A TETRAENE ALDEHYDE AS THE MAJOR SEX PHEROMONE COMPONENT OF
THE PROMETHEA MOTH (*Callosamia promethea* (Drury))

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ABSTRACT The promethea moth *Callosamia promethea* is one of three species of silkmoths from the genus *Callosamia* that occur in North America. Cross attraction of males to heterospecific calling females has been observed in the field and hybrid progeny have been produced by pairing heterospecifics in captivity. These observations suggest that all three species share or have considerable overlap in the sex attractant pheromones produced by females, so that other prezygotic isolating mechanisms such as diel differences in reproductive activity limit hybridization in the field. Coupled gas chromatography-electroantennogram detection and gas chromatography mass-spectrometry analyses of extracts of volatiles collected from female promethea moths supported the identification of (4*E*,6*E*,11*Z*,13*Z*)-hexadeca-4,6,11,13-tetraenal (4*E*,6*E*,11*Z*,13*Z*)-16:Ald as the compound in extracts that elicited the largest responses from antennae of males. The identification was confirmed by non-selective synthesis of several isomers as analytical standards, and stereoselective synthesis of (4*E*,6*E*,11*Z*,13*Z*)-16:Ald for testing in field trials. Male moths were strongly attracted to synthetic (4*E*,6*E*,11*Z*,13*Z*)-16:Ald, suggesting that this compound is the major and possibly the only component of the sex pheromone of these large saturniid moths. Based on the cross-attraction of heterospecifics, it is likely that this is also a major pheromone component of the other two North American *Callosamia* species as well.

KEY WORDS *Callosamia promethea*, Saturniidae, (4*E*,6*E*,11*Z*,13*Z*)-hexadeca-4,6,11,13-tetraenal, Sex pheromone

INTRODUCTION

Giant silk moths (Lepidoptera: Saturniidae) are among the largest and most attractive moths in North America. As a result, they are prized by collectors and their life histories are well known (Collins and Weast 1961; Tuskes et al. 1996). Populations of many saturniid moths are reported to have declined in the northeastern United States and several appear on state endangered species lists (Schweitzer 1988; Tuskes et al. 1996; Boettner et al. 2000). Hypotheses for the reported declines include habitat loss, disruption of mating by street lights, non-target effects of insecticides, and parasitization by the generalist parasitoid fly *Compsilura concinnata* (Schweitzer 1988; Holden 1992; Johnson et al. 1995; Tuskes et al. 1996; Boettner et al. 2000).

The promethea moth, *Callosamia promethea* (Drury) is one of the three species of silkmoths in the genus *Callosamia* that occur in North America (Ferguson 1972). It is the most widely distributed of the three species, occurring from Canada south to Florida and eastern Texas. In parts of its range it is sympatric and seasonally synchronic with either or both of the congeners *Callosamia angulifera* (Walker) and *Callosamia securifera* (Maassen). Although cross-attraction of male *C. angulifera* to female *C. promethea* has been observed (Skinner 1914; Toliver et al. 1979) and all of the possible male-female crosses (except ♂ *C. promethea* and ♀ *C. angulifera*) among the three species have been made in captivity (Haskins and Haskins 1958; Remington 1968; Peigler 1977, 1980), hybrids reportedly do not occur in the wild (see Peigler 1977). Differences in the diel patterns of reproductive behaviour among the three species are hypothesized to be a primary mechanism ensuring reproductive isolation in nature given that at least some

cross-attraction has been documented. Specifically, *C. securifera* is reported to be active from ~10:00-13:30 (Brown 1972, referred to as *C. carolina*), whereas *C. promethea* is active from ~15:30-18:30 (Rau and Rau 1929), and *C. angulifera* is active from 19:30-24:00 (Collins and Weast 1961).

Because of their crucial role in mate-location and recognition, often over long distances, lepidopteran sex pheromones are powerful, species-specific attractants (El-Sayed 2012). These characteristics make them valuable tools for sampling rare species and low-density populations. For example, a recent study demonstrated that pheromone lures were an excellent tool for detecting and surveying populations of an iconic and protected saturniid species native to Europe, the Spanish moon moth *Graellsia isabellae* (Graells) (Millar et al. 2010). With that study as proof of concept for using pheromones for sampling potentially threatened lepidopteran species, the objective of the work described here was to identify, synthesize, and bioassay the female-produced sex pheromone of the promethea moth. Given the documented cross-attraction between at least two of the three North American species, it is likely that the major component(s) of the sex pheromone will be shared by all three species.

METHODS AND MATERIALS

Insects Pupae of *C. promethea* were collected by Craig Mitzell from Marshall, Starke, Fulton, and Pulaski counties in northern Indiana in the winter of 2010 and shipped to the quarantine facility at the University of California, Riverside (USDA-APHIS permit # P526P-08-02964). Pupae were sorted by sex and placed on paper towelling inside

plastic boxes ($40 \times 27 \times 16$ cm) with loose fitting lids. Paper towelling was hung from the sides of the inside of the boxes, and additional towelling was draped across the top to allow emerging adults to hang upside down during wing expansion. Humidity was provided with an open 473 ml jar of deionized water and additional moisture was added periodically by spraying the cocoons with water. The boxes were held next to a window to provide natural light, augmented with two 32 watt fluorescent lights on a 14:10 L:D cycle. Room temperature was 22°C and room humidity was not controlled. Emerged males were placed in glassine envelopes inside a plastic bag with a damp piece of towelling in a refrigerator for storage. For all male moths used for electroantennogram analyses, the genitalia were removed prior to removal from the quarantine facility. Emerged females were used immediately for pheromone collection as described below, or were refrigerated for up to 3 d in a plastic bag with a damp paper towel to accumulate a number of females for analysis. Chilled females were removed from refrigeration in the morning of the day of use and sampled in the late afternoon (16:00 to 17:00 h). Single adult females to be used for pheromone collections were placed in screen cages constructed of 6.3 mm mesh hardware cloth (ca. 15 cm dia. $\times$ 14 cm high) and then placed inside a wooden double-sleeve rearing box (76 cm long $\times$ 43 cm deep $\times$ 33 cm high in front to 50 cm high in back). The glass top of the rearing box allowed visual confirmation of extrusion of the ovipositor, which indicated calling. Calling behavior was observed late in the photophase, approximately 11 h after sunrise, and only calling females were used for pheromone collections.

Solid Phase Micro-Extraction (SPME) of Sex Pheromone Glands SPME collections from

calling females were made using similar methods to those used for *Graellsia isabellae* (Millar et al. 2010). Calling females were grasped firmly by the abdomen with gentle pressure towards the tip of the abdomen to evert the sex pheromone gland, the scale-free tissue just anterior to the ovipositor. The exposed gland was wiped with the SPME fiber, with all surfaces being wiped at least twice. SPME collections were made using 100 μm polydimethylsiloxane fibers (Supelco, Bellefonte, PA). Approximately 5-7 mm of the SPME fiber was exposed and the fiber holder was fastened to the lab bench so that both hands could be used to manipulate the insect. The loaded SPME fiber was immediately analyzed by coupled gas chromatography-electroantennogram detection (GC/EAD), or by coupled gas chromatography-mass spectrometry (GC/MS).

Solvent Extraction of Sex Pheromone Glands Single female extracts were made from 3 calling females after their pheromone glands had been wiped with an SPME fiber. Sex pheromone glands were removed by forcing eversion of the gland, then clamping the abdomen just anterior to the gland using forceps to maintain the pressure in the gland, then slicing the gland off with a razor blade such that the gland remained inflated in the forceps. The gland was then soaked for 10 min in ca 50 μl of clean pentane, taking care not to submerge the cut end of the tissue. Three single female extracts were analyzed by coupled GC/EAD in their dilute form and subsequently combined and concentrated for GC/MS analysis.

Gas Chromatography-Electroantennogram (GC-EAD) and Gas Chromatography-Mass Spectrometry Analyses SPME wipe samples and solvent extracts of pheromone glands

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4 135 were analyzed first by GC-EAD. Details of the instrumentation and methods for making
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6 136 antennal preparations have been previously described in detail (Millar et al. 2010). GC-
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9 137 EAD analyses were conducted on a DB-5 column (30 m $\times$ 0.25 mm ID, 0.25 micron film;
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11 138 J&W Scientific, Folsom, CA, USA) programmed from 100°C/1 min then 10°C/min to
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14 139 275°C for 40 min. Retention indices were calculated relative to straight chain alkanes.
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16 140 Analyses were repeated on a DB-Wax column (30 m $\times$ 0.25 mm ID, 0.25 micron film;
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18 141 J&W Scientific) programmed from 100°C/1 min then 10°C per min to 250°C for 60 min.
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20 142 SPME wipe samples were analyzed on an Agilent 6890N GC equipped with an HP5-MS
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22 143 column (30 m $\times$ 0.25 mm ID, 0.25 micron film) and coupled to a 5975C mass selective
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24 144 detector (Agilent, Santa Clara, CA, USA). Analyses were run in splitless mode, with He
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26 145 as carrier gas, temperature programming from 40°C/1 min, 10°C/min to 280°C and hold
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29 146 20 min. The injector temperature was 250°C, and loaded fibers were desorbed for 30 sec.
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36 148 *Synthesis of pheromone candidates* All solvents were dried and distilled according to
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38 149 standard procedures (PureSolv-ENTM Innovative Technology, Inc.). Commercially
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40 150 available starting materials were purchased from Sigma-Aldrich Química (Madrid,
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42 151 Spain). Reactions involving air- or moisture-sensitive materials were carried out under
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44 152 Ar. Unless otherwise specified, solutions of crude products were dried over anhydrous
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46 153 Na₂SO₄ and concentrated under reduced pressure. Purification by flash or vacuum flash
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48 154 chromatography was carried out on silica gel 60A (230-400 mesh). IR spectra were
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50 155 recorded on a Nicolet Avatar 360 FT-IR spectrometer (Thermo Electron Inc., Madison,
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52 156 Wisconsin, USA). NMR spectra were recorded at 400 and 500 MHz for ¹H and 100 and
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54 157 125 MHz for ¹³C on a Varian Mercury 400 and Inova 500 spectrometer (Varian Inc., Palo
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Alto, CA, USA), respectively. GC analyses were determined on a TraceTM GC 2000 Thermo Finnigan gas chromatograph equipped with a HP-5 column (30 m × 0.25 mm ID × 0.25 μm film) (Agilent Technologies, Santa Clara, CA, USA). Low resolution mass spectra (MS) were obtained on a Fisons MD 800 instrument (Thermo Fisher Scientific, Waltham, Massachusetts, USA). High resolution mass spectra (HRMS) were recorded on an UPLC Acquity (Waters) coupled to a LCT Premier XE (Waters) spectrometer.

Non-stereoselective syntheses of (4*E*,6*E*,11*Z*/*E*,13*Z*)-hexadeca-4,6,11,13-tetraenals are shown in Figure 1, and described in detail in the online supplement.

Synthesis of (4E,6E,11Z,13E)-hexadeca-4,6,11,13-tetraenal. (Figure 2)

1-Bromo-5-chloropent-1-yne (2). *N*-bromosuccinimide (6.25 g, 34.75 mmol) was added in portions to a solution of 5-chloropent-1-yne (**1**) (3.24 g, 31.59 mmol) in acetone (95 ml). The mixture was stirred at room temperature until complete solution and then silver nitrate (0.27 g, 1.58 mmol) was added. The mixture was protected from light, stirred at room temperature for 30 min and quenched by pouring into water (150 ml). After extraction with ether (3x100 ml), the combined organic layers were washed with brine, dried and concentrated. The residue was purified by vacuum flash chromatography eluting with hexane to yield 1-bromo-5-chloropent-1-yne (**2**) (5.05 g, 88%) as a yellow oil. IR (film) ν : 2958, 1436, 1288, 851 cm^{-1} . ¹H NMR (400 MHz, CDCl₃): δ 3.64 (t, *J* = 6.3 Hz, 2H), 2.42 (t, *J* = 6.8 Hz, 2H), 1.97 (tt, *J*₁ = *J*₂ = 6.6 Hz, 2H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 78.56 (CBr), 43.61 (ClCH₂), 39.17 (C), 31.15 (CH₂), 17.27 (CH₂) ppm. EIMS, *m/z* (%): 184 ([*M*+2]⁺, 35), 182 ([*M*+1]⁺, 69), 182 ([*M*]⁺, 61), 147 (41), 145 (45),

180 120 (44), 119 (56), 118 (51), 117 (58), 101 (51), 75 (30), 73 (43), 66 (52), 65 (100), 63
181 (48), 62 (33).
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183 *9-Chloronona-3,5-diyne (3)*. An oven-dried 3-necked flask with a dry ice condenser was
184 charged under Ar atmosphere with CuCl (0.25 g, 2.48 mmol), NH₂OH·HCl (1.38 g, 19.84
185 mmol) and anhydrous MeOH (140 ml). The mixture was stirred for 10 min until a
186 homogenous solution was obtained, and then cooled to -78°C. An excess of 1-butyne (ca.
187 4 ml) was condensed into the mixture with the dry ice condenser, then *n*-propylamine
188 (3.85 ml, 46.84 mmol) was added followed by bromoalkyne **2** (5.00 g, 27.55 mmol) in
189 anhydrous MeOH (22 ml) via cannula over 10 min. The mixture was allowed to warm to
190 room temperature, stirred for 2 h and quenched by pouring into water (170 ml). After
191 filtering through a pad of Celite[®], the aqueous layer was extracted with hexane (5x75 ml)
192 and the combined organic layers were washed with brine, dried and concentrated. The
193 residue was purified by vacuum flash chromatography eluting with hexane to obtain 9-
194 chloronona-3,5-diyne (**3**) (3.26 g, 77%) as a colorless oil. IR (film) ν : 2915, 2879, 2840,
195 1455, 1429, 1315, 1290, 1063, 969, 845 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 3.64 (t, *J* =
196 6.3 Hz, 2H), 2.45 (t, *J* = 6.8 Hz, 2H), 2.26 (quart, *J* = 7.5 Hz, 2H), 1.97 (tt, *J*₁ = *J*₂ = 6.6
197 Hz, 2H), 1.15 (t, *J* = 7.5 Hz, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 79.37 (C), 75.45
198 (C), 66.40 (C), 64.57 (C), 43.61 (ClCH₂), 31.22 (CH₂), 16.81 (CH₂), 13.49 (CH₂), 13.03
199 (CH₃) ppm. EIMS, *m/z* (%): 157 ([M+2]⁺, 12), 156 ([M+1]⁺, 74), 155 ([M]⁺, 61), 154
200 (97), 126 (75), 119 (73), 117 (77), 115 (67), 105 (62), 104 (64), 103 (81), 92 (62), 91
201 (100), 89 (70), 79 (77), 78 (62), 77 (86), 75 (61), 65 (81), 63 (76), 51 (67).

(3Z,5Z)-9-Chloronona-3,5-diene (**4**). Cyclohexene (9.05 ml, 89.23 mmol) was added dropwise to a solution of $\text{BH}_3\cdot\text{SMe}_2$ (4.50 ml, 42.68 mmol) in THF (30 ml) at 0°C. After the addition was complete, a further 14 ml of THF was added to the white suspension and the mixture was stirred for 1 h at 0°C. Chlorodiyne **3** (3.00 g, 19.40 mmol) in THF (10 ml) then was slowly added to the slurry, and the mixture was stirred and allowed to warm to room temperature. After stirring for 5.5 h, glacial acetic acid (10 ml) was added, and the solution was heated to 55-60°C for 5 h. The mixture then was cooled to room temperature and 6M aqueous NaOH (33 ml) was added followed by careful addition of 35% H_2O_2 (10 ml; caution: very exothermic!), keeping the temperature below 40°C. After cooling to room temperature, the organic layer was decanted and the aqueous residue was extracted with Et_2O (4x30 ml). The combined organic layers were dried, concentrated, and purified by vacuum flash chromatography eluting with hexane to provide (3Z,5Z)-9-chloronona-3,5-diene (**4**) (2.48 g, 81%) as a colorless oil. IR (film) ν : 3037, 3003, 2963, 2931, 2871, 1446, 1306, 1067, 979, 866 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ 6.36-6.19 (m, 2H), 5.52-5.37 (m, 2H), 3.54 (t, $J = 6.6$ Hz, 2H), 2.34 (tdd, $J_1 = J_2 = 7.4$, $J_3 = 1.3$ Hz, 2H), 2.19 (ddq, $J_1 = J_3 = 7.5$, $J_2 = 1.5$ Hz, 2H), 1.87 (tt, $J_1 = J_2 = 7.0$ Hz, 2H), 1.00 (t, $J = 7.5$ Hz, 3H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ 134.70 (CH), 129.56 (CH), 125.11 (CH), 122.76 (CH), 44.58 (CH_2), 32.55 (CH_2), 24.80 (CH_2), 20.98 (CH_2), 14.31 (CH_3) ppm. EIMS, m/z (%): 160 ($[\text{M}+1]^+$, 30), 159 ($[\text{M}]^+$, 9), 158 (84), 116 (22), 109 (26), 96 (16), 95 (97), 93 (50), 91 (41), 82 (23), 81 (100), 80 (26), 79 (81), 77 (58), 68 (73), 67 (95), 65 (28), 55 (59), 53 (37), 51 (16).

(6Z,8Z)-Undeca-6,8-dien-1-yne (**5**). A mixture of lithium acetylide-ethylenediamine complex (1.85 g, 20.03 mmol) and NaI (0.11 g, 0.72 mmol) in anhydrous DMSO (50 ml) was stirred in a dry flask under Ar for 5 min at room temperature, then a solution of diene **4** (2.27 g, 14.31 mmol) in anhydrous DMSO (12 ml) was added dropwise. After 5 h, the solution was poured into water (200 ml) and extracted with pentane (5x50 ml). The combined organic layers were dried, concentrated, and purified by flash chromatography eluting with pentane to give (6Z,8Z)-undeca-6,8-dien-1-yne (**5**) (1.35 g, 64%) as a pale yellow oil. IR (film) ν : 3305, 3036, 3004, 2965, 2935, 2870, 2118, 1598, 1456, 1068 cm^{-1} . ^1H NMR (400 MHz, CDCl_3): δ 6.33-6.20 (m, 2H), 5.50-5.39 (m, 2H), 2.30 (tdd, $J_1 = J_2 = 7.6$, $J_3 = 1.2$ Hz, 2H), 2.23-2.15 (m, 4H), 1.95 (t, $J = 2.6$ Hz, 1H), 1.63 (tt, $J_1 = J_2 = 7.2$ Hz, 2H), 1.00 (t, $J = 7.5$ Hz, 3H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ 134.34 (CH), 130.53 (CH), 124.67 (CH), 122.95 (CH), 84.49 (C), 68.55 (CH), 28.55 (CH_2), 26.58 (CH_2), 20.97 (CH_2), 18.09 (CH_2), 14.33 (CH_3) ppm. EIMS, m/z (%): 148 ($[\text{M}]^+$, 6), 133 (17), 120 (17), 119 (77), 117 (18), 108 (14), 106 (22), 105 (62), 95 (20), 93 (45), 92 (33), 91 (100), 81 (29), 80 (39), 79 (86), 78 (29), 77 (63), 68 (14), 67 (76), 66 (16), 65 (30), 55 (48). HRMS: Calcd. for $\text{C}_{11}\text{H}_{17}$: 149.1330 $[\text{M}+1]^+$; found: 149.1331.

2-(1E,6Z,8Z)-Undeca-1,6,8-trienyl-1,3-dioxaborinane (**6**). A solution of 1M $\text{HBBBr}_2 \cdot \text{SMe}_2$ in anhydrous CH_2Cl_2 (3.84 ml, 3.84 mmol) was added dropwise to a flask charged with (6Z,8Z)-undeca-6,8-dien-1-yne (**5**) (0.57 g, 3.84 mmol) in anhydrous CH_2Cl_2 (28 ml) at 0°C under Ar. After stirring for 15 min at 0°C and 15 h at room temperature, 1M aqueous NaOH (8.5 ml) was added and the mixture was stirred for 15 min. The organic layer was then decanted, dried, and concentrated. The crude product

was taken up in pentane (20 ml) and 1,3-propanediol (0.30 ml, 4.18 mmol) was added. The mixture was stirred for 3 h at room temperature, then crystalline anhydrous Na₂SO₄ was added. After stirring 1 h, the slurry was filtered and the filtrate was concentrated. The residue was purified by vacuum flash chromatography on silica gel eluting with mixtures of hexane: Et₂O (1:0 to 1:1) to obtain compound **6** (0.55 g, 61%) as a yellow oil.

¹H NMR (400 MHz, CDCl₃): δ 6.50 (dt, *J* = 17.7, 6.5 Hz, 1H), 6.28-6.17 (m, 2H), 5.47-5.39 (m, 2H), 5.32 (dt, *J* = 17.7, 1.5 Hz, 1H), 4.02 (t, *J* = 5.5 Hz, 4H), 2.22-2.11 (m, 6H), 1.96 (quint, *J* = 5.5 Hz, 2H), 1.50 (tt, *J*₁ = *J*₂ = 7.5 Hz, 2H), 0.99 (t, *J* = 7.5 Hz, 3H) ppm.

¹³C NMR (100 MHz, CDCl₃): δ 151.20 (CH), 133.89 (CH), 131.72 (CH), 127.56 (CH-B), 123.92 (CH), 123.16 (CH), 61.85 (2CH₂O), 35.25 (CH₂), 28.60 (CH₂), 27.57 (CH₂), 27.18 (CH₂), 20.94 (CH₂), 14.35 (CH₃) ppm. HRMS: Calcd. for C₁₄H₂₄BO₂: 235.1869 [M+1]⁺; found: 235.1863.

1-tert-Butyldimethylsilyloxypent-4-yne (7). Imidazole (4.86 g, 71.33 mmol) and *tert*-butyldimethylsilyl chloride (8.60 g, 57.06 mmol) were added to a solution of 4-pentyn-1-ol (4.00 g, 47.55 mmol) in anhydrous CH₂Cl₂ (120 ml) and the mixture was stirred for 3 h at room temperature. Saturated aqueous NaHCO₃ (200 ml) was then added, and after stirring, the organic layer was separated and the aqueous layer was extracted with EtOAc (2x120 ml). The combined organic layers were washed with saturated aqueous NH₄Cl (200 ml) and brine (300 ml), dried, and concentrated. The residue was purified by vacuum flash chromatography eluting with hexane to yield *1-tert*-butyldimethylsilyloxypent-4-yne (**7**) (9.12 g, 97%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃): δ 3.70 (t, *J* = 6.0 Hz, 2H), 2.28 (td, *J* = 7.1, 2.7 Hz, 2H), 1.93 (t, *J* = 2.7

Hz, 1H), 1.73 (m, 2H), 0.90 (s, 9H), 0.06 (s, 6H) ppm. ^{13}C NMR (125 MHz, CDCl_3): δ 99.9 (C), 84.4 (CH), 61.6 (CH_2O), 31.7 (CH_2), 26.1 ($\text{C}(\text{CH}_3)_3$), 18.5 ($\text{C}(\text{CH}_3)_3$), 15.0 (CH_2), -5.1 (CH_3SiCH_3) ppm. HRMS: Calcd. for $\text{C}_{11}\text{H}_{23}\text{OSi}$: 199.1518 $[\text{M}+1]^+$; found: 119.1519. The ^1H and ^{13}C NMR spectra agreed with those previously reported (Sparling et al. 2009).

(4E)-1-tert-Butyldimethylsilyloxy-5-iodopent-4-ene (**8**). An oven-dried flask was charged with bis(cyclopentadienyl)zirconium hydridochloride (1.39 g, 5.40 mmol), and after flushing thoroughly with Ar, dry THF was added (15 ml). The suspension was chilled to 0°C and stirred for 15 min. A solution of pentyne **7** (0.98 g, 4.90 mmol) in dry THF (5 ml) was then added, the flask was shielded from light, and the mixture was stirred at 0°C for 30 min. After warming to room temperature, the reaction mixture was stirred for 3.5 h more. The resulting solution was cooled again to 0°C , and a solution of iodine (1.37 g, 5.40 mmol) in dry THF (7 ml) was added dropwise, and stirring was continued for 45 min. The mixture was warmed to room temperature, quenched by pouring into saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (40 ml), and the slurry was filtered through a pad of Celite[®] eluting with hexane (200 ml). The organic solution was washed with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (100 ml) and brine (150 ml), dried, and concentrated. The residue was purified by flash chromatography on silica gel eluting with mixtures of hexane:EtOAc (1:0 to 95:5) to provide *(4E)-1-tert-butyldimethylsilyloxy-5-iodopent-4-ene* (**8**) (1.25 g, 78%) as a yellow oil. ^1H NMR (400 MHz, CDCl_3): δ 6.52 (dt, $J = 14.0, 6.8$ Hz, 1H), 6.00 (dt, $J = 14.0, 1.4$ Hz, 1H), 3.60 (t, $J = 6.8$ Hz, 2H), 2.13 (td, $J = 6.8, 1.4$ Hz, 2H), 1.60 (quint, $J = 6.8$ Hz, 2H), 0.89 (s, 9H), 0.04 (s, 6H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ 146.15 (CH), 74.63

(CH), 61.94 (CH₂O), 32.44 (CH₂), 31.34 (C(CH₃)₃), 25.91 (C(CH₃)₃), 18.28 (CH₂), -5.34 (CH₃SiCH₃) ppm. HRMS: Calcd. for C₁₁H₂₄OSi: 327.0641 [M+1]⁺; found: 327.0652.

The ¹H and ¹³C NMR spectra agreed with those previously reported (Mukai et al. 1998).

(4E,6E,11Z,13Z)-Hexadeca-4,6,11,13-tetraenol (9). A solution of pentene **8** (0.58 g, 1.78 mmol) in THF (4 ml) was added to a solution of borinane **6** (0.38 g, 1.62 mmol) in THF (4 ml), followed by Pd(PPh₃)₄ (0.09 g, 0.08 mmol) and 1M aqueous KOH (3.4 ml, 3.40 mmol). The mixture was stirred under nitrogen at 60°C for 4.5 h, cooled to room temperature and then anhydrous Na₂SO₄ and hexane (40 ml) were added. The slurry was filtered through a pad of Celite[®], the filtrate was concentrated, and the residue was filtered through a pad of silica gel eluting with a mixture of hexane: EtOAc 9:1. The resulting product was concentrated, diluted with THF (10 ml), and 1M tetrabutylammonium fluoride in THF (1.8 ml, 1.80 mmol) was added. After stirring at room temperature for 15 h, the mixture was diluted with saturated aqueous NH₄Cl (20 ml), the organic layer was separated, and the aqueous layer was extracted with EtOAc (4x15 ml). The combined organic layers were dried, concentrated, and purified by flash chromatography eluting with mixtures of hexane:EtOAc (9:1 to 2:5) to provide *(4E,6E,11Z,13Z)-hexadeca-4,6,11,13-tetraenol (9)* (0.24 g, 63% over 2 steps) as a yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 6.30-6.15 (m, 2H), 6.08-5.96 (m, 2H), 5.62-5.52 (m, 2H), 5.48-5.40 (m, 2H), 3.66 (t, *J* = 6.5 Hz, 2H), 2.23-2.13 (m, 6H), 2.08 (dt, *J*₁ = *J*₂ = 7.2 Hz, 2H), 1.66 (tt, *J*₁ = *J*₂ = 7.0 Hz, 2H), 1.47 (tt, *J*₁ = *J*₂ = 7.5 Hz, 2H), 1.40 (bs, OH), 1.00 (t, *J* = 7.5 Hz, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 133.94 (CH), 132.51 (CH), 131.69 (CH), 131.46 (CH), 131.12 (CH), 130.64 (CH), 123.95 (CH), 123.14 (CH), 62.66

(CH₂O), 32.45 (CH₂), 32.27 (CH₂), 29.42 (CH₂), 29.05 (CH₂), 27.11 (CH₂), 20.95 (CH₂), 14.35 (CH₃) ppm. EIMS, *m/z* (%): 234 ([M]⁺, 4), 205 (4), 190 (19), 175 (32), 147 (33), 136 (17), 135 (77), 134 (31), 133 (32), 131 (37), 123 (15), 122 (63), 121 (55), 120 (23), 119 (81), 109 (21), 108 (42), 107 (75), 106 (30), 105 (78), 95 (55), 94 (40), 93 (92), 92 (46), 91 (91), 81 (72), 80 (67), 79 (100), 78 (36), 77 (76), 69 (37), 68 (21), 67 (89), 66 (31), 65 (41), 55 (77), 53 (49), 43 (30), 41 (76). HRMS: Calcd. for C₁₆H₂₇O: 235.2062 [M+1]⁺; found: 235.2071.

(4E,6E,11Z,13Z)-Hexadeca-4,6,11,13-tetraenal (**10**). A mixture of dry DMSO (0.16 ml) and dry CH₂Cl₂ (0.7 ml) was added dropwise under Ar to a cooled (-78°C) solution of oxalyl chloride (0.09 ml, 1.02 mmol) in dry CH₂Cl₂ (2 ml). A solution of tetraenol **9** (0.16 g, 0.68 mmol) in dry CH₂Cl₂ (0.7 ml) was then added and the mixture was stirred at -78°C for 1.5 h. Triethylamine (0.7 ml, 5.03 mmol) was added and the mixture was stirred for 30 min more at -78°C. The reaction was then warmed to room temperature and quenched with water (30 ml). The organic layer was separated and the aqueous layer was extracted with hexane (4x10 ml). The combined organic layers were dried, concentrated, and the residue was purified by flash chromatography on silica gel eluting with hexane:EtOAc 95:5 to give *(4E,6E,11Z,13Z)*-hexadeca-4,6,11,13-tetraenal (**10**) (0.05 g, 32%, ≥98% isomeric purity) as a pale yellow oil. The aldehyde was diluted immediately with hexane to a 0.03% solution, BHT (6.5 mg) was added as stabilizer, and the solution was sealed in a glass ampoule flushed with Ar to minimize degradation. NMR spectra were run in CDCl₃, previously filtered through a pad of solid NaHCO₃ to minimize possible acid-catalyzed trimerization of the aldehyde. ¹H NMR (400 MHz,

CDCl₃): δ 9.77 (t, J = 1.6 Hz, 1H), 6.30-6.15 (m, 2H), 6.08-5.95 (m, 2H), 5.63-5.51 (m, 2H), 5.48-5.39 (m, 2H), 2.53 (dt, J = 7.4, 1.6 Hz, 2H), 2.40 (dt, $J_1 = J_2$ = 7.0 Hz, 2H), 2.23-2.14 (m, 4H), 2.08 (dt, $J_1 = J_2$ = 7.3 Hz, 2H), 1.47 (tt, $J_1 = J_2$ = 7.4 Hz, 2H), 1.00 (t, J = 7.5 Hz, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 202.11 (CHO), 133.95 (CH), 133.32 (CH), 131.77 (CH), 131.61 (CH), 130.30 (CH), 129.41 (CH), 123.98 (CH), 123.11 (CH), 43.48 (CH₂), 32.24 (CH₂), 29.34 (CH₂), 27.08 (CH₂), 25.25 (CH₂), 20.94 (CH₂), 14.34 (CH₃) ppm. HRMS: Calcd. for C₁₆H₂₅O: 233.1905 [M+1]⁺; found: 233.1901.

Field Trials Five mm diam. rubber septa (Thomas Scientific, Swedesboro, NJ, USA) were extracted twice with hexane, allowed to dry, then loaded with 50 μ g of (4*E*,6*E*,11*Z*,13*Z*)-16:Ald in 50 μ l hexane. Septa were stored in a -20°C freezer until needed (replaced at ca. 14 d intervals in the field). Initial observations were made of moths responding to a septum atop a 1m tall PVC post near a suburban home (GPS 38.026205, -84.569868). Subsequent observations of moths responding to and/or being trapped in a 53 $\times$ 53 $\times$ 53 cm cube-shaped trap constructed of PVC pipe and fiberglass screen were made at the same location. Each vertical side of the trap had a central cone-shape surface that narrowed from 25 to a 12 cm diam. opening towards the inside of the trap (see Figure 5A) to limit egress. Between 15 May and 24 May 2012, moths were captured with a sweep net or trap at this location as they approached a septum.

Between 24 May 2012 and 13 June 2012 a cube trap baited with 50 μ g of (4*E*,6*E*,11*Z*,13*Z*)-16:Ald and an identical control trap baited with a septum dosed with 50 μ l hexane were hung from tulip poplar trees (*Liriodendron tulipifera* L.) at the University

of Kentucky Arboretum (GPS 38.016382,-84.502477). Tulip poplar is a reported host for both *C. angulifera* and *C. promethea* (Ferguson 1972; Tuskes et al. 1996). The openings of the traps were ca. 1 m above ground level. Treatment and control traps were separated by about 10 m. Positions of baited and control traps were switched 6 times over 20 d.

Between 17 July 2012 and 13 August 2012 baited and control traps were hung from trees at the University of Kentucky's North Farm (GPS 38.127451,-84.512887). Treatment and control traps were separated by at least 50 m, with 2000 m between pairs of traps. The surrounding farm areas had fence rows that included black cherry (*Prunus serotina*) and other reported host plants for *C. promethea*. Within a treatment-control pair of traps, positions were switched 7 times over 27 d. A voucher specimen, netted while responding to a pheromone lure, has been deposited in the insect collection of the Department of Entomology, University of Kentucky, Specimen Record-H11025.

RESULTS AND DISCUSSION

Identification of the Major Pheromone Component Analysis of SPME wipe samples of pheromone glands by GC-EAD showed one major antennal response to a small peak with a retention index of 1926 on a DB-5 GC column, and several minor responses (Figure 3). Subsequent GC-MS analysis of two other SPME wipe samples showed that the compound eliciting the largest response exhibited a small molecular ion at m/z 232 with losses of 18 (loss of water) and 44 mass units (loss of C_2H_4O) from the molecular ion (Figure 4). This molecular weight suggested a molecular formula of $C_{16}H_{24}O$, for a possible tetra-unsubstituted aldehyde. This was supported by the fact that the retention

index of the unknown was noticeably larger than that of (4*E*,6*E*,11*Z*)-hexadeca-4,6,11-trienal (1861; Millar et al. 2010). Furthermore, the increase of approximately 50 retention units for a conjugated diene on DB-5 (e.g., retention index of (11*Z*,13*Z*)-hexadecan-11,13-dienal: 1875)) versus hexadecanal (1821) on the DB-5 column suggested that the unknown might have two isolated conjugated diene systems. Additional retention index data from standards suggested that the unknown probably did not have a monoene and a conjugated triene because this would result in an increase of approximately 130-140 retention units versus hexadecanal. That is, (10*E*,12*E*,14*E*)- and (10*E*,12*E*,14*Z*)-hexadeca-10,12,14-trienals had retention indices of 1961 and 1967 respectively (~145 units more than hexadecanal), and (*Z*)-11-hexadecenal had a retention index of 1810 on this column (11 units less than hexadecanal). With this data suggesting a C16 tetraenal with two conjugated diene systems, we then turned to the literature on pheromones identified from saturniid species. In particular, the pheromone of *Antheraea polyphemus* Cramer had been reported as (6*E*,11*Z*)-hexadeca-6,11-dienal (Kochansky et al. 1975), whereas the pheromones of *Philosamia cynthia ricini* Boisduval (Bestmann et al. 1989) and *Graellsia isabellae* Graells had been identified as (4*E*,6*E*,11*Z*)-hexadeca-4,6,11-trienal (Millar et al. 2010). This suggested likely structures as being (4,6,9,11)- or (4,6,11,13)-hexadecatetraenals, each with 16 possible stereoisomers, and with the latter structure assessed as being easier to synthesize. We further assumed that based on these precedents, the diene closest to the aldehyde function might be in the 4,6 position, with (4*E*,6*E*)-stereochemistry. Close examination of the mass spectrum showed an even-mass ion at *m/z* 122 (14%) from possible cleavage between allylic carbon 8 and carbon 9, with a hydrogen transfer, providing a small fragment of evidence in support of a 4,6- dienal

structure. The remainder of the mass spectrum was dominated by ions which, from their masses, could only contain carbon and hydrogen, with one to five unsaturations. We were not able to glean any further solid information about the possible positions of the double bonds from the mass spectrum.

Further evidence for the (4*E*,6*E*) and the (Z11) stereochemistry was obtained by GC-EAD analysis of a mixed standard of (4*E*,6*E*,11*Z*)- and (4*Z*,6*E*,11*Z*)-hexadeca-4,6-11-trienals, available from work with *G. isabellae* (Millar et al. 2010). The (4*E*,6*E*,11*Z*)-isomer elicited large responses from antennae, whereas the responses to the (4*Z*,6*E*,11*Z*)-isomer were much smaller.

Thus, we first focused our attention on developing non-selective syntheses that would provide all four of the (4*E*,6*E*,11*Z*/*E*,13*Z*/*E*)-isomers as pairs of isomers (Figure 1, and supporting online information). The four isomers were differentiable on the DB-5 column (retention indices: (4*E*,6*E*,11*Z*,13*Z*) 1926; (4*E*,6*E*,11*E*,13*Z*) 1920; (4*E*,6*E*,11*Z*,13*E*) and (4*E*,6*E*,11*E*,13*E*) pair, 1906 and 1936), with the retention index and mass spectrum of the (4*E*,6*E*,11*Z*,13*Z*)-isomer matching that of the unknown. In sum, the available evidence all supported the pheromone structure as being (4*E*,6*E*,11*Z*,13*Z*)-hexadeca- 4,6,11,13-tetraenal, and so a stereoselective synthesis of this compound was developed to provide material for field testing.

Synthesis of (4E,6E,11Z,13Z)-hexadeca-4,6,11,13-tetraenal (10) (Figure 2). We required a short and efficient synthesis that could generate a conjugated (Z,Z)-diene and a second conjugated (E,E)-diene with high stereoselectivity. We envisioned that the former could be derived from Z-selective reduction of a conjugated diyne, whereas the latter could be

made from a palladium catalyzed Suzuki-type coupling of an *E*-vinyl halide and an *E*-vinyl boronate. Thus, bromination of chloroalkyne **1** with *N*-bromosuccinimide and AgNO₃ catalysis in acetone (Jurberg et al. 2010) provided bromoalkyne **2** in 88% yield. Then Cadiot-Chodkiewicz coupling (Bayer and Maier 2004) of bromoalkyne **2** with 1-butyne in the presence of CuCl, hydroxylamine hydrochloride, and *n*-propylamine in MeOH gave diyne **3** (77%) which was stereoselectively reduced to the corresponding diene (*Z,Z*)-**4** with dicyclohexylborane, prepared *in situ* (Brown et al. 1977). Acetylenation of **4** with lithium acetylide-ethylene diamine complex catalyzed with NaI in DMSO (Sonnet and Heath 1980) provided dienyne **5** in 64% yield. With this intermediate in hand, we initially tried to convert **5** into the corresponding iodoalkene by reaction with bis(cyclopentadienyl)zirconium hydride (Schwartz's reagent) followed by iodine (Ribe et al. 2000), but the reaction failed, possibly because of coordination of Zr with the conjugated double bond system. Thus, as an alternative, dienyne **5** was transformed into the corresponding borinane **6** by treatment with dibromoborane-dimethyl sulfide complex followed by hydrolysis and reaction with 1,3-propanediol (Organ and Ghasemi 2004) (61% overall yield from **5**), completing the first key intermediate. The second key intermediate, protected (*E*)-iodoalkenol **8**, was obtained by hydrido-zirconation of alkyne **7** with Schwartz's reagent followed by iodination to provide *tert*-butyldimethylsilyl-protected (*E*)-iodoalkenol **8** in 78% yield and high stereoselectivity. Suzuki reaction of borinane **6** with compound **8**, catalyzed by tetrakis(triphenylphosphine) palladium(0) and aqueous KOH, coupled the two intermediates to complete the tetraene framework. Tetrabutylammonium fluoride-induced deprotection then gave tetraenol **9** in 63% yield from **6** (2 steps). Finally, Swern

oxidation of alcohol **9** gave tetraenal **10**, which was immediately diluted in hexane, treated with BHT antioxidant and sealed in an ampoule to minimize degradation until the compound could be used in field trials.

Field Trials All direct observations of moth activity were made at the suburban home of one of the authors (KH). Direct observations were made of the responses of several male *C. promethea* responding to a tetraenal lure during late afternoon to early evening (17:20 and 19:00 h). Males exhibited slow, zigzagging flight with nearly continuous upwind progress towards the lure, followed by contact with the pheromone septum while continuing to fan their wings. After several seconds on the lure two males were observed to drop ca. 5 m downwind with an abrupt flight, only to reorient to the lure again. Two other moths were netted as they reoriented to the lure. One of these was pinned and has been deposited at the University of Kentucky's Insect Collection as a voucher specimen (Specimen Record-H11025). Male moths also were observed as they approached and entered a cube trap, and then contacted the pheromone lure (Figure 5A,B). No moths were observed to stop short and veer away from the trap while in flight, but sometimes they contacted the external screen sides before entering through the trap cone. Within the trap, a male would repeatedly contact the lure and often remained active for many minutes before coming to rest at the top or sides of the trap (Figure 5C). On one occasion a moth was observed to exit the trap after contacting the lure by flying directly downwind only to reorient and enter the trap a second time. At least two moths that assumed resting positions within the trap eventually escaped before morning. Thus, this trap design does not appear to limit the entrance of moths, but it also does not retain all moths. Nine

478 moths were caught in traps or netted in 10 d at this location. No control traps were used
479 at this location.

480 At the UK Arboretum, 7 male *C. promethea* were caught in baited traps over 20 d.
481 No moths were caught in control traps (binomial probability, $P=0.008$). No moths were
482 caught between 1 June and 13 June 2012, suggesting that the first of the expected two
483 seasonal flight periods was complete by early June. During mid-July to mid-August
484 2012, 9 male *C. promethea* were trapped in two baited traps and no moths were caught in
485 two control traps over 27 days ($P=0.002$). The entire trapping effort in both locations
486 (not including the suburban area) included 74 trapping days with 16 males captured in
487 traps baited with lures (0.22 males per baited trap per day) versus no males being caught
488 in control traps (16 vs. 0; binomial probability; $P=0.00002$).

489 We have no information on the natural density of these insects, or the
490 effectiveness of the pheromone relative to a female. The 50 μg loading rate was arbitrary
491 based on the initial success at the suburban area, and on the rather limited amounts of
492 synthetic pheromone available. A higher loading rate very likely would have captured
493 more males. To further place these results in perspective, Waldbauer and Sternburg
494 (1985) used caged females to trap male *promethea* moths in late June in Michigan and
495 reported the capture of 1.28 and 1.19 males per trap per day in 1983 and 1984,
496 respectively. For a non-pest species reported to have experienced population declines
497 (see Boettner et al. 2000), it may not be surprising for trap captures to be low when host
498 resources are dispersed over wide areas.

499 Thus, our analyses of a limited number of pheromone gland extracts and the
500 results of field trials with a synthetic compound support the hypothesis that the novel

structure (4*E*,6*E*,11*Z*,13*Z*)-hexadeca- 4,6,11,13-tetraenal is a major pheromone component of the promethea moth. It is possible that one or more of the minor components of pheromone gland extracts that elicited responses from the antennae of male moths may also be components of the full pheromone blend. Nevertheless, the tetraenal alone provides a useful tool for detection and monitoring of threatened and declining *Callosamia* moth populations.

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SUPPORTING ONLINE INFORMATION

Supporting information includes descriptions of the syntheses of isomeric mixtures of (4*E*,6*E*,11*Z*/*E*,13*E*)-hexadeca-4,6,11,13-tetraenal (**20a**), (4*E*,6*E*,11*E*/*Z*,13*Z*)-hexadeca-4,6,11,13-tetraenal (**20b**) and (4*E*,6*E*,11*E*,13*Z*/*E*)- hexadeca-4,6,11,13-tetraen-1-ol (**23**), and a video showing the responses of male moths to pheromone lures.

REFERENCES

- 523
- 524
- 525 Bayer A, Maier ME (2004) Synthesis of enamides from aldehydes and amides.
- 526 Tetrahedron 60:6665-6677
- 527 Bestmann HJ, Attygalle, AB, Schwarz, J, Garbe W, Vostrowsky O, Tomida I (1989)
- 528 Pheromones. 71. Identification and synthesis of female sex pheromone of eri-
- 529 silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae). Tetrahedron Lett
- 530 30:2911-2914
- 531 Boettner GH, Elkinton JS, Boettner CJ (2000) Effects of a biological control introduction
- 532 on three nontarget native species of saturniid moths. Conserv Biol 14:1798-1806
- 533 Brown HC, Mandal AK, Kulkarni SU (1977) Hydroboration. 45. New, convenient
- 534 preparations of representative borane reagents utilizing borane-methyl sulfide. J
- 535 Org Chem 42:1392-1398
- 536 Brown LN (1972) Mating behavior and life habits of the sweet-bay silk moth
- 537 (*Callosamia carolina*). Science 176:73-75
- 538 Collins MM, Weast RD (1961) Wild Silk Moths of the United States. Collins Radio Co.,
- 539 Cedar Rapids, Iowa, USA.
- 540 El-Sayed A (2012) The Pherobase: Database of Pheromones and Semiochemicals.
- 541 <http://www.pherobase.com>
- 542 Ferguson DC (1972) Fasc. 20.2B, Bombicoidea (in part). Pp. 243-275 in RB Dominick
- 543 et al. The Moths of North America North of Mexico. Classey, London, UK

- 544 Haskins CP, Haskins EF (1958) Note on the inheritance of behavior patterns for food
545 selection and cocoon spinning in F1 hybrids of *Callosamia promethea* × *C.*
546 *angulifera*. Behavior 13:89-95
- 547 Holden C (1992) Thousands of insects “enroll” at Yale. Science 256:313
- 548 Johnson KS, Scriber JM, Nitao JK, Smitley, DR (1995) Toxicity of *Bacillus*
549 *thuringiensis* var *kurstaki* to three nontarget Lepidoptera in field studies. Environ
550 Entomol 24:288-297
- 551 Jurberg ID, Odabchian Y, Gagosz Y (2010) Hydroalkylation of alkynyl ethers via a
552 gold(I)-catalyzed 1,5-hydride shift/cyclization sequence. J Am Chem Soc
553 132:3543-3552
- 554 Kochansky J, Tette J, Taschenberg EF, Cardé RT, Kaissling K-E, Roelofs WL (1975)
555 Sex pheromone of the moth *Antheraea polyphemus*. J Insect Physiol 21:1977-
556 1983
- 557 Millar JG, McElfresh JS, Romero C, Vila M, Mari-Mena N, López-Vaamonde C (2010)
558 Identification of the sex pheromone of a protected species, the Spanish moon
559 moth *Graellsia isabellae*. J Chem Ecol 36:923-932
- 560 Mukai C, Sugimoto Y-I, Hanoaka M (1998) A new procedure for highly stereoselective
561 and regioselective synthesis of 2-ethynyl-3-hydroxytetrahydropyran derivatives
562 based on alkyne- $\text{Co}_2(\text{CO})_6$ complex. Tetrahedron 54:823-850
- 563 Organ MG, Ghasemi H (2004) Metal-catalyzed coupling reactions on an olefin template:
564 The total synthesis of (13*E*,15*E*,18*Z*,20*Z*)-1-hydroxypentacos-13,15,18,20-
565 tetraen-11-yn-4-one 1-acetate. J Org Chem 69:695-700
- 566 Peigler RS (1977) Hybridization of *Callosamia* (Saturniidae). J Lepidop Soc 31:23-34

- 567 Peigler RS (1980) Demonstration of reproductive isolating mechanisms in *Callosamia*
568 (Saturniidae) by artificial hybridization. J Res Lepidoptera 19:72-81
- 569 Rau P, Rau N (1929) The sex attraction and rhythmic periodicity in giant saturniid
570 moths. Trans Acad Science St Louis 26:81-221
- 571 Ribe S, Kondru, RK, Beratan DN, Wipf P (2000) Optical rotation computation, total
572 synthesis, and stereochemistry assignment of the marine natural product pitiamide
573 A. J Am Chem Soc 122:4608-4617
- 574 Remington CL (1968) Suture-zones of hybrid interaction between recently joined biota.
575 Evol Biol 2:321-428
- 576 Schweitzer DF (1988) Status of Saturniidae in the northeastern USA: a quick review.
577 News Lepidop Soc 1:4-5
- 578 Skinner H (1914) *Callosamia promethea* and *angulifera* (Lepid.) Entomol News 25:468-
579 469
- 580 Sonnet PE, Heath RR (1980) Stereospecific synthesis of (Z,Z)-11,13-hexadecadienal, a
581 female sex pheromone of the navel orangeworm, *Amyelois transitella*
582 (Lepidoptera: Pyralidae). J Chem Ecol 6:221-228
- 583 Sparling BA, Simpson GL, Jamison TF (2009) Strategic use of nickel(0)-catalyzed
584 enyne-epoxide reductive coupling toward the synthesis of (-)-cyatha-3,12-diene.
585 Tetrahedron 65:3270-3280
- 586 Toliver ME, Sternburg JG, Waldbauer GP (1979) Daily flight periods of male
587 *Callosamia promethea* (Saturniidae). J Lepidop Soc 33:232-238

- 1
2
3
4 588 Tuskes PM, Tuttle JP, Collins MM (1996) The Wild Silk Moths of North America: A
5
6 589 Natural History of the Saturniidae of The United States and Canada. Cornell
7
8
9 590 University Press, Ithaca, New York
10
11 591 Waldbauer GP, Sternburg JG (1985) Adult emergence in two univoltine *Callosamia*
12
13 592 *promethea* populations: preponderance of the early emerging morph in the north
14
15
16 593 and the late emerging morph in the south (Lepidoptera: Saturniidae). Great Lakes
17
18
19 594 Entomol 18:139-142
20
21 595
22
23 596
24
25
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Figure Captions

Figure 1 Nonstereoselective syntheses of (4*E*,6*E*,11*Z*/*E*,13*Z*)-hexadeca- 4,6,11,13-tetraenals and (4*E*,6*E*,11*E*,13*Z*/*E*)-hexadeca- 4,6,11,13-tetraenals. Details of the syntheses are described in the online Supplementary Information.

Figure 2 Stereoselective synthesis of (4*E*,6*E*,11*Z*,13*Z*)-hexadeca- 4,6,11,13-tetraenal (10).

Figure 3 Representative coupled gas chromatography-electroantennogram analysis on a DB-5 column of a SPME wipe sample of the extruded pheromone gland of a female *Callosamia promethea* moth. Upper trace is the gas chromatogram, lower trace is the antennal response.

Figure 4 EI mass spectra of the component in the insect extract that elicited the largest response from male antennae in GC-EAD analyses (top) and synthetic (4*E*,6*E*,11*Z*,13*Z*)-hexadeca- 4,6,11,13-tetraenal (bottom).

Figure 5 A) Cube trap built for field bioassays; B) Male *Callosamia promethea* on a pheromone-baited rubber septum inside the cube trap; C) A male *Callosamia promethea* moth that was netted at a pheromone lure.

Figure 1

Figure 2

Figure 3

Figure 4

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Figure 1

Figure 2.

Figure 3
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