Glucosylated Suspensosides, Water-Soluble Pheromone Conjugates from the Oral Secretions of Male *Anastrepha suspensa*

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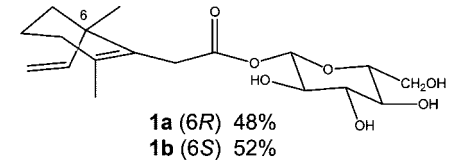
A diastereomeric mixture of the glycosylated pheromones (6*R*)- (**1a**) and (6*S*)-β-D-glucopyranosyl 2-(2,6-dimethyl-6-vinylcyclohex-1-enyl)acetate (**1b**), which we named respectively suspensoside A and suspensoside B, was isolated from the oral secretions of male Caribbean fruit flies, *Anastrepha suspensa.* The absolute stereochemical configurations were established using microsample NMR instrumentation, chiral gas chromatography, and chemical synthesis utilizing pure enantiomers of anastrephin, (3a*S*,4*R*,7a*S*)- (**4a**) or (3a*R*,4*S*,7a*R*)-4,7a-dimethyl-4-vinylhexahydrobenzofuran-2(3*H*)one (**4b**), as the aglycon precursor.

Volatile pheromones produced by male *Anastrepha suspensa* (Loew), the Caribbean fruit fly, are key factors in the natural aggregation strategy of this agricultural pest.[1−4] To attract conspecifics, males deposit aqueous oral secretions (~33 wt % sugar) at lek mating sites that emit, over many days, two diastereomeric pairs of the *trans*-fused γ-lactone 4,7a-dimethyl-4-vinylhexahydrobenzofuran-2(3*H*)-one: (3a*S*,4*R*,7a*S*)-(−)-anastrephin (**4a**), (3a*R*,4*S*,7a*R*)-(+)-anastrephin (**4b**), (3a*S*,4*S*,7a*S*)-(−)-epianastrephin (**4c**), and (3a*R*,4*R*,7a*R*)-(+)-epianastrephin (**4d**).[5] Since the γ-lactones (**4a**−**d**) have a greater potential for water to air (or lipid) phase transfer than the other structures with which they are in aqueous equilibria,[6,7] we became interested in elucidating precursory chemistry, such as hydrolysis, dehydration, and conjugation reactions, which enable the oral secretions to carry and release the volatile aggregation pheromones over prolonged periods. We describe herein the isolation of natural diastereomeric pheromone glucoconjugates named suspensoside A and suspensoside B, respectively (6*R*)- (**1a**) and (6*S*)-β-D-glucopyranosyl 2-(2,6-dimethyl-6-vinylcyclohex-1-enyl)acetate (**1b**), as well as the chemical synthesis used to confirm absolute stereochemical configuration.

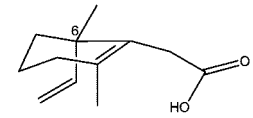
Results and Discussion

An initial structural analysis with HPLC-ESIMS of crude oral secretions from *A. suspensa* males revealed a component with prominent buffer-adduct ions of *m*/*z* 401.4 $[M + formate]^-$ and 374.4 $[M + NH_4]^+$ in negative- and positive-ionization modes, respectively (Figure 1). These results supported a molecular formula of $C_{18}H_{28}O_7$, which was later confirmed with HRESIMS. Based on column and mobile phase considerations, the retention time of this component, 20.3 ± 0.1 min, was diagnostic of nonpolar character. Yet, MS^n experiments showed the loss of *m*/*z* 162, which typifies the cleavage of a hexose monosaccharide structural unit.

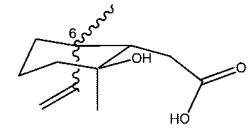
Degradation studies aided the characterization of both the glycon and aglycon of **1a** and **1b**. In the case of the glycon, base-catalyzed hydrolysis (1 M NaOH at 45 °C, 3 h) of the purified component yielded a hexose monosaccharide that coeluted with $^{13}C_6$ D-glucose during HPLC-ESIMS analysis (Figure S1, Supporting Information). Acidic methanolysis of the purified component (1 M HCl/MeOH, 80 °C, 18 h) followed by treatment with Tri-Sil (Pierce, Rockford, IL) in pyridine (80 °C, 0.5 h) yielded per-*O*-trimethylsilyated derivatives of the glycon. Subsequent analysis with GC-MS, as described in Merkle and Poppe,[8] agreed with that of an authentic standard and was used to confirm that the liberated hexose monosaccharide was glucose. With respect to the aglycon, the acid-catalyzed hydrolysis of the purified conjugate (1 M HCl at 80 °C, 3 h) resulted in **4a**−**d**, **3a**−**d**, and **2a** and **2b**; alternatively, the base-catalyzed hydrolysis described above resulted in only **2a** and **2b**. These products, which were identified on the basis of chromatographic and spectrometric agreement with purchased or synthetic standards (Figures S2 and S9, Supporting Information), suggested that the glucosylated component was either **1a**, **1b**, or a mixture of the diastereomers. It is interesting to note that all of these pheromone components are related through aqueous equilibria, and all occur in freshly collected oral secretions of males.



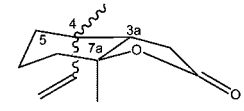
1a (6*R*) 48%
1b (6*S*) 52%



2a (6*R*) 48%
2b (6*S*) 52%



3a (1*S*, 6*R*, 2*S*) 55%
3b (1*R*, 6*S*, 2*R*) 45%
3c (1*S*, 6*S*, 2*S*) 55%
3d (1*R*, 6*R*, 2*R*) 45%



4a (3a*S*, 4*R*, 7a*S*) 55%
4b (3a*R*, 4*S*, 7a*R*) 45%
4c (3a*S*, 4*S*, 7a*S*) 55%
4d (3a*R*, 4*R*, 7a*R*) 45%

Microsample NMR studies were used to confirm that the glucosylated pheromone component naturally exists as a mixture of **1a** and **1b**. Using a novel a 1 mm triple-resonance high-temperature superconducting probe developed by Brey et al.,[9] we performed ^{1}H NMR, COSY, HMQC, and HMBC experiments on ~40 μg of isolated material in 10 μL of d_4-methanol (Table 1 and Figure 2). One salient ^{1}H NMR spectroscopic feature was the doubling of peaks diagnostic of a diastereomeric pair that was not resolved during chromatographic purification (Figure 3). The C-7 (δ_C 34.8) AB spin system clearly illustrates this relationship and

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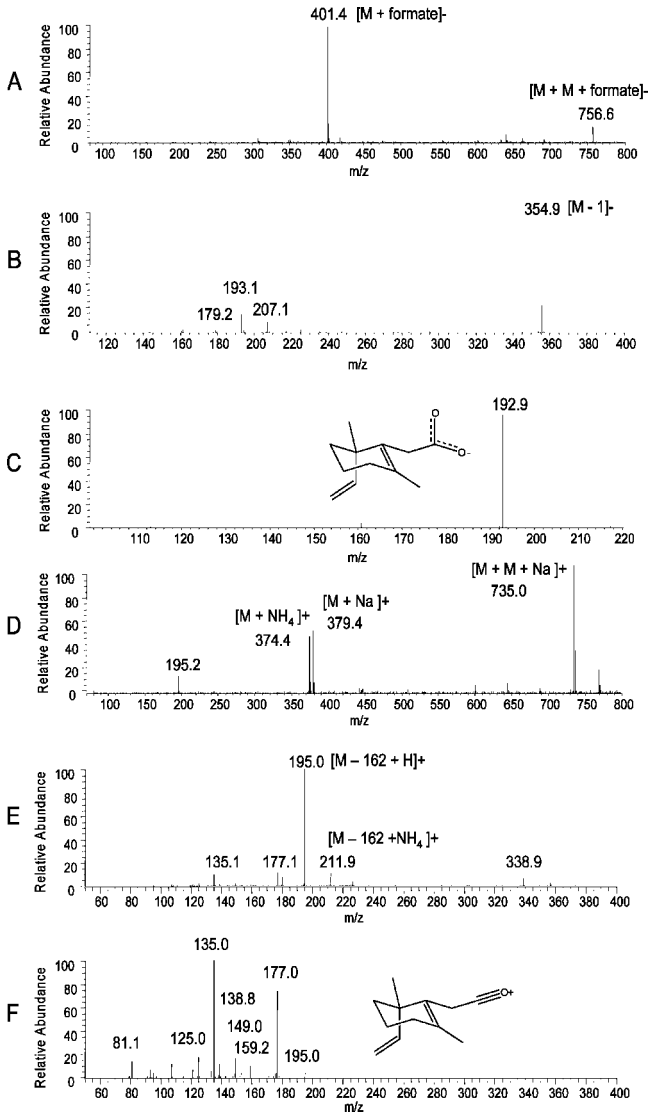


Figure 1. HPLC-(−)ESIMS of **1a** or **1b** over *m/z* 80−800 (A), MS^2 spectrum of *m/z* 401 [M + formate]$^-$ showing loss of formate (B), and MS^3 spectrum of the *m/z* (401→355) ions (C) that results from the carboxylate of the aglycon. HPLC-(+)ESIMS of **1a** or **1b** over *m/z* 80−800 (D), MS^2 spectrum of *m/z* 374 ([M + NH_4]$^+$) showing loss of a hexose monosaccahride (E), and MS^3 spectrum of the *m/z* (374→195) ions (F) that results from an acylium ion, consistent with the (+)ESIMS^n spectra of **3a**−**d** and **4a**−**d** (shown in the Supporting Information).

showed geminal coupling values ($^2J \approx 17.7$ Hz) consistent with those expected for methylene protons of this type (δ_H 3.01−3.24). The proton signals (δ_H 5.46−5.48) at the anomeric C-1′ position (δ_C 94.2) of the glycon also exhibited conspicuous peak doubling, as well as coupling ($^3J_{1'-2'} = 8.15$ Hz) associated with a diaxial arrangement of vicinal protons and a β-glucosidic orientation. Multiple-bond $^1H-^{13}C$ connectivity, assessed using HMBC, was observed between the anomeric proton at C-1′ and the most upfield carbon resonance, C-8 (δ_C 171.2). This result, in combination with base-catalyzed hydrolysis and mass spectrometry, was indicative of an *O*-glucosyl ester linkage to the aglycon. Three other quaternary carbons, not amenable to single-bond $^1H-^{13}C$ HMQC correlation experiments, were also detected with HMBC. The more deshielded methyl protons (δ_H 23.7) showed connectivity to an aliphatic carbon, C-6 (δ_C 41.9), and the more shielded methyl protons (δ_H 19.5) showed connectivity to a vinylic carbon, C-2 (δ_C 134.5); both groups of methyl protons also exhibited connectivity, albeit relatively weaker, to another vinylic carbon at C-1 (δ_C 127.0). The three vinylic protons (δ_H 4.91, 5.04, 5.71), as well as the two furthest upfield methylene proton systems (δ_H 1.46−1.53, 1.54−1.65), correlated with the aliphatic quaternary carbon at C-6 (δ_C 41.9). Another set of methylene protons (δ_H 1.98−2.12) showed analogous correlation to the two vinylic carbons at C-1 (δ_C 127.0) and C-2 (δ_C 134.5), which have no attached protons, but not to the aliphatic quaternary carbon at C-6 (δ_C 41.9). The methylene protons of the C-7 AB spin system (δ_H 3.01−3.24) were particularly useful in deciphering the core cyclohexene acetic acid structure of the aglycon, as they coupled to the four quaternary carbons (C-1, C-2, C-6, C-8) only, and not to the carbons at C-3, C-4, and C-5, which have methylene protons, respectively, at δ_H 1.98−2.12, 1.54−1.65, and 1.46−1.53. $^1H-^1H$ COSY was used to probe the three-bond vicinal (C-3/C-4, C-4/C-5, C-1′/C-2′, C-2′/C-3′, C-3′/C-4′, C-4′/C-5′, C-5′/C-6′), four-bond (C-3/C-5, C-2′/C-4′, C-3′/C-5′), vinylic (C-11/C-12), and geminal (C-3, C-4, C-5, C-7, C-6′) proton interactions at the specified carbons. In combination with COSY results, HMQC correlations were used to assign the glucose carbons (C-1′ through C-6′).

The relative stereochemical configurations of the aglycons and glucoconjugates were confirmed with chemical synthesis (Scheme 1). Briefly, the γ-hydroxy carboxylates (**3a** or **3b**) were quantitatively obtained from enantiomerically pure (>97% ee) **4a** or **4b** starting material via base-catalyzed hydrolysis (2-propanol: H_2O, 1:1 v/v, KOH). Methyl esterification, to form **5a** or **5b**, was then accomplished using methanolic CH_2N_2. Tertiary alcohol dehydration (E1), using triphenylphosphine and iodine,[10] resulted in the formation of **6a** or **6b** (∼65% yield) and the regeneration of **4a** or **4b** (∼25% yield), which were both purified using semipreparative normal-phase HPLC. Hydrolysis of **6a** or **6b** under mild basic conditions (K_2CO_3 aqueous in methanol, 15 °C) gave **2a** or **2b**. A stereoselective β-*O*-glucosylation of **6a** or **6b** with 2,3,4,6-tetra-acyl-α-D-glucopyranosyl bromide was achieved by refluxing them in ACN containing mercuric cyanide.[11] Another hydrolysis under mild basic conditions allowed for the removal of glycon acetyl groups and the formation of **1a** or **1b**.

The absolute stereochemical configurations of the natural pheromone glucoconjugates were verified by analyzing the derivatives of the base-catalyzed hydrolysates, using GC-FID with a β-Dex 120 chiral analytical column capable of enantiomeric resolution. Specifically, GC-amenable methyl esters of the natural aglycon were prepared with methanolic diazomethane for retention comparisons against synthetic **6a** and **6b**. Figure 4 illustrates that synthetic **6a** (t_R 20.64 ± 0.01 min) and **6b** (t_R 20.82 ± 0.01 min) resolve chromatographically and that the methylated natural algycon was nearly racemic (48 ± 8% **6a**; 52 ± 9% **6b**; mean ± SE, $n = 10$). For glucose determination, retention comparisons were made between the alditol trifluoroacetate derivatives[12] of purchased D-glucose (t_R 25.50 ± 0.1 min) or L-glucose (t_R 24.53 ± 0.1 min) standards and similarly derivatized glycon, which coeluted only with the former.

The pheromone aglycon is most likely conjugated enzymatically within the crop, or related digestive tissue,[13,14] as glucosidases and glucotransferases often function in an anabolic capacity[15,16] and abiotic *O*-glucosyl esterification of **2a** or **2b** was not observed under simulated physiologic conditions of 0.01 M $NaHCO_3$ buffer at crop pH (5.5) at 35 °C over a week. In other controlled experiments under these conditions, only 8 ± 3% of **1a** and **1b** was converted to **2a** or **2b**. The communicative function(s) of the glucoconjugates for *A. suspensa* is likely tied to the integrity of the *O*-glucosyl ester linkage. Enzymic, or abiotic, hydrolysis of the glucoconjugates in the oral secretions would prolong the emission of **4a**−**d** from lek sites. This is curious from an evolutionary perspective, because many fruits control the release of their volatile attractants from sugar-based matrixes in this manner.[17] Alternatively, pheromone activity of the glucoconjugates, or the enzymatically liberated aglycon, may be related to the tasting of oral secretion scent

Table 1. NMR Spectroscopic Data (600 MHz, CD_3OD) of Natural **1a** and **1b**

position	δ_c, mult	δ_H (J in Hz)	HMBC (H → C#)	COSY
1	127.0, qC		3, 7, 9, 10, 11	
2	134.5, qC		3, 4, 7, 9	
3	32.1, CH_2	1.98−2.12, m	4, 5, 9	3, 4, 5
4	18.5, CH_2	1.54−1.65, m	3, 5	3, 4, 5
5	37.5, CH_2	1.46−1.54, m	3, 4, 10, 11	3, 4, 5
6	41.9, qC		4, 5, 7, 10, 11, 12	
7	34.8, CH_2	3.07, d(17.7)[a]; 3.01, d(17.8)[b]		7
		3.18, d(17.7)[a]; 3.24, d(17.8)[b]		7
8	171.2, qC		1,7	
9	19.5, CH_3	1.66, s	3	
10	23.7, CH_3	1.12, s	5, 11	
11	146.4, CH	5.71, dd($^3J_{cis}$ = 10.6, $^3J_{trans}$ = 17.5)	5, 10	12
12	112.0, CH_2	4.91, dd ($^2J_{jem}$ = 1.6, $^3J_{trans}$ = 17.5)		11
		5.04, dd ($^2J_{jem}$ = 1.6, $^3J_{cis}$ = 10.6)		11
1′	94.2, CH	5.46, d (8.15)[a]; 5.48, d (8.15)[b]	2′	2′
2′	72.8, CH	3.32, d (8.15)		1′, 3′
3′	77.5, CH	3.34−3.38, m	2′, 5′	4′, 5′
4′	70.2, CH	3.34−3.38, m	3′, 5′, 6′	3′, 5′
5′	77.2, CH	3.34−3.38, m	3′, 4′, 6′	3′, 4′
6′	61.4, CH_2	3.68, dd	5′	5′
		3.81, dd		5′

[a,b] Proton signals of the 6*R* (**1a**) and 6*S* (**1b**) diastereomers, respectively.

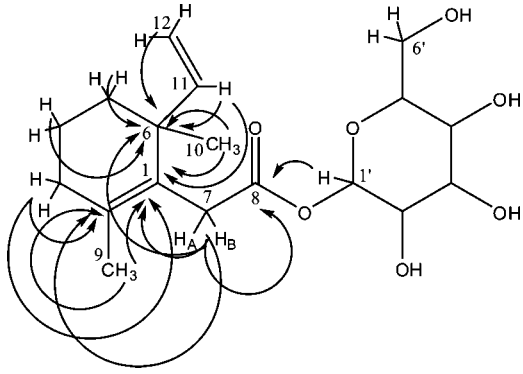


Figure 2. Selected multiple-bond $^1H-^{13}C$ correlation for **1a** and **1b** as revealed by HMBC.

markings by conspecifics, which is a common behavior within Tephritidae.[3] To substantiate the latter, future investigations will explore the physiological effects of feeding **1a** and **1b** to adult male and female *A. suspensa*.

Experimental Section

General Experimental Procedures. Enantiomerically pure (>97% ee) standards (>99%) of **4a**−**d** (lot # 882608-1:5) were purchased from Nitto Denko Co. (Osaka, Japan), and their spectroscopic and spectrometric properties are consistent with previous characterizations.[18−26] The external standard for HPLC-MS, (+)-sclareolide, was purchased from Sigma (St. Louis, MO). HRESIMS analysis was performed on a Bruker APEX II 4.7 T Fourier transform ion cyclone resonance mass spectrometer (Bruker Daltonics, Billerica, MA). Optical rotations were obtained using a Perkin-Elmer 241 polarimeter with a 0.5 mL cell. UV−vis spectra were acquired using a NanoDrop ND-1000 spectrophotometer. NMR data were collected at 27 °C using a Bruker Avance II 600 MHz spectrometer (Bruker 600 Ultrashield magnet) and a novel 1 mm triple-resonance superconducting probe with Z-gradients.[9] Chemical shift values were referenced to the residual CD_3OD solvent signal at δ_C 49.2, δ_H 3.31. Acquisition parameters for all NMR experiments are available with their respective spectra in Figures S10−S15, Supporting Information.

Gas Chromatography−Flame Ionization Detection. An Agilent 6890 N gas chromatograph equipped with a flame ionization detector (FID) was used for analyses of chirality. Holox (Charlotte, NC) high-purity helium was used as the carrier gas (1.4 mL/min). Cool on-column injection (1 μL) was at 83 °C and the detector maintained at 250 °C. GlasSeal connectors (Supelco Inc.) fused three silica columns in series: a deactivated silica column (*l* 8 cm, i.d. 0.53 mm) inserted into the injector, a deactivated fused silica column that served as a retention gap (*l* 10 m, i.d. 0.25 mm, df 0.25 μm), and a Supelco β-Dex 120 analytical column (*l* 30 m, i.d. 0.25 mm, df 0.25 μm). The oven program was as follows: isothermal at 80 °C for 5 min, heated at 7 °C/min to 150 °C, then isothermal for 35 min. Analyte t_R (min): **6a** 20.64 ± 0.01, **6b** 20.82 ± 0.01, L-glucose 24.53 ± 0.1, D-glucose 25.50 ± 0.1, **4b** 46.77 ± 0.02, **4a** 47.49 ± 0.02, **4d** 48.24 ± 0.02, and **4c** 48.76 ± 0.02.

Gas Chromatography−Mass Spectrometry. A Varian 3400 gas chromatograph and a Finnigan MAT Magnum ion trap mass spectrometer (GC-ITMS) were operated with 70 eV electron impact (EI) ionization or isobutane chemical ionization (CI). Full-scan spectra (*m*/*z* 40 to 400) were acquired at 0.85 s per scan. Cool on-column injection (1 μL) was at 40 °C with He carrier gas (1.4 mL/min). Transfer-line and manifold temperatures were 240 and 220 °C, respectively. The oven program was as follows: isothermal at 40 °C for 5 min, heated at 11 °C/min to 200 °C, isothermal for 10 min, heated at 25 °C/min to 250 °C, then isothermal for 15 min. The three-column system described above was used, except that the analytical column was a J&W DB-1 (*l* 30 m, i.d. 0.25 mm, df 0.25 μm). Analyte t_R (min): **6a** and **6b** 15.27 ± 0.01, **4a** and **4b** 17.15 ± 0.01, **3a**−**d** 17.22 ± 0.01, **4c** and **4d** 17.31 ± 0.01, and 2,3,4,6-tetra-acyl-α-D-glucopyranosyl bromide 21.28 ± 0.01.

HPLC−Mass Spectrometry. A Thermo Separation Products Spectra System P4000 pump, a ThermoFinnigan UV6000LP LDC photodiode array detector (PDA), a YMC-Pack ODS-AQ analytical column (*l* 250 mm, i.d. 4.6 mm, S 5 μm), and a Finnigan LCQ DecaXP Max mass spectrometer (HPLC-MS) were used. Mass spectra were obtained using electrospray ionization (±ESI) with a 5 kV spray voltage and a 275 °C capillary temperature. Sheath and sweep gas flow rates (arb) were 40 and 20, respectively. The mobile phase (1 mL/min) was split after the PDA; ~10% was directed to the MS and the remainder collected. Eluant composition was (a) 0.1% formic acid in ACN, (b) 10 mM ammonium formate, and (c) 10 mM ammonium formate in 90% ACN. The elution program was isocratic (4a:72b:24c) for 13.5 min, to 4:0:96 over 4.5 min, isocratic (4:0:96) for 17 min. Analyte t_R (min): **3c** and **3d** 10.3 ± 1.5, **3a** and **3b** 10.8 ± 1.6, **2a** and **2b** 12.4 ± 1.7, **1a** and **1b** 20.1 ± 0.4, **4c** and **4d** 21.8 ± 0.4, and **4a** and **4b** 21.9 ± 0.4.

Fractions containing synthetic or natural pheromone components were pooled, and the pH was adjusted to 7 with $NaHCO_3$ prior to being concentrated to dryness. The residue was dissolved in H_2O, diluted to 1 mL, transferred to 1 mL Supelco DSC-18 1 mL solid-phase extraction cartridges, and then flushed into 2 mL tubes. Salts were removed by rinsing the cartridges with H_2O (3 × 1 mL), and the analytes were eluted with three 1 mL rinses of 50% ACN into 2 mL tubes. The purified sample was then concentrated to dryness before being dissolved in d_4-methanol for NMR.

Insects and Natural Product Isolation. *A. suspensa* males were cultured as previously described.[5] After squeezing their abdomens with

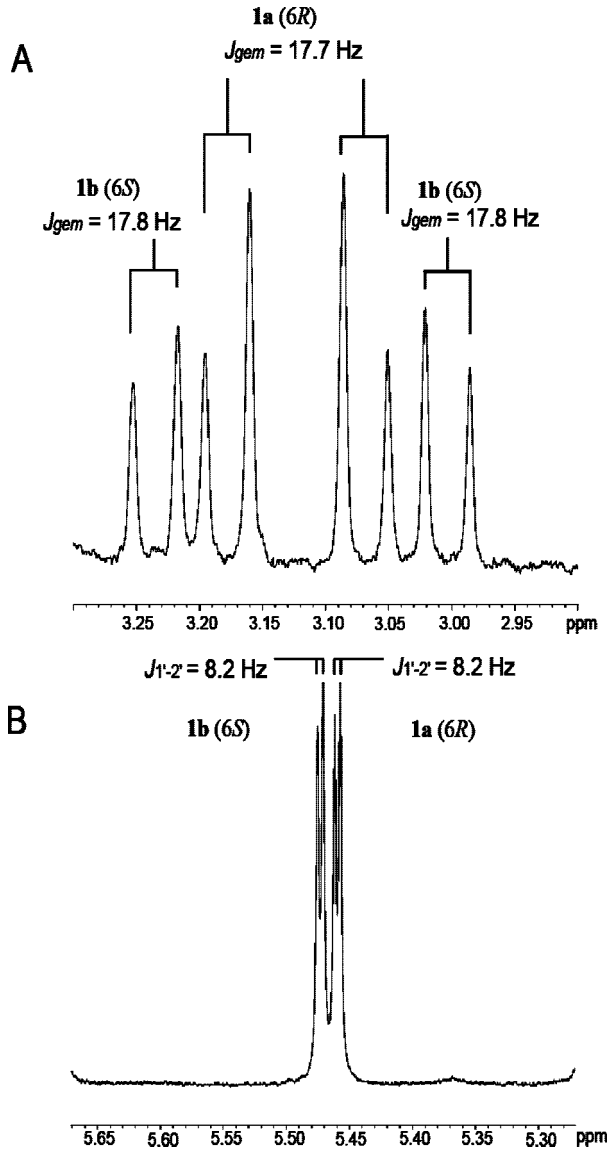
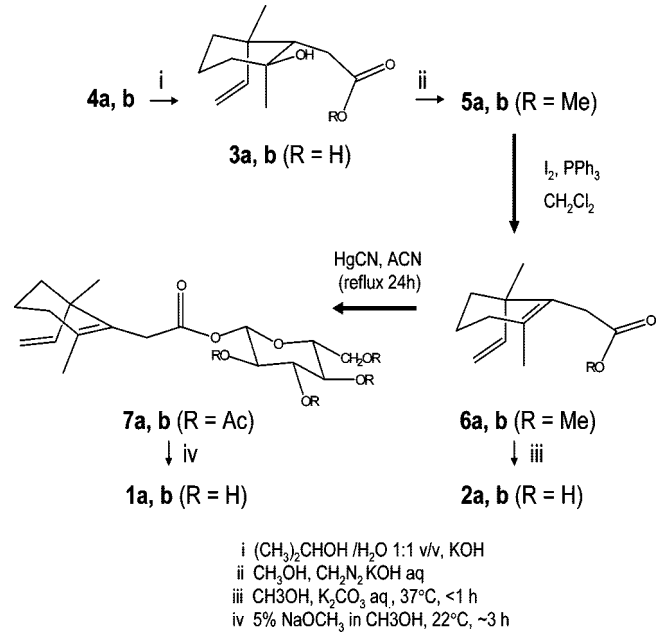


Figure 3. Within the ^{1}H NMR spectra of the natural pheromone glucoconjugates, we observed a diasteromeric relationship between **1a** and **1b** that was indicative of approximately racemic distribution of the aglycons **2a** and **2b**. (A) The C-7 AB spin system clearly illustrates this relationship and shows geminal coupling values of 17.7 and 17.8 Hz for **1a** and **1b**, respectively. (B) The protons at the anomeric position (C-1′) of the glycon also exhibited conspicuous peak doubling, as well as 8.2 Hz coupling associated with a diaxial arrangement of vicinal protons and a β-glucosidic linkage.

the fingertips until regurgitation, oral secretion from 11−14-day-old adults was harvested with a glass capillary (1 mm i.d.) that penetrated a vial under slight negative pressure at 4 °C. Collections were made 2 ± 0.5 h prior to sunset, pooled until ~0.6 mL was accumulated, and stored at −70 °C.

Oral secretion (0.5 mL) was thawed and diluted to 1 mL. These samples were extracted with hexane (1 mL) containing 0.8 nL of tetradecane internal standard and analyzed for **4a**−**d** by GC-MS. Alternatively, these samples were transferred to the solid-phase extraction cartridges as described above. The cartridges were flushed with water (3 × 1 mL), and then the analytes were eluted, with 3 × 1 mL rinses of 0.05% formic acid in 50% ACN, into 3 mL tubes. Eluants were concentrated to 0.5 mL and split. To one 0.25 mL subsample was added 120 μL of ACN containing 360 μg of external standard prior to HPLC-MS analysis. The remaining subsample was concentrated to dryness and dissolved in a methanolic solution of diazomethane. Extraction with hexane and subsequently H_2O (0.5

Scheme 1



mL each) yielded methyl esters (**5a**−**d** and **6a** and **6b**) amenable to GC within the organic phase. In pooled samples of freshly isolated male OS, the diastereomeric distribution of **4a** and **4b** to **4c** and **4d** and **3a** and **3b** to **3c** and **3d** was consistently ~2.5:1 and is in close agreement with other studies.[21,22] The lactone (**4a**−**d**) and acid (**3a**−**d**) pheromone forms, which were collectively present at ~35 ± 5 ng/μL, accounted for 28 ± 7% and 61 ± 6% (grand mean ± SE, n = 18)[27] of the pheromone analytes, respectively. By comparison, the "free" aglycon (**2a** and **2b**) and the glucoconjugates (**1a** and **1b**) represented the remaining 11 ± 4% and were found at

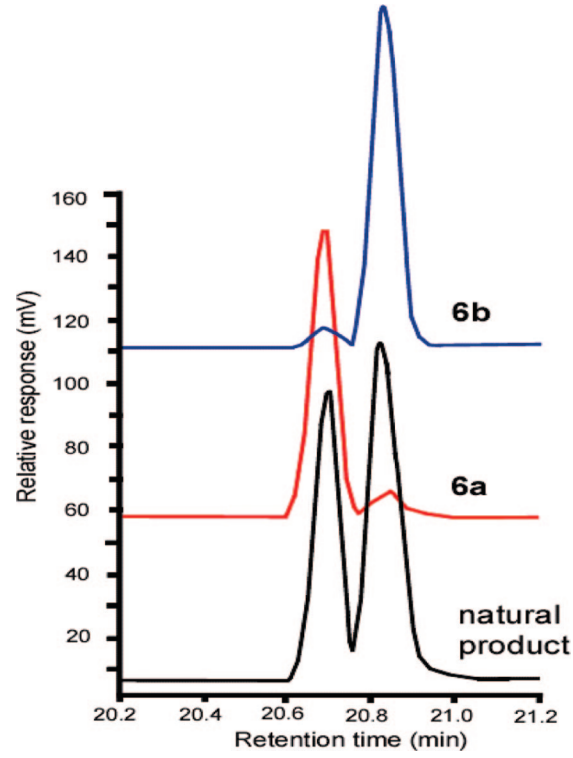


Figure 4. The natural aglycons were liberated from their conjugation to glucose via base-catalyzed hydrolysis. GC-FID analysis of the methyl-esterified aglycons were compared to synthetic standards and indicated an approximately racemic distribution (48 ± 8% **6a**: 52 ± 9% **6b**, n = 10).

~2.1 ± 0.5 and ~2.2 ± 0.5 ng/μL, respectively. In light of these amounts, an alternative method of isolation was developed to facilitate NMR studies on the natural glucosides; 1 ft × 1 ft × 1 ft plexiglass rearing cages were wiped with paper towels saturated with methanol/water (1:1). The towels were collected and rinsed with methanol in a Buchner funnel. The filtrate was evaporated to dryness, dissolved in 1 mL, and purified as described above.

Determination of D-Glucose. Corresponding alditol trifluoroacetates were prepared for GC-FID with the method of Linqvist and Jansson[12] by dissolving 0.1−1 mg of purchased D- or L-glucose sugar standards, or the dried base-catalyzed hydrolysate of the glucoside, in ~0.3 mL of 1 M NH_4OH containing $NaBH_4$ (1 mg/mL) for 30 min at 22 °C. The sample was then repeatedly quenched with methanol containing 10% acetic acid and evaporated to dryness. This process was repeated until neutralization. Acetonitrile (~250 μL), containing 10% trifluoroacetic acid, and ~100 μL trifluoroacetic anhydride were added to the dry sample prior to heat treatment (60 °C for 15 min). After cooling to 25 °C, the samples were dried with a gentle stream of N_2, dissolved in ~0.3 mL of dichloromethane, and analyzed. The resulting glycon derivative coeluted with that of D-glucose (t_R 25.50 ± 0.1 min), but not with that of L-glucose (t_R 24.53 ± 0.1 min).

***β*-D-Glucopyranosyl 2-(2,6-dimethyl-6-vinylcyclohex-1-enyl)acetate (1a and 1b).** 2,3,4,6-Tetra-acyl-α-D-glucopyranosyl bromide (103 mg, 0.25 mmol), **6a** or **6b** (20 mg, 0.1 mmol), and $Hg^{II}CN$ (57 mg, 0.25 mmol) were refluxed in anhydrous ACN/ether (1:1)[11] to form **7a** and **7b**; *O*-glucosylation was monitored by disappearance of the brominated glycon via GC-MS. Upon completion (~8 h), the reaction mixture was diluted with ether, washed with brine, dried with $MgSO_4$, and concentrated to a residue. A 5% methanolic solution of sodium methoxide at 22 °C removed the glycon acetyl groups in ~3 h. After neutralization with 10% acetic acid, the mixture, which contained **1a** or **b** in ~35% overall yield, was concentrated to dryness. Synthetic glucoside was purified by HPLC in the same manner as the natural isolate. Colorless; **1a** $[\alpha]_D^{25}$ −35, **1b** $[\alpha]_D^{25}$ 37 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (4.05) nm; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 357.2837 (calcd for $[M + H]^+$ $C_{18}H_{29}O_7$, 357.1914).

2-(2,6-Dimethyl-6-vinylcyclohex-1-enyl)acetic acid (2a and 2b). Methyl ester **5a** or **5b** was formed in quantitative yield when 40 mg (0.19 mmol) of **3a** or **3b**, respectively, was dissolved in a ~1 mL of methanolic solution of diazomethane. The solution was diluted with pentane prior to an extraction with H_2O (0.5 mL each). The organic phase, which contained **5a** or **5b**, was then dried ($MgSO_4$), filtered, and concentrated to ~200 μL prior to being dissolved in 0.5 mL of CH_2Cl_2. As reported previously,[10] this solution was then added dropwise to a solution of CH_2Cl_2 (2 mL) containing triphenylphosphine (58 mg, 0.22 mmol) and iodine (56 mg, 0.22 mmol) that has been stirring for ~10 min. Tertiary alcohol dehydration, which was monitored by GC-MS until completion (~2 h), resulted in the formation of 24 mg of **6a** or **6b** (12 mmol; 65% yield)[28] and 9 mg of **4a** or **4b** byproduct (5 mg; 25% yield). The reaction mixture was concentrated to an oily residue and flash chromatographed on silica gel (230−400 mesh) with hexane/ethyl acetate (8:2) to remove polar reaction components. Utilizing an equivalent mobile phase composition at 3 mL/min flow rate, HPLC with an refractive index detector and an Econosil 10 μm column (Alltech 6233) was used for final purification of **6a** or **6b** (t_R 6.1 ± 0.3 min) and **4a** or **4b** (t_R 10.8 ± 0.4 min). Hydrolysis of **6a** or **6b** under mild basic conditions (K_2CO_3(aq) in methanol, 15 °C) yielded **2a** or **2b** with >95% efficiency. Colorless; **2a** $[\alpha]_D^{25}$ −45, **2b** $[\alpha]_D^{25}$ 55 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (3.05) nm; ^1H NMR (600 MHz, methanol-d_4) δ 1.21 (3H, s, H_3−10), 1.44−1.51 (2H, m, H_2-5), 1.56−1.64 (2H, m, H_2-4), 1.68 (3H, s, H_3-9), 1.99−2.10 (2H, m, H_2-3), 3.08 (1H, d, ^2J = 17.7 Hz, H_A-7), 3.10 (1H, d, ^2J = 17.7 Hz, H_B-7), 4.95 (1H, dd, $^2J_{gem}$ = 1.6 Hz, $^3J_{trans}$ = 17.5 Hz, HC=CHH_{trans}-12), 5.10 (1H, dd, $^2J_{gem}$ = 1.6 Hz, $^3J_{cis}$ = 10.6 Hz, HC=CHH_{cis}-12), 5.65 (1H, dd, $^3J_{cis}$ = 10.6 Hz, $^3J_{trans}$ = 17.5 Hz, *H*C=CHH-11); HMBC (600 MHz, methanol-d_4) δ 18.7 (CH_2, C-4), 19.1 (CH_3, C-9), 23.5 (CH_3, C-10), 32.4 (CH_2, C-3), 35.8 (CH_2, C-7), 36.9 (CH_2, C-5), 42.5 (C, C-6), 113.0 (HC=*C*HH, C-12), 127.5 (C, C-1), 134.9 (C, C-2), 146.2 (H*C*=CHH, C-11), 172.6 (C=O, C-8); HRESIMS *m/z* 195.2232 (calcd for $[M + H]^+$ $C_{12}H_{19}O_2$, 195.1385); GC-CIMS of **4a** and **4b**, *m/z* (% rel int) 195 $[MH]^+$ (79), 177 (14), 135 (7), 135 (4); **5a** and **5b**, *m/z* (% rel int) 209 $[MH]^+$ (62), 195 (18), 177 (12), 135 (9); **6a** and **6b**, *m/z* (% rel int) 209 $[MH]^+$ (75), 177 (7), 153 (11), 135 (7).

2-(2-Hydroxy-2,-6-dimethyl-6-vinylcyclohexanyl)acetic acid (3a−d). Enantiomerically pure (>97% ee) **4a−d** (40 mg, 0.20 mmol) were hydrolyzed quantitatively to their corresponding γ-hydroxy carboxylates, **3a−d**, by the dropwise addition of 10 mM NaOH (2 mL) to 2 mL of 2-propanol that was rapidly stirred at 35 °C for 8 h. The reaction mixture was then concentrated in vacuo to 1 mL, adjusted to pH 6 with 0.1 M HCl, and flushed through Supelco DSC-18 1 mL solid-phase extraction cartridges. The cartridges were then rinsed with water (3 × 1 mL) to remove salt. Compounds **3a−d** were eluted with three 1 mL portions of 0.05% formic acid in 50% ACN, which were collected, combined, and concentrated to dryness. The spectroscopic properties agreed well with previous reports.[18,19] Colorless; **3a** $[\alpha]_D^{25}$ +27.3, **3b** $[\alpha]_D^{25}$ −25.6, **3c** $[\alpha]_D^{25}$ +42.1, **3d** $[\alpha]_D^{25}$ −39.9 (*c* 1.0, ethanol); UV (MeOH) λ_{max} (log ε) 220 (3.15) nm; ^1H NMR (600 MHz, methanol-d_4) **3a** and **3b** δ 1.05 (3H, s, H_3−10), 1.09 (3H, s, H_3-9), 1.29−1.87 (6H, m, H_2-3,4,5), 1.99 (1H, dd, ^3J = 3.8, 6.9 Hz, H-1), 2.35 (1H, dd, ^3J = 3.8 Hz, ^2J = 15.9 Hz, H_A-7), 2.45 (1H, dd, ^3J = 6.9 Hz, ^2J = 16.0 Hz, H_B-7), 5.02 (1H, dd, $^2J_{gem}$ = 1.9 Hz, $^3J_{trans}$ = 18.6 Hz, HC=CHH_{trans}−12), 5.07 (1H, dd, $^2J_{gem}$ = 1.8 Hz, $^3J_{cis}$ = 10.8 Hz, HC=CHH_{cis}-12), 6.04 (1H, dd, $^3J_{cis}$ = 10.7 Hz, $^3J_{trans}$ = 17.7 Hz, *H*C=CHH-11); **3c** and **3d** δ 0.95 (3H, s, H_3-10), 1.18 (3H, s, H_3-9), 1.25−1.85 (6H, m, H_2-3,4,5), 1.89 (1H, dd, ^3J = 4.8, 7.1 Hz, H-1), 2.14 (1H, dd, ^3J = 4.7 Hz, ^2J = 16.0 Hz, H_A-7), 2.28 (1H, dd, ^3J = 7.0 Hz, ^2J = 16.1 Hz, H_B-7), 4.91 (1H, dd, $^2J_{gem}$ = 1.8 Hz, $^3J_{trans}$ = 18.0 Hz, HC=CH-H_{trans}-12), 4.99 (1H, dd, $^2J_{gem}$ = 1.7 Hz, $^3J_{cis}$ = 10.7 Hz, HC=CH-H_{cis}-12), 5.77 (1H, dd, $^3J_{cis}$ = 10.6 Hz, $^3J_{trans}$ = 17.8 Hz, *H*C=CHH-11); HMBC (600 MHz, methanol-d_4) **3a** and **3b** δ 20.0 (CH_2, C-4), 21.5 (CH_3, C-9), 29.5 (CH_3, C-10), 34.0 (CH_2, C-7), 37.5 (CH_2, C-5), 39.5 (CH_2, C-6), 42.5 (C, C-3), 53.5 (CH_2, C-1), 75.1 (C, C-2), 110.5 (HC=*C*HH, C-12), 151.2 (H*C*=CHH, C-11), 175.5 (C=O, C-8); **3c** and **3d** δ 19.5 (CH_3, C-10), 20.5 (CH_2, C-4), 23.2 (CH_3, C-9), 34.5 (CH_2, C-7), 38.2 (CH_2, C-5), 38.0 (CH_2, C-6), 42.4 (C, C-3), 51.5 (CH_2, C-1), 73.5 (C, C-2), 112.5 (HC=*C*HH, C-12), 153.2 (H*C*=CHH, C-11), 179.1(C=O, C-8); HRESIMS *m/z* 213.2415 (calcd for $[M + H]^+$ $C_{12}H_{20}O_3$, 213.1491).

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Supporting Information Available: Spectroscopic data are available free of charge via the Internet at http://pubs.acs.org.

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