*-acyl-α-D-glucopyranosyl-*β*-D-fructofuranoside, 2,3,4-tri-*O*-acyl-α-D-glucopyranosyl-(6′-*O*-acetyl)-*β*-D-fructofuranoside, 2,3,4,6-tetra-*O*-acyl-α-D-glucopyranosyl-*β*-D-fructofuranoside, and 2,3,4,6-tetra-*O*-acyl-α-D-glucopyranosyl-(6′-*O*-acetyl)-*β*-D-fructofuranoside, where the major acyl constituents were 2-methylbutyryl, hexanoyl, 4-methylvaleryl, and heptanoyl groups. Bioassays of the individual sugar esters against adult sweetpotato whiteflies revealed that the majority of the activity was produced by tri- and tetra-substituted sucrose esters, while highly substituted sucrose or glucose esters were weakly toxic or completely inactive.

Keywords: *Glucose esters; sucrose esters; Petunia; structures; whitefly pesticides; chromatography; NMR; GC/MS*

INTRODUCTION

Very interesting biological properties have been observed for sugar esters produced by leaf trichomes of several members of the Solanaceae family of plants, such as tobacco, potato, and tomato. These sugar esters are sucrose esters or glucose esters that are composed of the lower aliphatic acids (C_2−C_{10}) esterified to two or more of the hydroxyl groups of glucose or sucrose. Sugar esters of *Nicotiana* species have shown antibiotic properties (Chortyk et al., 1993) and sucrose esters of *Nicotiana gossei* are active greenhouse whitefly insecticides (Buta et al., 1993). Another paper has described the plant growth inhibitory activity of the glucose esters of *Nicotiana miersii* (Matsuzaki et al., 1989). Sugar esters are believed to be responsible for observed entrapment or repulsion of insect predators on wild potato species (King et al., 1988, 1993 and references cited therein), have been related to aphid resistance (Neal et al., 1990), and have shown antifungal activity (Holley et al., 1987). Exudates from the trichomes of wild tomato leaves have revealed the presence of glucose esters in the polar lipids (Burke et al., 1987; Goffreda et al., 1990). As apparent from the above references, the most interesting Solanaceae plants are those of the *Nicotiana* family, whose species, including *Nicotiana tabacum*, the commercial tobacco plant, have been the source of a large and diverse group of both glucose esters and sucrose esters. The levels and compositions of both glucose and sucrose esters of 50 *Nicotiana* species have been characterized (Severson et al., 1991). Acids esterified to sucrose or glucose were generally methyl-branched and ranged from C_2 to C_8 aliphatic acids, with methyl groups on the 2, 3, or 4 carbon of the acids. The most predominant sucrose esters had acyl groups on the hydroxyl groups of the 2, 3, and 4 carbons of the glucose portion. One of the most interesting species has been *N. gossei*, mainly due to the fact that its sucrose esters have shown potent toxicity against whiteflies (Pittarelli et al., 1993). The activity was due to 2,3-di-*O*-acyl-1′,6′-di-*O*-acetylsucroses and 2,3-di-*O*-acyl-1′-*O*-acetylsucroses, where the acyl groups were mainly 5-methylhexanoyl and 5-methylheptanoyl groups. Subsequently, two glucose esters (2,3-di-*O*-acylglucose and 1-*O*-acetyl-2,3-di-*O*-acylglucose) were also identified in the active sugar esters isolate (Severson et al., 1994). Such potent insecticidal activities of natural sucrose esters against persistent and damaging whiteflies (over $200 million losses annually in the United States alone; *Sweetpotato Whitefly*, 1994) have shown that sugar esters are a new class of "natural" insecticides that should be developed for commercial use against soft-bodied arthropods. This is particularly urgent for the control of the whitefly, which has developed resistance to commercial insecticides.

Our recent discovery that *Petunia* cultivars also produce a sticky exudate of sugar esters that are toxic to sweetpotato whiteflies (Kays et al., 1994) has prompted our structural investigation of these sugar esters. This paper details our separation and identification of the

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specific sugar ester structures and determination of the structural types responsible for the observed whitefly toxicity.

MATERIALS AND METHODS

Plant Extraction. A composite sample (about 70 kg) of the above-ground part of a cross section of 114 *Petunia* × *hybrida* Hort. cultivars was collected from plants grown in 1994 at the University of Georgia Horticulture farm. Plants were mass-washed with methylene chloride, and the resulting extract was concentrated on rotary evaporators (35 °C), to yield about 80 g of crude extract. A portion of the extract was then partitioned between hexane and acetonitrile, using 186 mL of hexane and 100 mL of acetonitrile per 5 g of extract. The sugar esters isolate (acetonitrile fraction) represented about 40% of the crude extract. This isolate was then used for further analyses or chromatography.

Analyses of Aliphatic Acids Esterified to Sugar Esters. Approximately 450 μg of the total sugar ester isolate (or of individual sugar esters) was saponified with 120 μL of 1.0 N KOH in 80% methanol−water at room temperature, for 24−48 h. Three replicates of 30 μL aliquots of the saponificates were transferred to microvials and taken to dryness under nitrogen; 30 μL of butanol containing 1 N *p*-toluenesulfonic acid was added to each capped sample vial, which was heated for 45 min at 76 °C and then cooled to room temperature. After addition of 25 μL of 5% aqueous Na_2CO_3 to neutralize the excess acid, 50 μL of distilled isooctane was added. The fatty acid butyl esters partitioned into the isooctane−butanol phase.

Gas chromatography (GC) analyses of butyl esters were conducted with an HP 5890 gas chromatograph equipped with 0.32 mm × 30 m bonded SE-54 fused silica capillary column (0.5 μm film thickness), prepared in our laboratory according to the method of Arrendale and Martin (1988). The oven temperature was held at 35 °C for 2 min and then increased at 4 °C/min to 220 °C. Injection port and FID detector temperatures were 200 and 275 °C, respectively. The carrier gas was hydrogen, with a linear velocity of 60 cm s^{-1} (at 100 °C). One microliter of each sample was injected in the splitless mode using a 0.5 min purge activation time. Relative levels of the acids were determined from the chromatographic data, using response data obtained from a mixture of fatty acid standards. The butyl esters were characterized by GC/MS data (HP 5989A GC/MS/Unix system) and by GC retention time data (using an HP 3396 Series III integrator) for an authentic standard (C_2−C_{10} acids) and blanks treated in the same manner as the samples.

Chromatographic Separation of *Petunia* Sugar Esters on Silicic Acid (SA). A portion of the total petunia sugar ester isolate (3.7 g) was separated on 120 g of 100 mesh silicic acid (Mallinkrodt AR 2847) contained in a glass chromatography column equipped with a stopcock and a 500 mL solvent reservoir with a ball joint, to allow use of a clamp and air or nitrogen pressure. The column was prepared by slurrying silicic acid with methylene chloride. The sugar isolate, dissolved in about 20 mL of chloroform, was deposited on the SA. The column was eluted with 600 mL volumes of increasing percentages (0.5, 1, 1.5, 2, 2.5, 3, etc., up to 40%) of acetone in methylene chloride. Over 130 fractions (200 mL) were collected. Fractions were concentrated on a rotary evaporator (35 °C), and aliquots were removed for GC and GC/MS analyses. Appropriate fractions were then subjected to the chromatotron separation. Pure SE5 eluted in 12% acetone−methylene chloride fractions, while pure SE6 eluted in 16% acetone−CH_2Cl_2 fractions.

Chromatotron Separation of *Petunia* Sugar Esters. Spinning thin-layer chromatography was performed on a chromatotron Model B 7924T (Harrison Research Inc., Palo Alto, CA). A circular (9-½ in.) glass plate (rotor) was coated with a 2 mm layer of silica gel 60 (EM Science) and oven-dried. The SA samples were applied as methylene chloride solutions, and the plate was dried. The eluting solvents consisted of 100 mL volumes of increasing percentages of acetone in methylene chloride, pumped at a rate of 3 mL/min. Fractions (10 mL) were collected. GE1 was separated with 6 × 100 mL portions of increasing percentages (0.5−1.75%) of acetone in methylene chloride, GE2 was separated with 4−7% acetone−CH_2Cl_2, SE3 with 18−23% acetone−CH_2Cl_2, and SE4 with 25−31% acetone in CH_2Cl_2.

GC. The sugar esters in the original isolate, in the SA fractions, and in the chromatotron fractions were characterized by GC as their volatile trimethylsilyl (TMS) ether derivatives. Sugar esters were reacted with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and dimethylformamide (DMF) in GC autosampler vials, which were sealed and heated at 75 °C for 1 h (Severson et al., 1984). One microliter samples were injected into a 0.32 mm × 30 m glass capillary GC column, coated with 0.1 μm of DB 5HT (J&W Scientific Co.). The GC oven temperature was programmed from 100 to 160 °C at 15 °C/min and then at 4 °C/min to 310 °C; the injection port was at 250 °C, and the flame ionization detector of the instrument (Hewlett-Packard 5890 with electronic pressure control) was set to 320 °C. The carrier gas (H_2) flow rate was set at 35 cm·s^{-1} (at 100 °C).

MS. Total *Petunia* sugar esters isolates, as well as silicic acid and the chromatotron liquid chromatography fractions, were analyzed as their TMS derivatives with a Hewlett-Packard 5989A GC/MS system. The GC column and conditions were the same as for the GC analyses. Total ion chromatograms were obtained. The GC/MS interface temperature was 280 °C, the ion source temperature was 250 °C, and the electron impact (EI) ionization energy was 70 eV for each analysis. Other MS conditions for the analyses were scan range of 40−650 Da, 0.88 scans/s, and electron multiplier voltage of 1866 V.

Magnetic Resonance Spectroscopy. All NMR data were acquired on a Bruker AMX400 spectrometer (400.13 MHz, ^1H). Typically, 2D ^1H homonuclear shift correlation experiments (COSY, correlation spectroscopy, TOCSY, total correlation spectroscopy) were carried out using a spectral width of 3.2 kHz for both dimensions. TOCSY data were obtained using a spin lock time of 60 ms with spin lock power of 8 kHz. For 2D ^1H−^{13}C heteronuclear experiments, a spectral width of 16 kHz was used in the ^{13}C dimension for HMQC (heteronuclear multiple quantum coherence) or 20.8 kHz for HMBC (heteronuclear shift correlation multiple bond correlation). ^1H−^{13}C coupling constants of 150 and 10 Hz were used in HMQC and HMBC experiments, respectively.

Whitefly Bioassay. Individual glucose or sucrose esters (10.0 mg) were placed into 20 mL scintillation vials and dissolved in 500 μL of methanol. Water (9.5 mL) was added and the vial sonicated for 10 min. Methanol (5%)−water was used as control. Adult whiteflies (*Bemisia tabaci* Gennadius B. Strain) were knocked from sweetpotato plants onto yellow sticky strips (Olson Products Inc., Medina, OH) on damp paper towels in flat plastic boxes, in a bioassay first devised by G. W. Pittarelli (personal communication). Each strip was 3 cm × 14 cm with two 3 cm square areas of sticky surface exposed, onto which approximately 30 adults per square adhered. Two strips were used per treatment. Treatment applications were replicated on different dates. The strips were sprayed with test compound solutions (2 mL), using an airbrush (Badger 2000), with a fine-mist nozzle setting from a distance of 30 cm, in a laboratory fume hood. Four concentrations, ranging from 0.0125 to 0.100%, were tested. Counts for mortality were made 2 h after spraying using a binocular microscope.

RESULTS AND DISCUSSION

In our initial work, we determined that the sugar esters of *Petunia* were composed of glucose and sucrose esters, with 2-methylbutyric, malonic, hexanoic, and 4-methylvaleric acids being the major aliphatic acids attached to the sugar moieties (Kays et al., 1994). An examination of the gas chromatogram of the total *Petunia* sugar ester isolate (Figure 1) reveals the complexity of the mixture, which is composed of lesser quantities of glucose esters (GE) and major quantities of sucrose esters (SE), as determined by GC/MS analy-

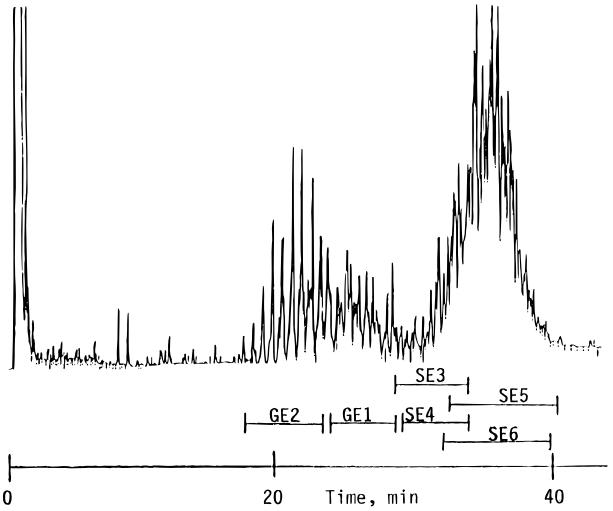

Figure 1. Gas chromatogram of total sugar esters isolated from *Petunia*.

Table 1. Initial GC/MS Characterization of Components of Silicic Acid Chromatography Fractions

fraction no.	eluting solvent	compd identification
3−6	0.25% acetone−CH_2Cl_2	fatty alcohols, sterols
8−9	1% acetone−CH_2Cl_2	diacyl glycerides
10−13	1.5% acetone−CH_2Cl_2	tetra-acyl glucoses
15−23	2.5−3.5% acetone−CH_2Cl_2	oleanolic acid, monoglycerides
31−36	5−5.5% acetone−CH_2Cl_2	triacyl glucoses
52−58	11−12% acetone−CH_2Cl_2	tetra-acyl monoacetyl sucrose
66−75	15−18% acetone−CH_2Cl_2	tetra-acyl sucrose
89−100	23−25% acetone−CH_2Cl_2	triacyl monoacetyl sucroses
103−117	29−33% acetone−CH_2Cl_2	triacyl sucroses

ses. Obviously, extensive chromatographic separations were required to isolate the individual GE or SE structural "types". (It should be explained that a "type" refers to a number of possible structures having the same substitution pattern on the sugar molecule, but with many possible aliphatic acid substituents. That is, a 2,3,4-triacylsucrose ester "type" could be any number of sucroses, with different acids, such as hexanoic, heptanoic, butyric, etc., esterified to the hydroxyl groups of C2, C3, and C4 of glucose.) The scheme to fractionate the total *Petunia* sugar esters isolate was based on previously successful approaches, involving a large scale SA column liquid chromatographic separation of the crude sugar ester isolate, followed by thin-layer chromatography of the appropriate SA fractions, which already contained partially separated sugar ester types. The successful SA separation was achieved by using a "solvent gradient" approach, where very small increases were made in the percentages of the polar solvent (acetone), such as 2%, then 2.5−3.0%, etc. This effected a slow elution, but an excellent separation of the sugar ester structural types, which differed from each other by the presence or absence of one alkyl substituent. The eluting SA fractions were analyzed by GC and GC/MS. The eluting solvents and fractions for the SA chromatography and the corresponding GE and SE types found are shown in Table 1. Using our past experience on mass spectral fragmentations of the GE and SE (Severson et al., 1994), it became apparent from the MS data that six distinct types were eluted, consisting of two glucose esters and four sucrose esters, designated GE1, GE2, SE3, SE4, SE5 and SE6 types. It was surprising to note that the SA produced excellent separation of SE5 and SE6. Thus, all in all, the SA separation, using small increases of acetone in methylene chloride, turned out to be a very successful approach, yielding two well-separated sucrose ester structural types.

To achieve further purification, GE1, GE2, SE3, and SE4 were subsequently separated by thin-layer chromatography on a chromatotron, which is an apparatus that spins a circular glass plate coated with SA. The sample and solvents are applied close to the center of the plate, and the centrifugal force and eluting solvents move the sugar esters to the edge of the plate, where the solution spins off the plate and can be collected with a fraction collector. In the chromatotron separation, the critical element was the use of a still less polar solvent−gradient system than was used to elute the compound from the SA column. Thus, for example, GE1, which eluted from the SA column with 1.5−2% acetone in CH_2Cl_2, was separated with sequential portions of 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75% acetone−CH_2Cl_2 solvents. Collected fractions were concentrated and then characterized by GC/MS. MS data showed that GE1 was comprised of tetra-acylglucose esters. The gas chromatogram of GE1, the tetra acylglucose esters, showed that there were a large number of compounds for this type of GE. As explained above, each compound has different acids attached at the 2-, 3-, 4-, and 6-positions of glucose, but each compound is a 2,3,4,6-tetrasubstituted glucose. Comparisons of the individual GE and SE gas chromatograms to that of the total *Petunia* sugar esters isolate (Figure 1) revealed the relative elution order and quantities of the isolated types in the total isolate. Thus, SE5 and SE6 apparently account for the majority of the sugar esters of *Petunia*.

The GC/MS data of the purified GE and SE were analyzed to deduce structural types. Thus, the MS data of the TMS derivatives of glucose ester type 1 compounds (GE1) (gas chromatogram in Figure 1) gave M − 15 ions at *m/z* 601, 615, and 629, indicating a series of glucose esters increasing by 14 amu. The base peak in all spectra was *m/z* 85, a C_5 acylium ion, with *m/z* 99 (C_6 acylium ion) and *m/z* 113 (C_7 acylium ion) being observed as the series increased in mass. From the M − 15 ions and carbon numbers of the acyl groups present, the data indicated a glucose moiety esterified with four acyl groups present. Thus, for example, glucose esterified with two C_5 acids and two C_6 acids gave M + 616; M − 15 = 601. Also, α and β isomers were evident from the MS spectra; therefore, the anomeric carbon of glucose (C1) was not esterified. Consequently, glucose esters type 1 (GE1) were a series of 2,3,4,6-tetra-acylglucose esters.

For GE2, the MS data showed M − 15 ions at *m/z* 575, 589, and 603, indicating a series of glucose esters with three acyl groups present. Acylium ions for C_5, C_6, C_7, and C_8 acyl groups were again observed. Again, due to the observed α and β isomers, the C1 hydroxyl was not esterified. Thus, it was clear that these glucose esters (GE2) were a series of triacylglucoses esterified at three of the four (2, 3, 4, 6) positions. However, the exact positions remained to be determined by NMR. The other four sugar ester fractions (subsequently called sucrose esters) appeared to be sucrose esters with three to five acyl substituents. The MS data for sugar esters type 3 (SE3) showed a series of ions: *m/z* 487, 501, 515 and 529, indicative of a triacylglucose esterified with mainly C_5, C_6, and C_7 acyl groups. Also observed was an ion at *m/z* 421, which indicates an acetyl group, and three TMS groups on the fructose moiety. A strong

Table 2. Relative Distribution[a] of Aliphatic Acids in *Petunia* Sugar Esters

	relative ratios						
acid	total isolate	GE1[b]	GE2[b]	SE3[b]	SE4[b]	SE5[b]	SE6[b]
acetic	0.22	0.03	0.07	0.40	0.10	0.58	0.10
isobutyric	0.02	0.04	0.01	0.02	0.00	0.04	0.04
n-butyric	0.02	0.01	0.00	0.01	0.00	0.03	0.03
2-Me-butyric	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3-Me-butyric	0.15	0.01	0.11	0.11	0.09	0.12	0.11
valeric	0.04	0.06	0.06	0.03	0.00	0.06	0.06
3-Me-valeric	0.00	0.00	0.00	0.01	0.00	0.00	0.01
4-Me-valeric	0.31	0.45	0.29	0.28	0.22	0.40	0.30
hexanoic	0.41	0.54	0.20	0.27	0.19	0.35	0.38
5-Me-hexanoic	0.04	0.03	0.00	0.02	0.04	0.01	0.03
4-Me-hexanoic	0.01	0.00	0.00	0.02	0.00	0.01	0.01
heptanoic	0.20	0.23	0.29	0.19	0.26	0.17	0.22
6-Me-heptanoic	0.02	0.02	0.03	0.02	0.10	0.01	0.02
octanoic	0.07	0.05	0.20	0.04	0.20	0.04	0.04
unknown	0.00	0.00	0.00	0.02	0.00	0.02	0.00
malonic	0.16	0.00	0.00	0.07	0.05	0.00	0.00

[a] Based on integrated peak areas, with 2-methylbutyric acid ester assigned a value of 1.00. [b] GE1, tetra-acylglucose; GE2, triacylglucose; SE3, triacylmonoacetylsucrose; SE4, triacylsucrose; SE5, tetra-acylmonoacetylsucrose; SE6, tetra-acylsucrose.

rearrangement ("R") ion at *m*/*z* 407 indicated that the acetyl group was not on C1′. As observed from other types of SE (Severson et al., 1994), a strong R also indicates that C6 was not esterified. Therefore, these compounds were identified as 2,3,4-tri-*O*-acylmonoacetylsucrose esters. The location of the acetyl group on fructose could not be determined.

The MS data for sucrose esters type 4 (SE4) (gas chromatogram, Figure 1) indicated a triacylglucose moiety, because the same series of ions were observed as for SE3. An ion at *m*/*z* 451 and a strong R ion at *m*/*z* 437 indicated that the fructose moiety had four TMS groups; therefore, these SE were identified as 2,3,4-tri-*O*-acylsucrose esters.

Sucrose esters type 5 (SE5) gave MS data that indicated that these SE were tetra-acylmonoacetylsucrose esters. This conclusion was reached on the following basis. MS data for these compounds showed a series of ions: *m*/*z* 499, 513, 527, 541, 555 and base peak of *m*/*z* 85 (C_5 acyl) indicative of a tetra-acyl glucose moiety and an ion at *m*/*z* 421 indicating a monoacetyl tri-TMS fructose. The R ion at 407 was very weak in these spectra, but this was to be expected, as esterification at C6 inhibits the formation of the R ion. Therefore, SE5 compounds had four acyl groups on the glucose portion of the molecule and one acetyl group on the fructose portion, most likely on C6′.

MS ions for sucrose esters type 6 (SE6) were very similar to SE5 data. The same series of MS ions for the glucose moiety were observed as for SE5. Instead of *m*/*z* 421, an ion at *m*/*z* 451, indicating a tetra-TMS fructose, was present. This led to the conclusion that SE6 compounds were a series of tetra-acylsucrose esters.

The next step was to determine the spectrum of acids that were esterified to the hydroxyl groups of the sugar esters. These were determined by saponification of the individual SE and GE compounds. The resulting acids were converted to their butyl esters, which were separated by GC and quantitated. The peak areas of the esters were converted to ratios, assigning the area to the largest peak, 2-methyl butyric, a value of 1.00. The resulting data are shown in Table 2. In this manner, one can more easily determine, by visual inspection, the three or four most abundant acids for each sugar ester. For the acetyl-substituted SE (SE3 and SE5), acetic acid was the next most abundant acid. For all sugar esters, hexanoic and 4-methylvaleric acids were the next most abundant acids, whereas heptanoic acid was present in slightly lesser quantities in all esters. The malonic acid in this 1994 isolate was found in lesser quantities than in the 1992 isolate, which had contained more flower parts. Also, malonic acid was only found in SE3 and SE4 compounds. The presence of malonic acid was completely unexpected as this acid had never been found in any of the sugar esters isolates of previously studied Solanaceae plants.

Even though MS data had provided a wealth of structural identification, the exact positions of the acid substituents could only be determined by NMR experiments. Accordingly, all six sugar esters were subjected to a number of ^1H and ^{13}C experiments, yielding the shift data (Tables 3 and 4) and the resulting identifications (Table 5). Standard NMR techniques (Nakanishi, 1990; Derome, 1990) were employed, and the NMR

Table 3. ^1H-NMR Shift (δ) Data for *Petunia* Sugar Esters

compd	G1	G2	G3	G4	G5	G6	F1	F3	F4	F5	F6
GE1	5.19	4.66	5.42	4.97	4.11	4.05					
GE2	5.18	4.68	5.40	4.93	3.88	3.32 3.41					
SE3	5.47	4.69	5.41	4.96	4.08	3.43 3.46	3.09 3.29	3.88	3.79	3.70	4.05 4.30
SE4	5.47	4.70	5.37	4.97	4.11	3.37 3.44	3.09 3.31	3.99	3.71	3.58	3.56
SE5	5.44	4.72	5.40	4.95	4.38	4.02	3.08 3.31	3.94	3.78	3.73	4.05 4.26
SE6	5.50	4.74	5.41	4.97	4.41	4.05	3.07 3.30	4.02	3.70	3.58	3.55

Table 4. ^{13}C-NMR Shift (δ) Data for *Petunia* Sugar Esters

compd	G1	G2	G3	G4	G5	G6	F1	F2	F3	F4	F5	F6
GE1	89.21	71.13	69.41	68.19	68.19	61.98						
GE2	88.85	71.13	69.55	68.53	69.21	60.06						
SE3	88.47	70.36	69.93	68.52	69.63	59.89	61.26	105.04	74.72	73.84	79.47	65.44
SE4	88.43	70.37	70.13	68.48	69.71	59.71	61.49	104.81	75.15	73.77	83.08	62.60
SE5	88.36	70.21	69.61	68.26	67.50	62.03	61.34	105.04	74.70	73.81	79.40	65.33
SE6	88.36	70.22	69.77	68.15	67.26	61.84	61.49	104.8	75.07	73.80	83.07	62.66

Table 5. Identified *Petunia* Glucose and Sucrose Esters

GE1	2,3,4,6-tetra-*O*-acyl-α-D-glucopyranose
GE2	2,3,4,tri-*O*-acyl-α-D-glucopyranose
SE3	2,3,4-tri-*O*-acyl-α-D-glucopyranosyl-(6′-*O*-acetyl)-β-D-fructofuranoside
SE4	2,3,4-tri-*O*-acyl-α-D-glucopyranosyl-β-D-fructofuranoside
SE5	2,3,4,6-tetra-*O*-acyl-α-D-glucopyranosyl-(6′-*O*-acetyl)-β-D-fructofuranoside
SE6	2,3,4,6-tetra-*O*-acyl-α-D-glucopyranosyl-β-D-fructofuranoside

Table 6. Adult Whitefly Bioassays of *Petunia* Sugar Esters

	% mortality[a]					
concn %	GE1[b]	GE2[b]	SE3[b]	SE4[b]	SE5[b]	SE6[b]
0.100	2	47	86	91	37	79
0.050	3	36	89	96	34	89
0.025	2	10	88	97	38	80
0.0125	1	6	70	85	26	62

[a] Mean of four replicates, control was 5% methanol−water, with percent mortality = 0.2%. [b] GE1, tetra-acylglucose; GE2, triacylglucose; SE3, triacylmonoacetylsucrose; SE4, triacylsucrose; SE5, tetra-acylmonoacetylsucrose; SE6, tetra-acylsucrose.

signal assignments were made as follows. Primary scalar correlation of ^{1}H spins were obtained by COSY experiments for all compounds. TOCSY spectra were then used to extend the scalar correlation further within the sugar rings and to confirm the assignment made by COSY spectra. Chemical shifts of protonated carbons in the sugar rings were determined in the HMBC spectra based on the ^{1}H assignments. The chemical shift of the quaternary carbon (F2) in fructose moiety of sucrose was assigned by the long-range correlation of F3 proton to the carbon in HMBC experiments. The connection of the two sugar moieties of sucrose was determined according to the cross peak of G1 proton to F2 carbon in HMBC. The positions of acyl groups in the sugar rings were determined by the long-range couplings in HMBC spectra between sugar protons and the carbonyl carbons of acyl groups. It was gratifying to see that all structures, proposed on the basis of the MS data, were confirmed by NMR.

At the conclusion of this work, a paper appeared on the isolation of sucrose esters of *Petunia hybrida* (Ohya et al., 1996). Only one of their isolated sucrose esters, the 2,3,4,6-tetra-acyl-SE, was identical to our findings. One of the other SE contained a malonic acid esterified to the 1′ carbon hydroxyl of fructose, whereas the SE identified in our studies contain malonyl groups in the SE3 and SE4 structures, which have acyl groups only on the glucose portion. It is possible that genetic differences exist between Japanese petunias, grown in a greenhouse, compared to our field-grown petunias.

Now that the structures and acid compositions of the individual sugar esters had been determined, one significant question remained: which were the most toxic compounds against whiteflies? Accordingly, the compounds were bioassayed against adult silverleaf whiteflies. In this procedure, adult whiteflies were trapped on strips of sticky tape and sprayed with various concentrations (0.0125−0.10%) of the compound. Percent mortality was determined after 2 h. The results (Table 6) were quite surprising. The most active compounds were the triacylsucroses and the triacylmonoacetylsucroses. Slightly less active were the tetra-acyl sucroses, while the tetra-acylmonoacetylsucroses and the triacylglucose esters were very weak. The tetra-acylglucoses were completely inactive. Up to now, we had assumed that all glucose esters would be as active as SE. The complete inactivity of the tetra-acylglucose esters was unexpected. Apparently, not only a specific acylation pattern but also a certain amount of polarity is required in the sugar ester structures to give whitefly toxicity. Thus, esterification of four of the five hydroxyls in glucose produces a very nonpolar structure. Even esterification of three of five of the hydroxyls, as in GE2, has greatly reduced the toxicity. In SE4, the most active sucrose ester, there are still five free hydroxyl groups and three esterified, while the next active compound (SE3) still has four free and four esterified hydroxyl groups. Thus, a medium-polarity sugar ester structure with a defined acylation pattern is a requirement for whitefly toxicity. This is a very valuable structure−activity clue for future syntheses of laboratory-prepared sucrose esters.

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