Synthesis and bioactivity of (13Z,15E)-octadecadienal: A sex pheromone component from Micromelalopha siversi Staudinger (Lepidoptera: Notodontidae)

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Abstract

BACKGROUND: Micromelalopha siversi (Staudinger) (Lepidoptera: Notodontidae) is a defoliator of poplar trees, Populus spp. (Salicaceae). In our previous study, 13,15-octadecadienal has been conformed as a female-produced candidate sex pheromone component of M. siversi, but the Z/E stereochemistry of the 1,3-diene system has not been identified so far.

RESULTS: Four unsaturated aliphatic aldehydes, Z13,E15-18:Ald, Z13,Z15-18:Ald, E13,E15-18:Ald, and E13,Z15-18:Ald, were synthesized from the commercially available 12-bromo-1-decanol mainly by alkylation of lithium alkyn, normal Wittig or Wittig–Schlosser olefination, and hydroboration-protonolysis. According to gas chromatography (GC) analysis of pheromone gland extracts, Z13,E15-18:Ald was the main component, and a small amount of Z13,Z15-18:Ald was also detected, with a ratio of approximately 7:3. However, the results of GC-electroantennographic detection (GC-EAD) showed that Z13,E15-18:Ald was the only compound with electrophysiological activity, whereas Z13,Z15-18:Ald elicited no activity. In the field, traps baited with only Z13,E15-18:Ald resulted in much superior results to those with Z13,Z15-18:Ald as well as the Z13,E15-18:Ald and Z13,Z15-18:Ald binary mixture.

CONCLUSIONS: Based on geometrically selective synthesis and bioactivity tests, the active sex pheromone component of M. siversi has been identified as Z13,E15-18:Ald, the pheromone component that has not been identified in Lepidoptera before. The synthetic component was attractive to male moths in preliminary field traps, which provides novel technologies to monitor and control this pest.

Supporting information may be found in the online version of this article.

Keywords: Micromelalopha siversi; sex pheromone; Z13,E15-18:Ald; chemical synthesis

1 INTRODUCTION

Poplar (Populus sp.) represents the most widely distributed and adaptable tree species in the world. An increasing amount of land is being used to plant poplars, particularly in China, South Korea, and the USA. Meanwhile, many countries with limited natural forests use poplars from plantations as an important timber source.1 In addition, poplar plantations are also being investigated as renewable sources of energy for environmental improvement,2–4 especially for important short rotation coppice (SRC) plantations, which contain and absorb vast quantities of atmospheric carbon dioxide.5–8

Micromelalopha siversi (Staudinger) (Lepidoptera: Notodontidae), which is mainly distributed in China, is one of the defoliators that severely damage poplar plantations.9 In addition, M. siversi larvae usually injure the mesophyll, causing balding of poplar branches, weakening the host and curtailing growth. Over recent decades, the biological characteristics of M. siversi have been extensively studied.10,11 Commonly, M. siversi has three to four generations in northeastern China, and five to seven generations in south-central China, but the generations overlap extensively. The females have a clear circadian rhythm-related calling behavior during the scotophase, but not during the light period.12 After mating, females will deposit their eggs on the poplar leaves. The larvae have five instar stages, and the mature larvae will pupate in the deciduous layer overwinter.13

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Outbreaks of this leaf-feeding pest across China have resulted in the wide application of natural insecticides or artificially synthesized pyrethroids, negatively affecting biodiversity as well as natural enemies within the ecosystem. Therefore, more environmentally acceptable approaches are required to control M. siversi effectively.

At present, mass trapping or mating disruption using species-specific sex pheromone traps are effective approaches to control numerous Lepidoptera species. The sex pheromone component plays an important role in contacting and promoting the chemical communication and reproductive behavior of M. siversi. A 13,15-octadecadienal, an unsaturated aliphatic aldehyde, was found to be the sex pheromone component of M. siversi by our group in 2019. However, the stereochemistry concerning the double bond at C-13 and C-15 has not yet been characterized.

In this study, the total synthesis of Z13,E15-18:Ald, Z13,Z15-18:Ald, E13,E15-18:Ald, and E13,Z15-18:Ald is presented. Moreover, electrophysiological and behavior tests were carried out to characterize the active sex pheromone component of M. siversi and to prepare an effective lure to monitor and control M. siversi.

2 MATERIALS AND METHODS

2.1 Insects and pheromone extraction

M. siversi pupae were obtained from Suiping (Henan Province, China), separated by sex and reared under the following conditions, temperature of 26 ± 1 °C, light/dark cycle of 14 h/10 h, together with relative humidity (RH) of 70 ± 5%. The adults were raised with 10% honey solution that was put onto cotton. On days 1 or 2 following emergence, the female moths were adopted to extract pheromone, whereas the male counterparts were utilized in gas chromatography-electroantennographic detection (GC-EAD) analysis. For the virgin calling female moths, their abdominal tips were cut, followed by extraction with distilled hexane for 30–40 min. Thereafter, the resultant hexane extracts were placed in 2-mL glass vials (Agilent Technologies, Palo Alto, CA, USA) and preserved in a refrigerator (Haier, Qingdao, Shandong, China) at −20 °C for subsequent chemical analyses.

2.2 GC-EAD analysis

An Agilent 7890A GC containing a flame ionization detector (FID) was used to perform coupled GC-EAD. A Y splitter (5181–3397, Agilent Technologies) along with an HP-5 capillary column (inside diameter 30 m × 250 μm, thickness of film 0.25 μm; Agilent Technologies) was used in analyses. As the effluent of the GC column, the carrier gas (hydrogen and nitrogen) was separated at a ratio of 1:1 to simultaneously detect between the FID and the EAD apparatuses. A 1-μL splitless sample was added at 220 °C (inlet temperature). The oven was held at 60 °C for 2 min, then programmed to 250 °C at a heating rate of 8 °C min⁻¹. This final temperature was maintained for 10 min. An EAD probe with high resistance, an Intelligent Data Acquisition Controller (CS-55), type IDAC-02, along with a Signal Interface Box (Syntech, Buchenbach, Germany) were used to detect antennal depolarization. The basic segment of the moth at 1–2 days of age was cut with caution to prepare freshly resected male antenna, which was later added to a glass capillary filled with 0.9% normal saline housing 0.39-mm silver wires (Sigmund Cohn Corp, Mt. Vernon, NY, USA). Electrodes were connected to a combi Probe (PRG-3, Syntech). The humid air filtered with charcoal flowed through the glass tube at a flow rate of 1 L min⁻¹. The glass tube was connected to the GC transfer line, which was designed to track the temperature of GC oven. A compound that was able to elicit an antennal response at least five times was considered to show electroantennographical activity.

2.3 Gas chromatography–mass spectrometry analysis

The chemical analysis was carried out using an Agilent GC coupled with a mass spectrometry system (TRACE GC 2000). The GC system was equipped with a DB-5 ms column (30 m × 0.25 mm × 0.25 μm). Samples of 1 μL from different solutions were injected manually into the system at an injector temperature of 230 °C under the splitless mode. The oven was held for 2 min at 80 °C, then heated to 190 °C at a heating rate of 15 °C min⁻¹, and maintained at that temperature for 10 min. The carrier gas (helium) was injected at a flow rate of 1.2 mL min⁻¹, filament bias voltage 70 eV, and ion source temperature of 250 °C. The scanning mode was adopted to obtain spectra (range of mass m/z 35–500). Compound retention times (RTs) were compared with synthesized standards to identify the compounds. The NIST11 library (Scientific Instrument Services, Inc., Ringoes, NJ, USA) was used to obtain mass spectra for reference.

2.4 Gas chromatography coupled with flame ionization detection

GC-FID analysis was performed on an Agilent 7890A equipped with a 30 m × 0.25 mm × 0.25-μm HP-FFAP column (Agilent Technologies). Samples of 1 μL from different solutions were injected manually into the system at the split mode (ratio 1:40) with an injector temperature of 220 °C. The oven was held for 2 min at 100 °C, then heated to 190 °C at a heating rate of 15 °C min⁻¹, maintained for 10 min, then further heated to 225 °C at a heating rate of 8 °C min⁻¹, and maintained for 10 min. Carrier gas (nitrogen) was injected at a flow rate of 1.0 mL min⁻¹. An FID operating at 230 °C was used for detection. The pheromone components were quantified relative to the external standard (1 μL aliquot of 5 ng μL⁻¹ n-tridecane).

2.5 Chemicals

All reactants used for synthesizing the four diastereomers of 13,15-octadecadienial were purchased from Sigma-Aldrich (St Louis, MO, USA). The solvents used to prepare gland extracts and to carry out chromatographic analyses were purchased from HPLC grade and provided by Sigma-Aldrich, while those used in synthesizing compounds were at Pro analysis grade and provided by Aladdin (Shanghai, China). A Bruker NMR spectrometer (Bruker, Fällanden, Switzerland) was used to record the NMR spectra in CDCl₃ (1H and 13C at 500 and 125 MHz, respectively), with tetramethylsilane as the internal standard.

2.6 Synthesis

The general synthetic procedures for diastereomers of 13,15-octadecadienial (1–4) were as follows (Schemes 1–3).

2.6.1 1,1-Dioctoxy-12-iododecane (6)

Pyridinium chlorochromate (860 g, 400 mmol) was used to oxidize 12-bromodecanol 5 (52.8 g, 200 mmol) in CH₂Cl₂ (800 mL) under ambient temperature for 4 h. The solvent removal was conducted at reduced pressure, while petroleum was used to wash the residual. Typically, solvent removal in vacuum resulted in a dark oil. SiO₂ was used to chromatograph the residue, while the crude 10-bromodecanol was produced through eluting using hexane:ethyl acetate (50:1, v/v) (Scheme 1).
Triethyl orthoformate (44.5 g, 300 mmol) was mixed with p-sulphonic acid monohydrate (0.57 g, 3 mmol) and the resultant mixture was placed into 300 mL of anhydrous ethanol solution containing crude 10-bromodecanal at 0 °C. Later, the resultant mixture was allowed to stand overnight at 0 °C. Next, water and K2CO3 solution were added to the mixture to prepare the basic solution. Diethyl ether was used to extract the mixture, followed by brine washing, MgSO4 drying and solvent removal at reduced pressure. Finally, chromatographic analysis of the obtained residue was conducted on silica with hexane and ethyl acetate (30:1, v/v), resulting in crude 1,1-diethoxy-12-bromodecanal being obtained.

Later, the as-obtained product was stirred using sodium iodide (90 g, 600 mmol) within dry acetonitrile (500 ml) in the presence of reflux to convert to iodide. After solvent removal at reduced pressure, water (200 mL) was used to dilute the mixture, while petroleum was used to extract the product. The extracts were rinsed with water, brine and 1% Na2S2O3 solution, followed by Na2SO4 drying, concentration at reduced pressure, and chromatographic analysis of the residue on SiO2. After eluting using hexane:ethyl acetate (30:1, v/v) a colorless oil was obtained, which was 1,1-diethoxy-12-iododecan (6) in 80% yield (61.4 g) according to 12-bromodecanol. 1H NMR (500 MHz, CDCl3, ppm): 1.19 (6H, t, J = 7.0 Hz, 2CH3), 1.29 (16H, m, 8CH2), 1.60 (2H, m, H-2), 1.82 (2H, m, H-11), 3.18 (2H, t, J = 7.0 Hz, H-12), 3.48 (2H, m, CH2O), 4.47 (1H, t, J = 6.0 Hz, OCHO). 13C NMR (125 MHz, CDCl3, ppm): 102.9 (OCHO), 60.8 (CH2O), 33.6 (C-2), 33.5 (C-11), 30.5 (C-10), 29.5 (C19), 29.5 (C18), 29.4 (C17), 29.4 (C16), 28.5 (C15), 28.5 (C14), 24.7 (C13), 15.3 (C12), 15.3 (C13), 15.3 (C14), 7.3 (C-12).

**Scheme 1.** The synthetic route of 15,15-diethoxypentadec-2-yn-1-ol 7. PCC, pyridinium chlorochromate; HC(OEt)3, triethoxy methane; p-TsOH·H2O, p-toluenesulfonic acid monohydrate; NaI, sodium iodide.

**Scheme 2.** The synthetic routes of the (13Z,15E)- and (13Z,15Z)-octadecadienal isomers. EMD, electrolytic manganese dioxide; PentylPPh3Br, pentyl(triphenyl)phosphonium bromide; n-BuLi, n-butyllithium; THF, tetrahydrofuran; KHMDS, potassium bis(trimethylsilyl)amide; (Cyclohexyl)2BH, dicyclohexylborane; (CO2H)2, oxalic acid.
2.6.2 15,15-Diethoxypentadec-2-yn-1-ol (7)
n-Butyllithium (2.5 M in hexane) (160 mL, 400 mol) was slowly added to a solution of propargyl alcohol (11.2 g, 200 mol) in hexamethyl phosphoril triamide (HMPT) and tetrahydrofuran (THF; 800 mL, 1:1, v/v) at −40 °C in the presence of argon. After stirring for 30 min, 1,1-diethoxy-12-iododecane (6; 38.4 g, 100 mmol) in HMPT:THF mixed solution (50 mL, 1:1, v/v) was added over 20 min, followed by overnight stirring. Later, water was added to quench the reaction mixture, while ethyl acetate was added for extraction. Afterwards, water was added to wash the organic layer, then Na2SO4 was used for drying and the crude product was obtained after evaporation. The colorless oil 15,15-diethoxypentadec-2-yn-1-ol (7) was obtained (23.4 g, 75% yield) after elution with hexane:ethyl acetate (10:1, v/v). 1H NMR (500 MHz, CDCl3, ppm): 1.19 (6H, t, J = 7.0 Hz, 2CH3), 1.25–1.31 (16H, m, 8CH2), 1.46–1.52 (2H, m, H-11), 1.57–1.61 (2H, m, H-2), 2.12–2.21 (2H, m, H-12), 3.45–3.51 (2H, m, CH2O), 3.60–3.66 (2H, m, CH2O), 4.24 (2H, t, J = 2.0 Hz, H-15); 4.47 (1H, t, J = 6.0 Hz, OCHO). 13C NMR (125 MHz, CDCl3, ppm): 102.9 (OCHO), 86.5 (C-13), 78.2 (C-14), 60.8 (CH2O), 60.8 (CH2O), 51.3 (C-15), 33.5 (C-2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.4 (CH2), 29.4 (CH2), 29.0 (CH2), 28.8 (CH2), 28.6 (CH2), 24.7 (C-3), 18.7 (C-12), 15.3 (CH3), 15.3 (CH3).

2.6.3 15,15-Diethoxypentadec-2-ynal (8)
Electrolytic manganese dioxide (EMD; 52.1 g, 600 mmol) was placed into a dry hexane solution (300 mL) of 15,15-diethoxypentadec-2-yn-1-ol (7; 10.0 g, 32.0 mmol). The resultant solution was subjected to 4 h of stirring under ambient temperature, then the manganese-containing residues were filtered off. The protected aldehyde 8 (a pale-yellow oil) was obtained after elution with hexane:ethyl acetate (20:1, v/v), resulting in a 93% yield (9.24 g). 1H NMR (500 MHz, CDCl3, ppm): 1.20 (6H, t, J = 7.0 Hz, 2CH3), 1.26–1.33 (16H, m, 8CH2), 1.38–1.42 (2H, m, H-11), 1.57–1.60 (2H, m, H-2), 2.40 (2H, t, J = 7.5 Hz, H-12), 3.45–3.53 (2H, m, CH2O), 3.60–3.66 (2H, m, CH2O), 4.47 (1H, t, J = 6.0 Hz, OCHO), 9.17 (1H, s, CHO). 13C NMR (125 MHz, CDCl3, ppm): 177.3 (CHO), 102.9 (OCHO), 94.5 (C-13), 77.2 (C-14), 60.8 (OCH2O), 60.8 (OCH2O), 33.6 (C-2), 29.5 (CH2), 29.5 (CH2), 29.3 (CH3), 29.3 (CH3), 28.8 (CH2), 28.7 (CH2), 27.5 (CH2), 24.7 (C-3), 19.1 (C-12), 15.3 (CH3), 15.3 (CH3).

2.6.4 (E)-18,18-Diethoxyoctadec-3-en-5-yne (9)
Propyl triphenylphosphonium bromide (4.62 g, 12.0 mmol) was suspended in THF (100 mL), followed by 30 min of stirring with n-BuLi (4.8 mL, 2.5 M in hexane). The resulting mixture was cooled to −70 °C, followed by the addition of aldehyde 8 (3.1 g, 10.0 mmol) in THF (5 mL). The mixture was stirred vigorously until the yellow coloration disappeared, and an additional amount of n-BuLi (2.5 M, 4.8 mL, 12.0 mmol) was added. The reaction mixture was stirred at −30 °C for 5 min, whereupon a solution of hydrogen chloride in ethyl ether (1 M, 13.0 mmol, 13.0 mL) and then potassium tertbutoxide (2.04 g, 18.2 mmol) in 2-methyl-2-propanol (1.35 g, 18.2 mmol) was added. Later, the mixed solution was subjected to 2 h of stirring under ambient temperature, water washing till neutrality, and MgSO4 drying. After evaporation of the solvent, the crude product (E-9 Z-9 = 7:1) was subjected to 10% silver nitrate SiO2 with hexane:ethyl acetate (60:1, v/v), yielding 61% (2.65 g) of the E-9 as a colorless oil. 1H NMR (500 MHz, CDCl3, ppm): 0.99 (3H, t, J = 7.5 Hz, CH3), 1.19 (6H, t, J = 7.0 Hz, CH3), 1.42–1.51 (16H, m, 8CH2), 1.60–1.68 (2H, m, H-2), 2.60–2.70 (2H, m, H-12), 3.45–3.51 (2H, m, CH2O), 3.60–3.66 (2H, m, CH2O), 4.24 (2H, t, J = 2.0 Hz, H-15); 4.47 (1H, t, J = 6.0 Hz, OCHO), 9.17 (1H, s, CHO). 

Scheme 3. The synthetic routes of the (13E,15E)- and (13E,15Z)-octadecadienal isomers. LiAlH4, lithium aluminium hydride; EMD, electrolytic manganese dioxide; PentylPPh3Br, pentyl(triphenyl)phosphonium bromide; n-BuLi, n-butyllithium; THF, tetrahydrofuran; KHMDS, potassium bis(trimethylsilyl)amide; (Cyclohexyl)2BH, dicyclohexylborane; (CO2H)2, oxalic acid.
A solution of compound 9 (2.0 g, 5.95 mmol) in THF (10 mL) was added dropwise to dicyclohexylborane solution (12.0 mmol). The suspension was stirred at −15 °C for 2 h, followed by stirring and heating of the obtained mixture under ambient temperature and further stirred under ambient temperature for 2 h until no dicyclohexylborane precipitate was observed. The resulting solution was mixed with 2 mL of glacial acetic acid and stirred at 50 °C for 2 h. Subsequently, 3 mL of 6 M sodium hydroxide and 3 mL of 35% hydrogen peroxide were added in succession to oxidize the resultant dicyclohexylborinate. The mixture was stirred for an additional 30 min and poured into 15 mL of ice water, extracted with hexane and dried with MgSO4. The mixture was stirred for 3 h and added to ambient temperature and further stirred under ambient temperature, and then 5 g of Celite was applied to dry the integrated organic phases. The crude product was purified by flash chromatography at medium pressure (hexane:ethyl acetate 60:1) to obtain an isomeric purity of >96% and a 62% yield based on 9 (1.02 g, colorless oil). 1H NMR (500 MHz, CDCl3, ppm): δ: 0.99 (3H, t, J = 7.5 Hz, CH3), 1.26–1.30 (16H, m, 8CH2), 1.60–1.64 (2H, m, H-3), 2.10–2.16 (4H, m, H-12 and H-17), 2.41 (2H, td, J = 7.5, 1.5 Hz, H-2), 3.50 (2H, m, OCH2), 4.47 (1H, t, J = 7.5 Hz, CH3), 5.44 (1H, t, J = 13.5, 6.5 Hz, H-16), 6.59 (1H, t, J = 11.0 Hz, H-14), 9.76 (1H, t, J = 2.0 Hz, CHO). 13C NMR (125 MHz, CDCl3, ppm): δ: 22.1 (C-3), 20.8 (C-9), 29.3 (CH2), 29.3 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 29.5 (CH2), 3.52 (2H, m, OCH2), 3.60 (2H, m, OCH2), 6.01 (1H, t, J = 7.5 Hz, CH3), 1.20 (6H, t, J = 7.0 Hz, 2CH3), 1.25–1.28 (16H, m, 8CH2), 1.47–1.53 (2H, m, H-5), 1.57–1.61 (2H, m, H-14), 2.30–2.35 (2H, m, H-4), 3.46–3.50 (2H, m, OCH2), 3.61–3.64 (2H, m, OCH2), 4.47 (1H, t, J = 6.0 Hz, CH3), 6.11 (1H, m, H-2), 6.85 (1H, dt, J = 15.5, 6.5 Hz, H-3), 9.49 (1H, d, J = 8.0 Hz, CHO). 13C NMR (125 MHz, CDCl3, ppm): δ: 194.2 (CHO), 159.1 (C-10), 133.6 (C-16), 132.1 (C-13), 123.4 (C-14), 123.0 (C-15), 43.9 (C-2), 29.6 (C-12), 29.5 (CH2), 29.5 (CH2), 29.4 (CH2), 29.3 (CH2), 29.3 (CH2), 29.1 (CH2), 27.4 (CH2), 22.1 (C-3), 20.8 (C-17), 14.2 (CH2). GC-MS (70 eV, m/z): 264, 235, 221, 147, 135, 121, 109, 95, 81, 67, 55.

A THF solution (10 mL) of compound 7 (10.0 g, 32.1 mmol) was added dropwise to a suspension of LiAlH4 (1.22 g, 32.1 mmol) in THF (20 mL). The resultant mixed solution was subjected to 2 h of stirring under ambient temperature, and then 5 g of Celite and Na2SO4·10H2O mixture (1:1, v/v) was carefully added to quench the reaction, followed by slurry filtering. Next, 20 mL of hexane was used to wash the Celite bed three times. MgSO4 was used to dry the integrated organic phase before solvent evaporation at reduced pressure. EMD was used to oxidize the crude product. The dienal 10 was prepared according to a previous description for preparing compound 8 based on compound 7, producing a 90% yield in two steps (9.02 g, pale-yellow oil). 1H NMR (500 MHz, CDCl3, ppm): δ: 1.20 (6H, t, J = 7.0 Hz, 2CH3), 1.25–1.30 (16H, m, 8CH2), 1.47–1.53 (2H, m, H-5), 1.57–1.61 (2H, m, H-14), 2.30–2.35 (2H, m, H-4), 3.46–3.50 (2H, m, OCH2), 3.61–3.64 (2H, m, OCH2), 4.47 (1H, t, J = 6.0 Hz, CH3), 6.11 (1H, m, H-2), 6.85 (1H, dt, J = 15.5, 6.5 Hz, H-3), 9.49 (1H, d, J = 8.0 Hz, CHO). 13C NMR (125 MHz, CDCl3, ppm): δ: 194.2 (CHO), 159.1 (C-10), 133.6 (C-16), 132.1 (C-13), 123.4 (C-14), 123.0 (C-15), 43.9 (C-2), 29.6 (C-12), 29.5 (CH2), 29.5 (CH2), 29.4 (CH2), 29.3 (CH2), 29.3 (CH2), 29.1 (CH2), 27.4 (CH2), 22.1 (C-3), 20.8 (C-17), 14.2 (CH2). GC-MS (70 eV, m/z): 264, 235, 221, 147, 135, 121, 109, 95, 81, 67, 55.
(CH₃), 29.5 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 25.6 (CH₂), 24.7 (CH₂), 15.3 (CH₃), 15.3 (CH₃), 13.6 (C-18).

2.6.10 (13E,15E)-Octadecadienal (3)

The dienal 3 was prepared from compound 11 (2.03 g, 6.0 mmol) as described before (deprotection of the acetal group by means of oxalic acid dihydrate) at an isomeric purity of >95%, producing 95% yield (1.51 g).¹H NMR (500 MHz, CDCl₃, ppm) δ: 0.99 (3H, t, J = 7.5 Hz, CH₃), 1.25–1.29 (16H, m, 8CH₂), 1.61 (2H, quint, J = 7.0 Hz, H-3), 2.02–2.09 (4H, m, H-12 and H-17), 2.41 (2H, td, J = 7.5, 1.5 Hz, H-2), 5.54–5.63 (2H, m, H-13 and H-16), 5.97–6.02 (2H, m, H-14 and H-15), 9.76 (1H, t, J = 2.0 Hz, CHO). ¹³C NMR (125 MHz, CDCl₃, ppm) δ: 20.29 (CHO), 133.8 (C-16), 132.5 (C-13), 130.3 (C-14), 129.4 (C-15), 43.9 (C-2), 32.6 (C-12), 29.5 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 25.6 (C-3), 22.1 (C-17), 13.6 (CH₃). GC-MS (70 eV, m/z): 264, 235, 221, 207, 143, 121, 109, 95, 81, 67, 55.

2.6.11 (13E,15Z)-1,1-Diethoxy-octadecadienal (11')

Compound 11' was prepared from compound 10 (1.56 g, 5 mmol) according to a previous description for preparing compound 9 based on compound 8. A mixture of Z-11' and E-11 at 20:1 was obtained during this process, which was chromatographed (10% silver nitrate SiO₂, hexane:ethyl acetate, 60:1) to remove the colorless oil 11 (1.14 g, 68% yield).¹H NMR (500 MHz, CDCl₃, ppm) δ: 0.99 (3H, t, J = 7.5 Hz, CH₃), 1.20 (6H, t, J = 7.5 Hz, 2CH₂), 1.26–1.28 (16H, m, 8CH₂), 1.37 (2H, m, H-11), 1.60 (2H, m, H-3), 2.06 (2H, q, J = 8.0 Hz, H-17), 2.17 (2H, q, J = 7.5 Hz, H-12), 3.45–3.51 (2H, m, OCH₂), 3.60–3.66 (2H, m, OCH₂), 4.47 (1H, t, J = 6.0 Hz, CH), 5.29 (1H, dt, J = 10.5, 7.5 Hz, H-13), 5.65 (1H, tdt, J = 14.5, 7.0 Hz, H-16), 5.91 (1H, dt, J = 14.5, 7.0 Hz, H-14), 6.27 (1H, ddq, J = 14.0, 11.0, 1.5 Hz, H-15). ¹³C NMR (125 MHz, CDCl₃, ppm) δ: 134.7 (C-16), 131.6 (C-13), 128.0 (C-14), 125.4 (C-15), 102.9 (O-CH-O), 60.8 (CH₂-O), 60.8 (CH₂-O), 33.6 (C-2), 32.9 (C-12), 29.6 (CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.5 (CH₂), 29.5 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 24.7 (CH₂), 20.9 (CH₂), 15.3 (CH₂), 14.3 (C-18).

2.6.12 (13E,15Z)-Octadecadienal (4)

The dienal 4 was prepared from compound 11' (1.0 g, 2.95 mmol) as previously described (for the deprotection of acetal group by oxalic acid dihydration) at an isomeric purity of >95%, resulting in 96% yield (0.75 g).¹H NMR (500 MHz, CDCl₃, ppm) δ: 0.99 (3H, t, J = 7.5 Hz, CH₃), 1.26–1.30 (16H, m, 8CH₂), 1.61 (2H, quint, J = 7.5 Hz, H-3), 2.08 (2H, q, J = 7.5 Hz, H-17), 2.17 (2H, q, J = 7.5 Hz, H-12), 2.41 (2H, td, J = 7.5, 1.5 Hz, H-2), 5.29 (1H, dt, J = 11.0, 7.5 Hz, H-13), 5.65 (1H, dt, J = 14.5, 7.0 Hz, H-16), 5.91 (1H, t, J = 11.0 Hz, H-14), 6.29 (1H, ddq, J = 15.0, 11.0, 1.5 Hz, H-15), 9.76 (1H, t, J = 2.0 Hz, CHO). ¹³C NMR (125 MHz, CDCl₃, ppm) δ: 202.9 (CHO), 134.7 (C-16), 131.6 (C-13), 128.0 (C-14), 125.5 (C-15), 43.9 (C-2), 29.5 (CH₂), 29.5 (CH₂), 29.5 (CH₂), 29.5 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 22.1 (C-3), 20.9 (C-17), 14.3 (CH₃).

2.7 Field attractiveness test

Field attractiveness tests were conducted in poplar plantations located at Shanghai (Shandong, China) and Suipin (Henan, China) from August to September 2019.

The delta-shaped traps, each with a sticky board baited with the synthetic compound (1000 μg) at different ratios and 1% butylated hydroxytoluene was injected into a polyvinyl chloride (PVC) capillary tube (inner diameter 0.1 cm, outer diameter 0.18 cm, length 10 cm) (Pherobio Technology Co. Ltd, Beijing, China), were hung from a plastic pole at a height of 3.0 m above the ground at intervals of 30–40 m. The pheromone compounds were dissolved in distilled hexane, then 10 μL of the mixed solution was added to the PVC tubes, with one PVC tube containing distilled hexane solution acting as the reference. In previous tests, no activity was elicited by the

Figure 1. (a) GC analysis of a mixture of synthetic compounds. (b) GC analysis of the pheromone extracts of female M. siversi. 1, (13Z,15E)-octadecadienal; 2, (13Z,15Z)-octadecadienal; 3, (13E,15E)-octadecadienal; 4, (13E,15Z)-octadecadienal.

Z13,E15-18:Ald: A sex pheromone component from Micromelalopha siversi www.soci.org Pest Manag Sci 2021; 77: 264–272 © 2020 The Authors. Wiley Online Library Journal: Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.
2.8 Statistical analysis

One-way analysis of variance was adopted for data analysis, and the means were compared through Tukey’s honestly significant difference test (SPSS 17.0, Inc., Chicago, IL, USA). The significance level was α = 0.05 for each test.

3 RESULTS

3.1 Synthesis of the four stereoisomers of 13,15-octadecadienial

According to our procedure, the aldehyde derivative, 1,1-diethoxy-12-iododecane 6, was obtained by three steps (80% yield through 12-bromo-1-decanol). The acetylenic compound 1 was obtained by the alkylation of lithium propargyl alcohol with 6 in HMPT: THF, with a yield of 75% (Scheme 1).

Aldehyde 8 was acquired through oxidizing intermediate 7 with electrolytic manganese dioxide (EMD), resulting in a 93% yield (Scheme 2). E-18,18-diethoxyoctadec-3-en-5-yne 9 was generated from a process that started with the Wittig–Schlosser reaction (Scheme 2). Aldehyde 8 was converted to (E)-18,18-diethoxyoctadec-3-en-5-yne 9 (E:Z-9:Z′ = 7:1) with n-propyl triphenylphosphonium bromide using n-BuLi as the base. Z-18,18-diethoxyoctadec-3-en-5-yne 9′ (Z′:E-9′ = 16:1) was prepared by the normal Wittig reaction. Aldehyde 8 was subjected to a (Z)-selective Wittig reaction using an ylide prepared from pentytriphenylphosphonium bromide, with potassium bis(trimethylsilyl)amide used as the base (Scheme 2). 9 and 9′ were subjected to (Z)-selective reduction of the triple bond separately by the hydroboration–protonolysis process using dicyclohexylborane in hexane. The acetal group was removed under acidic conditions (aqueous oxalic acid). After purification by column chromatography using silica gel impregnated with 10% silver nitrate, the (Z)-diene 1 (63% based on 9, isomeric purity >95%) and (Z,Z)-diene 2 (62% based on 9, isomeric purity >96%) were obtained.

The normal (E,E-11′/E,E-11 = 20:1, 68% yield) and Schlosser–Wittig conditions (E,E-11′/E,E-11′ = 10:1, 66% yield) were adopted for the separate formation of the protected E,E-11 and E,Z-11′ dienals (Scheme 3). The isomeric purity levels of E,E-3 and E,Z-4 increased to >95% following deprotection and purification.

Finally, Z13,E15-18:Ald, Z13,Z15-18:Ald, E13,E15-18:Ald, and E13,Z15-18:Ald were successfully synthesized in eight steps.

3.2 Chromatographic analysis

The GC spectra of pheromone extracts were compared with those of the synthesized compounds (Z13,E15-18:Ald 1: Retention time 20.55 min; Z13,Z15-18:Ald 2: Retention time 20.98 min; E13,E15-18:Ald 3: Retention time 21.22 min; E13,Z15-18:Ald 4: Retention time 20.77 min) (Fig. 1(a),(b)). It was found that Z13,E15-18:Ald 1 was the major component (~14.72 ± 8.68 ng/gland) and existed together with a very small amount of Z13,Z15-18:Ald 2 at a ratio of about 7:3.

Figure 2. Response of M. siversi male antennae to synthetic standards detected by GC-EAD. 1, (13Z,15E)-octadecadienial; 2, (13Z,15Z)-octadecadienial.

Figure 3. The number of M. siversi male moths trapped (mean ± SE) in lures containing the synthesized compounds (dose = 1000 μg). (a) In Shanghe, Shandong (116.57° 34.14′ E, 37.20°17.38′ N) from 26 August to 4 September 2019. Five duplicates were set for every treatment (F(3,16) = 23.543, P < 0.001). (b) In Suipin, Henan (113.23°40.18′ E, 33.15°26.23′ N) from 1 to 14 September 2019. Five duplicates were set for every treatment (F(4,20) = 8.866, P < 0.001). Columns designated with different lowercase letters indicate significant differences (P < 0.05).

Field experiment 1 was conducted from 26 August to 4 September 2019 in Shanghe, Shandong, China. The lures baited with (13Z,15E)-octadecadienial and (13Z,15Z)-octadecadienial (ratios 1:0, 1:1, 0:1 and 0:0) were used as the base (Scheme 2). The number of moths trapped was determined twice weekly. The lures baited with the synthesized compounds at 1:0, 4:1, 3:2, 0:1 were baited with the synthesized compounds at 1:0, 4:1, 3:2, 0:1 and 0:0 ratios. Five duplicates were set for each treatment and the number of moths trapped was determined twice weekly.

Field experiment 2 was conducted from 1 to 14 September 2019 in Suipin, Henan, China. To illustrate bait effectiveness, all lures were baited with the synthesized compounds at 1:0, 4:1, 3:2, 0:1 and 0:0 ratios. Five duplicates were set for each treatment and the number of moths trapped was determined twice weekly.

The number of moths trapped was determined twice weekly.
The male antennal response to a mixture of Z13,E15-18:Ald 1 and Z13,Z15-18:Ald 2 was tested (Fig. 2). As suggested by the GC-EAD results, Z13,E15-18:Ald 1 was the only electrophysiologically active compound (Retention time 22.02 min), whereas Z13, Z15-18:Ald 2 elicited no activity (22.33 min) (Fig. 2).

3.3 Field attractiveness test
In field experiment 1, the attractiveness of Z13,E15-18:Ald and Z13,Z15-18:Ald was tested and showed significant differences among all treatments (F(3,16) = 23.543, P < 0.001) (Fig. 3(a)). Traps baited with Z13,Z15-18:Ald alone caught a small number of males and there was no difference compared with the control. However, the number of catches increased significantly when Z13,E15-18:Ald was added. Traps baited with Z13,E15-18:Ald alone caught more males than those baited with Z13,Z15-18:Ald alone and the binary blend (1:1). The results show that the presence of Z13,E15-18:Ald significantly affects the attractiveness of the traps.

In field experiment 2, to further verify the attractiveness of Z13,E15-18:Ald and the binary blends of Z13,E15-18:Ald and Z13,Z15-18:Ald were tested in another field experiment (F(1,40) = 8.866, P < 0.001) (Fig. 3(b)). The traps baited with Z13,Z15-18:Ald alone caught fewer males among the tested lures at different ratios. Meanwhile, the numbers of catches of the binary blends were almost the same as those in traps baited with Z13,15-18:Ald. The traps baited with Z13,E15-18:Ald alone caught the most males among the tested lures. These results indicate that Z13,E15-18:Ald is the key sex pheromone component of M. siversi. Although Z13,Z15-18:Ald exists in the pheromone extracts of M. siversi, it might make no difference to the attractiveness of Z13,E15-18:Ald.

4 DISCUSSION
In Lepidoptera, sex pheromone components play an important role in chemical communication and reproductive behavior. There are considerable differences in the chemical structures of the species-specific pheromones. Typically, the varied pheromones are mainly categorized by their chemical structures.19,20 Type I pheromone components of 10 species from Notodontid have been described, most of which contain C16 (Z,Z)-dienes or conjugated enyne moieties.21 In Thaumetopoea japonica, the sex pheromone represents a mixture composed of Z11,Z13-16:Ald and Z11,Z13-16:OH.21 For T. pityocampa, T. wilkinsoni, and T. processionea, Z13,Y11-16:Ac represents the critical sex pheromone.22–24 Similarly, Z13,Y11-16:Al is the critical sex pheromone in Heterocampa guttivitta,25 while Z11,13-15: Ald and Z11,Z13-16:Ac are the critical sex pheromones in Notodontia dromedaries and N. torva, respectively.26,27 Interestingly, there were no traces of C16-dienes or conjugated enynes in the pheromone gland extracts of M. siversi, where 13,15-octadecadienyl was the only active component detected. Therefore, M. siversi appears to be the only species of Notodontidae that uses octadecadienyl as the sex pheromone component. In this study, Z13,E15-18:Ald, Z13,Z15-18: Ald, E13,E15-18:Ald, and E13,Z15-18:Ald were synthesized by geometrically selective approaches. Based on the electrophysiological and field experimental results, Z13,E15-18:Ald was found to be an attractant component in the blend of sex pheromones of M. siversi.

The unsaturated aliphatic aldehyde 13,15-octadecadienyl consists of a terminal formyl group and a 1,3-diene system, which act as the critical functional groups for the recognition of the sex pheromone components of M. siversi. As far as we know, there are few reports on the unsaturated dienyl type I pheromone at C-13 and C-15 in Lepidoptera. Typically, only Z13,Z15-18:Ald is suggested to be a component of the sex pheromone in Thaumetopoea solitaria.28

The formyl group was first introduced as an acetal derivative at the early synthesis period, and this avoided isomerizing the adjacent diene system from oxidation.29 According to our procedure, the acetal derivative 1,1-dioethoxy-12-lodo decadecane was obtained in three steps.

The presence of the 1,3-diene system is quite common in sex pheromones of Lepidoptera. For instance, the derivatives of (5Z,7E)- and (5E,7Z)-dodecadienyl are the active sex pheromone components of pine caterpillars.30–33 In addition, (11Z,13Z)-hexadecadienyl acetate synergizes the codlemone attraction to male Cydia pomonella, and it is also the sex pheromone of Cydia toreuta (Grote) and Melissopus latiferreanus.34–37

Wittig olefination is an effective protocol to construct the 1,3-diene system of sex pheromone components.38–41 In the normal Wittig reaction, the unstabilized ylides are produced primarily through those erythro betaine intermediates, resulting in the production of Z-alkene products.42–44 By contrast, the E-alkene products are mainly produced by the Wittig–Schlosser reaction via the threo betaine intermediates.45–47 Accordingly, in this study, the E configuration of the enyne and diene were constructed by the Wittig–Schlosser condition, whereas the Z configurations were prepared through the normal Wittig reaction.48,49

Although Z13,Z15-18:Ald was presented in the pheromone extracts of female M. siversi, it elicited no electrophysiological activity. In the field experiments, traps baited with the ZZ isomer alone caught a few males, which might result from the presence of 3.6% of ZE isomer in the bait. On the whole, the ZZ isomer appeared to have no influence on the number of catches when it was mixed with the ZE isomer at various ratios. More research is warranted for verification of these results.

5 CONCLUSION
Our results indicate that Z13,E15-18:Ald, a heretofore undescribed natural product, is the most active sex pheromone component of M. siversi. Traps baited with this synthesized sex pheromone component can be used in M. siversi monitoring or even in mating disruption. Further work will focus on the development of more convenient and efficient traps.

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SUPPORTING INFORMATION
Supporting information may be found in the online version of this article.

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