This article was downloaded by: [Southern Taiwan University of Science and Technology] On: 27 October 2014, At: 01:06 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Bioscience, Biotechnology, and Biochemistry Publication details, including instructions for authors and subscription information: <u>http://www.tandfonline.com/loi/tbbb20</u>

Synthesis and Characterization of Hexadecadienyl Compounds with a Conjugated Diene System, Sex Pheromone of the Persimmon Fruit Moth and Related Compounds

Takanobu NISHIDA^a, Le Van VANG^a, Hiroyuki YAMAZAWA^a, Ryuji YOSHIDA^a, Hideshi NAKA^b, Koji TSUCHIDA^b & Tetsu ANDO^a

^a Graduate School of Bio-Applications and Systems Engineering (BASE), Tokyo University of Agriculture and TechnologyKoganei, Tokyo 184-8588, Japan

^b Laboratory of Applied Entomology, Faculty of Agriculture, Gifu UniversityGifu 501-1193, Japan

Published online: 22 May 2014.

To cite this article: Takanobu NISHIDA, Le Van VANG, Hiroyuki YAMAZAWA, Ryuji YOSHIDA, Hideshi NAKA, Koji TSUCHIDA & Tetsu ANDO (2003) Synthesis and Characterization of Hexadecadienyl Compounds with a Conjugated Diene System, Sex Pheromone of the Persimmon Fruit Moth and Related Compounds, Bioscience, Biotechnology, and Biochemistry, 67:4, 822-829, DOI: <u>10.1271/bbb.67.822</u>

To link to this article: <u>http://dx.doi.org/10.1271/bbb.67.822</u>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Synthesis and Characterization of Hexadecadienyl Compounds with a Conjugated Diene System, Sex Pheromone of the Persimmon Fruit Moth and Related Compounds

Takanobu Nishida,¹ Le Van Vang,¹ Hiroyuki Yamazawa,¹ Ryuji Yoshida,¹ Hideshi Naka,² Koji Tsuchida,² and Tetsu Ando^{1,†}

¹Graduate School of Bio-Applications and Systems Engineering (BASE), Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan ²Laboratory of Applied Entomology, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan

Received November 7, 2002; Accepted December 4, 2002

Hexadecadien-1-ol and the derivatives (acetate and aldehyde) with a conjugated diene system have recently been identified from a pheromone gland extract of the persimmon fruit moth (Stathmopoda masinissa), a pest insect of persimmon fruits distributed in East Asia. The alcohol and acetate showed their base peaks at m/z 79 in a GC-MS analysis by electron impact ionization, but the aldehyde produced a unique base peak at m/z 84, suggesting a 4,6-diene structure. To confirm this inference, four geometrical isomers of each 4,6-hexadecadienyl compound were synthesized by two different routes in which one of two double bonds was furnished in a highly stereoselective manner. Separation of the two isomers synthesized together by each route was facilely accomplished by preparative HPLC. Their mass spectra coincided well with those of natural components, indicating that they were available for use as authentic standards for determining the configuration of the natural pheromone. Furthermore, other hexadecadienyl compounds, including the conjugated diene system between the 3and 10-positions, were synthesized to accumulate the spectral data of pheromone candidates. 5,7-Hexadecadienal interestingly showed the base peak at m/z80; meanwhile, the base peaks of its alcohol and acetate were detected at m/z 79 like the corresponding 4,6dienes. The base peaks of all 6,8-, 7,9-, and 8,10-dienes universally appeared at m/z 67 like 9,11-, 10,12-, and 13,15-dienes, the spectra of which have already been published. Although 3,5-hexadecadienal was not prepared, base peaks at m/z 67 and 79 were recorded for the alcohol and acetate, respectively.

Key words: sex pheromone; persimmon fruit moth; 4,6-hexadecadienyl acetate; 4,6-hexadecadienal; conjugated diene

The persimmon fruit moth, *Stathmopoda masinis-sa* Meyrick (Lepidoptera; Oecophoridae), is a pest insect of persimmon fruits distributed in East Asia.

This species has two generations a year and causes serious damage from infestation by the larvae causing the fall of immature fruit. It is not easy to monitor the population to predict the best time for pesticide spraying because the adults are not sufficiently attracted by a light trap. If the synthetic pheromone of S. masinissa is available, it will serve as a monitoring tool and be further utilized to decrease the population density directly by disrupting sexual communication instead of by killing with insecticides. New programs of integrated pest management for S. masinissa led us to search for the chemical structure of the natural pheromone. We have recently identified hexadecadien-1-ol and its two derivatives, acetate and aldehyde, from a pheromone gland extract of virgin females by using gas chromatography combined with mass spectrometry (GC-MS). Comparing with some authentic dienyl compounds, the chromatographic behavior of the three hexadecadienyl components indicated the conjugation of two double bonds.¹⁾ Their exact positions, however, have not been experimentally confirmed because the content of the natural components was too low to be applied to chemical reactions such as ozonolysis.

Lepidopteran sex pheromones have been identified in more than 500 species.²⁾ Among them, compounds with a conjugated diene system in a C₁₀-C₁₈ straight chain are one of the most important groups. A series of these conjugated dienes has been analyzed by GC-MS, and some characteristic fragment ions, which are informative for estimating the double-bond positions, have been observed in their spectra as measured by electron impact ionization (EI).³⁾ However, none of the spectra reported for the hexadecadienyl compounds, including the diene structure between the 9-position and the terminal methyl group, coincided with those of the pheromone components of *S*. *masinissa*. In particular, the m/z values of their base peaks were different. As shown in Table 1, the base

[†] To whom correspondence should be addressed. Tel/Fax: +81-42-388-7278; E-mail: antetsu@cc.tuat.ac.jp

Functional group	Base peak (m/z)									
		Natural pheromone								
	9,11-diene	10,12-diene	11,13-diene	12,14-diene	13,15-diene	(4,6-diene)				
Alcohol	67	67	95	81	67	79				
Acetate	67	67	95	81	67	79				
Aldehyde	67	67	95	81	67	84				

 Table 1. Base Peaks in the Mass Spectra of Some Synthetic Hexadecadienyl Compounds with a Conjugated Diene System and of Natural

 Pheromone Components of S. masinissa

^a Spectral data of the compounds including (E)-double bonds previously measured with an ESCO EMD-05B spectrometer (70 eV).³⁾



Fig. 1. Fragmentation Pathways Proposed for the Characteristic Fragment Ions Which Were Detected in the EI-MS Analysis of *n*-Aldehydes with a Conjugate Diene System at the 4,6-Position (A) or 5,7-Position (B).

peaks of the synthetic dienes were detected at m/z 67, 81, or 95, and the three functional derivatives with the diene structure at the same position showed the base peaks at the same m/z value. The base peaks of the natural components are different from those of the synthetic dienes, indicating that the conjugated diene system of the S. masinissa pheromone is located at the 8,10-position or at a position lower than that.¹⁾ Furthermore, the base peak of the natural hexadecadienal (m/z 84), which differs from those of the other two components (m/z 79), suggests the possibility of a 4,6-diene structure for the aldehyde component. 4,6-Hexadecadienal can be expected to produce a stable fragment ion with a 2,3-dihydropyranyl structure ($C_5H_8O^+$, m/z 84) by cyclization after cleavage of the C-C bond between the 5and 6-positions (Fig. 1A).

Sex pheromones with the 4,6-diene structure have not been identified from lepidopteran insects and their spectral data have not been reported to the best of our knowledge.²⁾ Another aldehyde with a 5,7diene structure can be expected to predominantly produce a homologous fragment ion (C₆H₁₀O⁺) at m/z 98 (Fig. 1B). While we have not found spectral data for 5,7-hexadecadienal, the EI-mass spectrum of 5,7-dodecadienal has been published.⁴⁾ An abundant ion was actually detected at m/z 98, supporting the fragmentation speculated in Fig. 1. We therefore decided to synthesize every geometrical isomer of 4,6-hexadecadienyl compounds in order to resolve the unknown configuration of the *S. masinissa* pheromone. We are also interested in the mass spectra of other positional isomers, not only to examine the validity of the foregoing fragmentation pathway, but also to support future research concerning a dienyl pheromone. This paper deals with the synthesis and characterization of a series of hexadecadienyl compounds with a conjugated diene system on the side of the terminal functional group.

Materials and Methods

Instruments. ¹H- and ¹³C-NMR spectra were recorded by a Jeol Alpha 500 Fourier transform spectrometer at 500.2 and 125.7 MHz, respectively, for CDCl₃ solutions containing TMS as an internal standard. ¹H-¹H-COSY and HSQC spectra were measured with the same spectrometer, using the usual pulse sequences and parameters. GC-MS was conducted in the EI mode with an HP 5973 mass spectrometer equipped with a DB-23 capillary column $(0.25 \text{ mm ID} \times 30 \text{ m}, \text{ J & W Scientific})$. The column temperature program was 50°C for 2 min and 10°C/min to 220°C. The ionization voltage was 70 eV. High-resolution (HR) mass spectra were measured by a Jeol JMS-700 mass spectrometer, using the same GC column and conditions as those for the measurements by the foregoing quadrupole machine. HPLC involved a Jasco PU-980 liquid chromatograph equipped with an integrator (System Instrument Chromatocorder 21J), a UV spectrometric detector (Jasco UV-970) operated at 240 nm, and an ODS column (Grand Pack ODS, 2.0 cm ID × 25 cm; Senshukagaku, Tokyo, Japan). As the solvent, 10% H₂O in methanol was used at a flow rate of 4.5 ml/min.

Abbreviations used for the chemical structures. The chemical structures of the synthetic pheromone candidates are abbreviated as follows: Z = (Z)-double bond, E = (E)-double bond, number before hyphen = position of double bond, number after hyphen = carbon number of straight chain, OH = alcohol, OAc = acetate, and Ald = aldehyde.

Synthesis of (4E,6Z)- and (4E,6E)-4,6-hexadecadienyl compounds (Scheme 1). After one hydroxyl group of 1,4-butanediol 1 had been protected as a tetrahydropyranyl (THP) ether, another hydroxyl group was oxidized with pyridinium chlorochromate (PCC) in CH_2Cl_2 to produce aldehyde 2, which was converted into (E)-2-alkenal 3 in three steps: a coupling reaction with methoxycarbonylmethylenetriphenylphosphorane, reduction with $LiAl(OC_2H_5)_2H_2$, and oxidation with PCC. In dry THF, 3 was coupled with a phosphorane derived from *n*-decyltriphenylphosphonium bromide, using *n*-butyl lithium as a base, to obtain a mixture of THP ethers of E4,Z6-16:OH and E4,E6-16:OH in a ratio of ca. 3:2. After removing the THP group by heating with catalytic *p*-toluenesulfonic acid in ethanol, two geometrical isomers were separated by preparative HPLC (*t*_Rs: E4,Z6-16:OH, 33.9 min; E4,E6-16:OH, 45.2 min). An aliquot of each isolated isomer was separately acetylated with acetic anhydride in pyridine to yield E4,Z6-16:OAc and E4,E6-16:OAc, and then oxidized with PCC to yield E4,Z6-16:Ald and E4,E6-16:Ald. ¹H-NMR (δ , ppm): E4,Z6-16:OH, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 1.68 (2H, tt, J=7, 7 Hz), 2.15 (2H, dt, J=7, 7 Hz), 2.20(2H, dt, J=7, 7 Hz), 3.67 (2H, t, J=7 Hz), 5.33 (1H, dt, J=11, 7 Hz), 5.66 (1H, dt, J=15, 7 Hz), 5.94 (1H, dd, J=11, 11 Hz), 6.35 (1H, dd, J=15, 11 Hz);E4,E6-16:OH, 0.88 (3H, t, *J*=6.5 Hz), ~1.26 (14H, broad s), 1.67 (2H, tt, J=7, 7 Hz), 2.05 (2H, dt, J=7, 7 Hz), 2.16 (2H, dt, J=7, 7 Hz), 3.66 (2H, t, J = 6.5 Hz), 5.58 (2H, m), 6.02 (2H, m); E4,Z6-16:OAc, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 1.74 (2H, tt, J=7, 7 Hz), 2.05 (3H, s), 2.16 (4H, tt, J=7, 7 Hz), 4.08 (2H, t, J=6.5 Hz), 5.34 (1H, dt, J=11, 7 Hz), 5.63 (1H, dt, J=15, 7 Hz), 5.93 (1H, dd, J=11, 11 Hz), 6.33 (1H, dd, J=15, 11 Hz); E4,E6-16:OAc, 0.88 (3H, t, J=6.5 Hz), ~1.26 (14H, broad s), 1.71 (2H, tt, J=7, 7 Hz), 2.04 (3H, s), 2.05 (2H, m), 2.13 (2H, dt, J=7, 7 Hz), 4.06 (2H, t, J=6.5 Hz), 5.56 (2H, m), 6.00 (2H, m); E4,Z6-16:Ald, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 2.15 (2H, dt, J=7, 7 Hz), 2.44 (2H, dt, J= 7, 7 Hz), 2.56 (2H, td, J=7, 2 Hz), 5.36 (1H, dt, J=11, 7 Hz), 5.63 (1H, dt, J=15, 7 Hz), 5.92 (1H, dd, J=11, 11 Hz), 6.35 (1H, dd, J=15, 11 Hz), 9.78 (1H, t, J=2 Hz); E4,E6-16:Ald, 0.88 (3H, t, J=6.5Hz), ~1.26 (14H, broad s), 2.18 (2H, dt, J=7, 7Hz), 2.46 (2H, dt, J=7, 7 Hz), 2.62 (2H, td, J=7, 2 Hz), 4.06 (2H, t, J = 7 Hz), 5.56 (2H, m), 6.00 (2H, m), 9.78 (1H, t, J = 2 Hz).

Synthesis of (4Z,6Z)- and (4Z,6E)-4,6-hexadecadienyl compounds (Scheme 2). After THP protection of the hydroxyl group, 3-chloro-1-propanol 4 was converted into acetylene compound 5 via a coupling reaction with a lithium acetylideethylenediamine complex in DMSO. Formylation of



Scheme 1. Synthetic Route to the (4*E*,6*Z*)- and (4*E*,6*E*)-Isomers of 4,6-Hexadecadien-1-ol and Its Derivatives, Acetate and Aldehyde.

a, 2,3-dihydropyran/p-TsOH; b, PCC/CH₂Cl₂; c, Ph₃P = CHCO₂CH₃/ benzene; d, LiAl(OC₂H₃)₂H₂/ether; e, Ph₃P = CH(CH₂)₈CH₃/THF; f, p-TsOH/EtOH; g, HPLC; h, Ac₂O/pyridine.

the lithium acetylide of 5 gave 2-alkynal 6, which was coupled with *n*-decylidenetriphenylphosphorane in THF to synthesize engne compound 7 with a C_{16} straight chain. Highly selective and specific conversion of the triple bond was accomplished by hydroboration with dicyclohexylborane and successive protonolysis, and a mixture of THP ethers of Z4,Z6-16:OH and Z4,E6-16:OH in a ratio of ca. 9:1 was obtained. After removing the THP group, two geometrical isomers were separated by preparative HPLC (t_{R} s: Z4,Z6-16:OH, 41.0 min; Z4,E6-16:OH, 34.2 min). An aliquot of each purified isomer was separately acetylated with acetic anhydride in pyridine to yield Z4,Z6-16:OAc and Z4,E6-16:OAc, and then oxidized with PCC to yield Z4,Z6-16:Ald and Z4,E6-16:Ald. ¹H-NMR (δ , ppm): Z4,Z6-16:OH, 0.88 (3H, t, J = 6.5 Hz), ~1.26 (14H, broad s), 1.66 (2H, tt, J=7, 7 Hz), 2.16 (2H, dt, J=7, 7 Hz), 2.27(2H, dt, J=7, 7 Hz), 3.65 (2H, t, J=6.5 Hz), 5.46(2H, m), 6.27 (2H, m); Z4,E6-16:OH, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 1.68 (2H, tt, J =7, 7 Hz), 2.15 (2H, dt, J=7, 7 Hz), 2.20 (2H, dt, J=7, 7 Hz), 3.67 (2H, t, J=7 Hz), 5.30 (1H, dt, J=11, 7 Hz), 5.66 (1H, dt, J = 15, 7 Hz), 5.98 (1H, dd, J =11, 11 Hz), 6.29 (1H, dd, J=15, 11 Hz); Z4,Z6-16:OAc, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 1.72 (2H, tt, J=7, 7 Hz), 2.05 (3H, s), 2.16 (2H, dt, J=7, 7 Hz), 2.25 (2H, dt, J=7, 7 Hz), 4.07 (2H, t, J=6.5 Hz), 5.44 (2H, m), 6.25 (2H, m); Z4,E6-16:OAc. 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 1.74 (2H, tt, J=7, 7 Hz), 2.05 (3H, s), 2.16 (4H, tt, J=7, 7 Hz), 4.08 (2H, t, J=6.5 Hz), 5.28 (1H, dt, J=11, 7 Hz), 5.66 (1H, dt, J=15, 7 Hz), 5.96 (1H, dd, J=11, 11 Hz), 6.29 (1H, dd, J=15, 11 Hz); Z4,Z6-16:Ald, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 2.13 (2H, dt, J=7, 7 Hz), 2.44 (2H, dt, J= 7, 7 Hz), 2.58 (2H, td, J=7, 2 Hz), 5.44 (2H, m), 6.25 (2H, m), 9.78 (1H, t, J=2 Hz); Z4,E6-16:Ald, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (14H, broad s), 2.15 (2H, dt, J=7, 7 Hz), 2.44 (2H, dt, J=7, 7 Hz), 2.56 (2H, td, J=7, 2 Hz), 5.28 (1H, dt, J=11, 7 Hz), 5.66 (1H, dt, J=15, 7 Hz), 5.96 (1H, dd, J=11, 11 Hz),6.29 (1H, dd, J=15, 11 Hz), 9.78 (1H, t, J=2 Hz).

Synthesis of 3,5-, 5,7-, 6,8-, 7,9-, and 8,10-hexadecadienyl compounds. According to a procedure similar to that shown in Scheme 1, each positional isomer of the 4,6-hexadecadienyl compounds was synthesized by changing the carbon chain length of the starting diol and that of the alkyl halides for phosphoranes used in the Wittig reaction as follows: 1,3-propanediol and 1-bromoundecane for the 3,5dienes, 1,5-pentanediol and 1-bromononane for the 5,7-dienes, 1,6-hexanediol and 1-bromooctane for the 6,8-dienes, 1,7-heptanediol and 1-bromoheptane for the 7,9-dienes, and 1,8-octanediol and 1-bromohexane for the 8,10-dienes. Separation of the (E, Z)and (E, E)-isomers of each hexadecadien-1-ol was also achieved by preparative HPLC. The hexadecadien-1-ols with E, Z configuration were converted into their acetate and aldehyde derivatives in the same manner as that used for the 4,6-diene, except for E3,Z5-16:Ald. This aldehyde was not yielded by the PCC oxidation of E3,Z5-16:OH. ¹H-NMR (δ , ppm): E3,Z5-16:OH, 0.88 (3H, t, J = 6.5 Hz), ~1.26 (16H), 2.13 (4H, tt, J = 7, 7 Hz), 2.38 (2H, dt, J = 7, 7Hz), 3.68 (2H, t, J = 6.5 Hz), 5.37 (1H, dt, J = 11, 7Hz), 5.62 (1H, dt, J = 15, 7 Hz), 5.96 (1H, dd, J = 11, 11 Hz), 6.43 (1H, dd, J=15, 11 Hz); E5,Z7-16:OH, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (12H), 1.47 (2H, tt, J=7, 7 Hz), 1.59 (2H, tt, J=7, 7 Hz), 2.15 (4H, m), 3.64 (2H, t, J=6.5 Hz), 5.31 (1H, dt, J=11, 7 Hz),5.64 (1H, dt, J=15, 7 Hz), 5.93 (1H, dd, J=11, 11 Hz), 6.31 (1H, dd, J=15, 11 Hz); E6,Z8-16:OH, E7,Z9-16:OH and E8,Z10-16:OH, 0.88 (3H, t, J= 6.5 Hz), ~1.3 (12H), 1.41 (2H, m), 1.57-1.58 (2H, tt, J=7, 7 Hz), 2.13 (4H, m), 3.64 (2H, t, J=6.5 Hz), 5.30-5.31 (1H, dt, J=11, 7 Hz), 5.63-5.64 (1H, dt, J=15, 7 Hz), 5.94 (1H, dd, J=11, 11 Hz),6.29-6.30 (1H, dd, J=15, 11 Hz); E3,Z5-16:OAc, 0.88 (3H, t, J = 6.5 Hz), ~1.26 (16H), 2.05 (3H, s), 2.15 (4H, tt, J=7, 7 Hz), 2.43 (2H, dt, J=7, 7 Hz), 4.11 (2H, t, J = 6.5 Hz), 5.37 (1H, dt, J = 11, 7 Hz), 5.60 (1H, dt, J=15, 7 Hz), 5.95 (1H, dd, J=11, 11 Hz), 6.39 (1H, dd, J=15, 11 Hz); E5,Z7-16:OAc, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (12H), 1.49 (2H, tt, J=7, 7 Hz), 1.65 (2H, tt, J=7, 7 Hz), 2.05 (3H, s), 2.15 (4H, dt, J=7, 7 Hz), 4.07 (2H, t, J=6.5 Hz), 5.32 (1H, dt, J=11, 7 Hz), 5.63 (1H, dt, J=15, 7 Hz), 5.94 (1H, dd, J=11, 11 Hz), 6.31 (1H, dd, J=15, 11 Hz); E6,Z8-16:OAc, E7,Z9-16:OAc and E8,Z10-16:OAc, 0.88-0.89 (3H, t, J = 6.5 Hz), ~1.3 (12H), 1.39 (2H, m), 1.61–1.63 (2H, tt, J=7, 7 Hz), 2.04 (3H, s), 2.12-2.13 (4H, m), 4.05 (2H, t, J= 6.5 Hz), 5.30–5.31 (1H, dt, J=11, 7 Hz), 5.63–5.64 (1H, dt, J=15, 7 Hz), 5.94 (1H, dd, J=11, 11 Hz),6.29–6.30 (1H, dd, J=15, 11 Hz); E5,Z7-16:Ald, 0.88 (3H, t, J = 6.5 Hz), ~1.27 (12H), 1.54 (2H, tt, J=7, 7 Hz), 1.65 (2H, tt, J=7, 7 Hz), 2.16 (4H, dt, J=7, 7 Hz), 2.44 (2H, td, J=7, 2 Hz), 5.33 (1H, dt, J=11, 7 Hz), 5.62 (1H, dt, J=15, 7 Hz), 5.93 (1H, dd, J=11, 11 Hz), 6.32 (1H, dd, J=15, 11 Hz), 9.76



Scheme 2. Synthetic Route to the (4Z,6Z)- and (4Z,6E)-4,6-Isomers of 4,6-Hexadecadien-1-ol and Its Derivatives, Acetate and Aldehyde.

a, 2,3-dihydropyran; b, LiC \equiv CH \cdot EDA /DMSO; c, HCO₂Et /*n*-BuLi /THF; d, Ph₃P = CH(CH₂)₈CH₃ /THF; e, BH(C₆H₁)₂ /THF; f, *p*-TsOH /EtOH; g, HPLC; h, Ac₂O /pyridine; i, PCC / CH₂Cl₂.

(1H, t, J=2 Hz); E6,Z8-16:Ald, E7,Z9-16:Ald and E8,Z10-16:Ald, 0.88 (3H, t, J=6.5 Hz), ~1.28 (10H, broad s), 1.42-1.44 (2H, m), 1.63 (2H, tt, J=7, 7 Hz), 2.13-2.14 (4H, m), 2.44 (2H, td, J=7, 2 Hz), 5.31-5.32 (1H, dt, J=11, 7Hz), 5.62-5.63 (1H, dt, J=15, 7 Hz), 5.93-5.94 (1H, dd, J=11, 11 Hz), 6.30-6.31 (1H, dd, J=15, 11 Hz), 9.76-9.95 (1H, t, J=2 Hz).

Results

Synthesis

Four geometrical isomers of 4,6-hexadecadien-1-ol and their functional derivatives, acetate and aldehyde, were synthesized by two routes, modifying the previous methods for dodecadienyl compounds with a conjugated diene system,⁵⁾ in which one of two double bonds was furnished in a highly stereoselective manner and the other by a Wittig reaction rather nonspecifically. Namely, in the route for Scheme 1 for the (4E, 6Z)- and (4E, 6E)-isomers, the E configuration at the 4-position was derived from stable (E)-2-alkenal 3. In the route for Scheme 2 for the (4Z, 6Z)- and (4Z, 6E)-isomers, the Z configuration at the 4-position was strictly established by hydroboration of the triple bond in enyne compound 7. By a Wittig reaction to form the double bond at the 6positon, the Z configuration was more facilely formed than the E configuration. Separation of the two geometrical isomers was accomplished by preparative HPLC with an ODS column as shown in Fig. 2. The $t_{\rm R}$ s of the (4*E*,6*Z*)- and (4*Z*,6*E*)-isomers were almost the same, but the chromatographic behavior of the two isomers prepared by each route was quite different. By the route similar to that in Scheme 1, the 3,5-, 5,7-, 6,8-, 7,9-, and 8,10-hexadecadienyl compounds were synthesized, and two geometrical isomers were also separated by the ODS column. The chemical structure of each isomer was confirmed by ¹H-NMR data, these being analyzed by the ¹H-¹H-COSY spectrum.

	Chemical shift (δ ppm)										
	C^1	C^2	C^3	C ^a —	C ^b =	C ^e —	$C^d =$	C ^e —	\mathbf{C}^{f}		
a = 3	62.5	32.3	_	29.2	133.4	126.3	128.3	130.7	27.7		
a = 3	62.4	32.5	_	23.8	130.8	124.4	123.3	132.8	27.5		
$\Delta \delta$ [(E4,Z6) – (Z4,Z6)]			_	5.4	2.6	1.9	5.0	-1.9	0.2		
a = 3	62.5	32.3	_	28.9	131.1	131.1	130.0	133.1	32.6		
a = 3	62.4	32.6	_	24.1	128.7	129.4	125.3	135.4	32.9		
$\Delta \delta$ [(E4,E6) – (Z4,E6)]			_	4.8	2.4	1.7	4.7	-2.3	-0.3		
a = 2	63.8	_	_	32.2	131.6	128.1	128.2	128.6	27.7		
a = 3	64.0	29.2	_	28.3	132.6	126.5	128.2	130.9	27.7		
a = 4	64.4	28.2	25.7	32.4	133.6	126.2	128.4	130.6	27.7		
a = 5	64.5	28.5	25.5	32.7	134.0	126.0	128.5	130.4	27.7		
a=6	64.6	28.6	25.8	32.8	134.3	125.8	128.5	130.3	27.7		
a = 7	64.6	28.6	25.9	32.8	134.5	125.8	128.6	130.2	27.7		
a=3	201.9	43.4	—	25.4	131.5	126.9	127.9	131.3	27.7		
	$ \begin{array}{r} a = 3 \\ a = 3 \\ 6)] \\ a = 3 \\ a = 3 \\ 6)] \\ a = 2 \\ a = 3 \\ a = 4 \\ a = 5 \\ a = 6 \\ a = 7 \\ a = 3 \\ \end{array} $	$ \begin{array}{c c} \hline C^{1} \\ \hline a=3 & 62.5 \\ a=3 & 62.4 \\ \hline 60] \\ a=3 & 62.5 \\ a=3 & 62.4 \\ \hline 60] \\ a=2 & 63.8 \\ a=3 & 64.0 \\ a=4 & 64.4 \\ a=5 & 64.5 \\ a=6 & 64.6 \\ a=7 & 64.6 \\ a=3 & 201.9 \\ \hline \\ \hline$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		

Table 2. ¹³C-NMR Assignments for Hexadecadienyl Compounds with a Conjugated Diene System $[-C^{a}H_{2}-C^{b}H = C^{c}H-C^{d}H = C^{e}H-C^{f}H_{2}-]^{a}$

^a In each compound, the chemical shifts of C¹⁴, C¹⁵, and C¹⁶ are 31.5-31.9, 22.6-22.7, and 14.1 ppm, respectively.



Fig. 2. Separation of the Geometrical Isomers of 4,6-Hexadecadien-1-ol in an ODS Column; (4E,6Z)- and (4E,6E)-Isomers (A) and (4Z,6E)- and (4Z,6Z)-Isomers (B).

¹³C-NMR analysis of hexadecadienyl compounds The signals of four olefinic protons in the conjugate diene system were separately recorded with the NMR measurements of E4,Z6-16:OH and Z4,E6-16:OH. Therefore, their corresponding olefinic carbon signals were unambiguously assigned by the HSQC spectra. For E4,E6-16:OH and Z4,Z6-16:OH, however, the ¹³C assignments could not be established even by 2D NMR experiments because the signals of H⁴ and H⁷ overlapped each other and those of H^5 and H^6 also overlapped. Therefore, the olefinic carbon signals were assigned by utilizing an empirical rule proposed for unbranched conjugated dienes;^{5,6)} *i.e.*, when an *E* configuration of the $C^{b} = C^{c}$ bond in a $C^{a}-C^{b}=C^{c}-C^{d}=C^{e}$ diene system is changed to a Z configuration without the conversion of the $C^d = C^e$ bond, the signals of C^a, C^b, C^c, and C^d shift upfield by ca. 5, 2.5, 2, and 5 ppm, respectively, and the signal of C^e shifts downfield by ca. 2 ppm. Table 2 shows, in addition to the signal assignments for the four isomers, differences in the chemical shifts ($\Delta\delta$) between the two geometrical isomers, these being obtained by subtracting the values of Z4,Z6-16:OH from those of E4,Z6-16:OH and by subtracting the values of Z4,E6-16:OH from those of E4,E6-16:OH. This table also lists the ¹³C assignments proposed for six positional isomers of the hexadecadienyl acetates with an E, Z configuration. The spectra of the 3,5-, 4,6-, and 5,7-dienes are distinguishable by considering an affect of the different distances between the acetoxyl group and the diene system, while the spectra of the 6,8-, 7,9-, and 8,10-dienes resemble each other. The acetoxyl group of the latter three dienes is located far from the conjugated system.

Mass spectra of hexadecadienyl compounds

Four geometrical isomers of the 4,6-hexadecadienyl compounds show almost the same mass spectra, these being similar to those of the natural pheromone components of S. masinissa.¹⁾ The base peak of the synthetic aldehyde was detected at m/z84, as expected by the fragmentation indicated in Fig. 1A, and the acetate and alcohol produced their base peaks at m/z 79, the same value as that for the natural components. Table 3 shows GC-MS data for the (E, Z)-isomers of the hexadecadienyl compounds synthesized in this experiment, spectra for the five hexadecadienals being shown in Fig. 3. Although 3,5hexadecadienal was not synthesized, the base peaks of the alcohol and acetate derivatives were observed at m/z 67 and 79, respectively. 5,7-Hexadecadienal shows an interesting spectrum with the base peak at m/z 80, while the alcohol and acetate produced the base peak at m/z 79. The base peaks of the 6,8-, 7,9-, and 8,10-dienes were universally detected at m/z 67, as was true for the 9,11-, 10,12-, and 13,15-dienes (Table 1).3)

As a homologue of the ion at m/z 84 of 4,6-hexadecadienal, 5,7-hexadecadienal produced an abundant ion at m/z 98 (Fig. 1B). Another fragment ion at m/z 112, further enlarged by 14 mass units, was characteristically detected in the spectrum of 6,8-hexadecadienal. Analysis by an HR-MS instrument confirmed the formulae of these ions as follows: C_5H_8O , obs. m/z 84.0517 (calc. 84.0575); $C_6H_{10}O$, obs. m/z

	t _R	$t_{\rm R}$ Relative intensity (%) of ions at indicated m/z											
	(mîn)	67	79	80	81	84	95	98	109	112	M-60	M-18	М
Alcohol													
3,5-diene	20.64	100	68	41	64	25	46	36	22	4	_	1	58
4,6-diene	20.93	45	100	32	35	11	28	18	9	8	_	2	23
5,7-diene	21.03	86	100	67	59	24	46	29	13	5	_	14	10
6,8-diene	21.01	100	81	44	72	9	44	26	21	5	_	2	28
7,9-diene	21.00	100	72	38	71	8	48	21	19	3	_	2	26
8,10-diene	21.07	100	58	31	69	6	48	14	19	3	_	1	25
Acetate													
3,5-diene	20.25	28	100	96	26	5	11	1	5	0	21	_	10
4,6-diene	20.31	25	100	98	31	1	14	1	4	0	25	_	10
5,7-diene	20.46	51	100	98	32	4	21	1	6	0	6	_	28
6,8-diene	20.48	100	88	60	59	2	45	2	13	1	6	_	16
7,9-diene	20.58	100	88	60	67	1	47	1	14	0	8	_	36
8,10-diene	20.64	100	75	50	74	1	52	1	14	0	5	_	28
Aldehyde													
4,6-diene	18.95	85	92	28	55	100	64	10	16	1	_	1	19
5,7-diene	18.94	52	78	100	31	5	26	61	9	2	_	1	18
6,8-diene	19.16	100	68	22	61	56	43	21	18	19	_	1	20
7,9-diene	19.20	100	51	20	68	11	42	76	18	3	_	1	20
8,10-diene	19.25	100	42	20	72	4	40	23	15	6	_	1	21

Table 3. GC-MS Data of Synthetic Hexadecadienyl Compounds with an E, Z Configuration in the Conjugated Diene System⁶

^a Capillary column (0.25 mm ID × 30 m); temperature program: 50°C for 2 min, 10°C/min to 220°C, and 220°C for 10 min.

98.0710 (calc. 98.0732); and $C_7H_{12}O$, obs. m/z112.0832 (calc. 112.0888). The 6,8-diene also produced the ion at m/z 84 with moderate intensity. The ion at m/z 98 also appeared in the spectrum of 7,9hexadecadienal with a slightly stronger intensity than that of the 5,7-diene. These ions, shown in Fig. 1 modified by extra dehydrogenation, might have been produced along a similar pathway.

5,7-Hexadecadienal produced another characteristic fragment ion at m/z 192 (M – 44) as the corresponding ion to 5,7-dodecadienal at m/z 136,⁴) indicating the diagnostic ion at m/z M – 44 for 5,7dienals. The abundance of the ions at m/z 193 of 6,8hexadecadienal and at m/z 137 of 6,8-dodecadienal⁴) indicates that the ion at m/z M – 43 is diagnostic for 6,8-dienals. These fragment ions are helpful leads for finding the double-bond positions of alkdienals. On the other hand, hexadecadien-1-ols and their acetates lack such a diagnostic ion. Mass spectra of the 4,6dienes resemble those of the 5,7-dienes, and spectra of the 6,8-dienes resemble those of the 7,9- and 8,10dienes.

Discussion

Mass spectra of the synthetic dienes confirmed that the natural pheromone components produced by *S. masinissa* were 4,6-hexadecadienyl compounds. Among the four geometrical isomers, the (4E,6Z)isomers showed the same chromatographic behavior as the natural components did in two kinds of capillary GC columns, indicating that the female sex pheromone consisted of E4,Z6-16:OH, E4,Z616:OAc, and E4,Z6-16:Ald. The results of electrophysiological experiments support this conclusion. Antennae of the male moths more strongly responded to these compounds than to the other geometrical isomers. The synthetic compounds induced mating behavior of the males in a laboratory bioassay, and furthermore, a preliminary field test interestingly revealed that a lure baited only with E4,Z6-16:OAc could attract the males. We will report these results in detail in another paper.¹⁾ It should be emphasized here that successful determination of the *S. masinissa* pheromone is attributable to the new synthetic standards under an experimental situation conducted with a limited amount of the female extract.

In order to determine the double-bond positions, a GC-MS analysis used to be carried out on the compound(s) derived by a chemical reaction of the pheromone component, such as aldehydes by ozonolysis and an adduct of dimethyl disulfide for a monoene compound.⁷⁾ For a conjugated diene, conversion with 4-methyl-1,2,4-triazoline-3,5-dione has been proposed.⁸⁾ The Diels-Alder cyclo-addition product between a diene and dienophile shows fragment ions reflecting the double-bond positions of the parent diene. However, when the pheromone content of the females is very low or a rearing method for the insect has not been established, it is difficult to supply a sufficient amount of the natural pheromone for the reaction. In this case, it is preferable to presume the structure by a GC-MS analysis without any chemical reactions, and we have found that each compound, including a conjugated diene system near the terminal methyl group, produced diagnostic ions unrelated

T. NISHIDA et al.



Fig. 3. Mass Spectra of Synthetic 4,6-, 5,7-, 6,8-, 7,9- and 8,10-Hexadecadienals with an E,Z Configuration (A-E).

to the functional group at the opposite site in a C_{12} - C_{16} chain.³⁾ Additionally, this study has revealed that the positional isomers of C_{16} aldehydes, including the diene structure near the formyl group, show some characteristic fragment ions such as at m/z 84 and 98, although C_{16} alcohols and the acetate derivatives, including the diene structure near the functional group, did not present any diagnostic fragment ions. Hexadecadienals are long-chain compounds, and there are many positional isomers. It is not easy to distinguish the mass spectra of the 8,10- and 9,11hexadecadienals, the diene structure of which is located around the center of the C_{16} chain, but other positional isomers can be identified by their distinct spectra.

The HR-MS analysis confirmed the fragment for-

mulae of the ions at m/z 84 (C₅H₈O) derived from 4,6-hexadecadienal and at m/z 98 (C₆H₁₀O) derived from 5,7-hexadecadienal. These ions include an oxygen atom and might be made by the fragmentation occurring at the side of the functional group as shown in Fig. 1. Therefore, it is expected that these fragment ions are also abundantly produced from 4,6- and 5,7-dienes with another chain length. In addition to the conjugated dienes, we measured the mass spectra of some monounsaturated compounds and observed the base peaks at m/z 84 and 98 for 4hexadecenal and 5-hexadecenal, respectively. Abundant ions at m/z 84 and 98 have been also recorded for 4-dodecenal and 5-dodecenal, respectively, among a series of positional isomers of C₁₂ aldehydes.⁹⁾ These ions are characteristic of the aldehydes and not of the alcohol and acetate derivatives. In this study, mass spectra were measured by EI at 70 eV, and the positional isomers showed different spectra. This result suggests that migration of the double bond did not occur easily after this rather drastic ionization. It is important in future work to determine how strong instrumental conditions influence the intensity of the diagnostic ions.

While 4,6,10- and 4,6,11-hexadecatrienyl compounds have been identified from female moths as pheromone components,^{10,11)} the 4,6-hexadecadienyl compounds are new pheromone components. Many conjugated dienes have been identified as lepidopteran sex pheromones, and their double bonds are usually located on one side of the terminal methyl group.²⁾ The chemical structure of the *S. masinissa* pheromone, however, suggests the possibility of more pheromone compounds with a conjugated diene system near the functional group. The EI mass spectra reported in this study are informative for future pheromone research.

References

- Naka, H., Vang, L. V., Inomata, S., Ando, T., Kimura, T., Honda, H., Tsuchida, K., and Sakurai, H., Sex pheromone of the Japanese persimmon moth, *Stathmopoda masinissa* (Lepidoptera: Oecophoridae): identification and laboratory bioassay of (4E,6Z)-4,6-hexadecadienyl compounds. J. *Chem. Ecol.*, submitted.
- Arn, H., Tóth, M., and Priesner, E., List of sex pheromones of Lepidoptera and related attractants. OILB-SROP/IOBC-WPRS, Secrétariat Général, F-84143 Montfavet, France, ISBN 92-9067-044-4, pp. 179 (1992). Online version can be accessed on http://www.pherolist.slu.se/
- 3) Ando, T., Ogura, Y., and Uchiyama, M., Mass spec-

tra of lepidopterous sex pheromones with a conjugated diene system. *Agric. Biol. Chem.*, **52**, 1415–1423 (1988).

- Ando, T., Katagiri, Y., and Uchiyama, M., Mass spectra of dodecadienic compounds with a conjugated double bond, lepidopterous sex pheromones. *Agric. Biol. Chem.*, 49, 413-421 (1985).
- Ando, T., Kurotsu, Y., Kaiya, M., and Uchiyama, M., Systematic syntheses and characterization of dodecadien-1-ols with conjugated double bond, lepidopterous sex pheromones. *Agric. Biol. Chem.*, 49, 141-148 (1985).
- Ando, T., Kusa, K., Uchiyama, M., Yoshida, S., and Takahashi, N., ¹³C NMR analyses on conjugated dienic pheromones of Lepidoptera. *Agric. Biol. Chem.*, 47, 2849–2853 (1983).
- Buser, H.-R., Arn, H., Guerin, P., and Rauscher, S., Determination of double bond position in monounsaturated acetates by mass spectrometry of dimethyl disulfide adducts. *Anal. Chem.*, 55, 818-822 (1983).
- Young, D. C., Vouros, P., and Holick, M. F., Gas chromatography-mass spectrometry of conjugated dienes by derivatization with 4-methyl-1,2,4-triazoline-3,5-dione. J. Chromato., 522, 295–302 (1990).
- Horiike, M., and Hirano, C., Mass spectrometric indication of double bond position in dodecenals without any chemical derivatization. *Biomed. Envi*ron. Mass Spectrom., 17, 301-306 (1988).
- Beevor, P. S., Cork, A., Hall, D. R., Nesbitt, B. F., Day, R. K., and Mumford, J. D., Components of female sex pheromone of cocoa pod borer moth, *Conopomorpha cramerella. J. Chem. Ecol.*, **12**, 1–23 (1986).
- Bestmann, H. J., Attygalle, A. B., Schwarz, J., Garbe, W., Vostrowsky, O., and Tomida, I., Pheromones, 71. Identification and synthesis of female sex pheromone of eri-silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae). *Tetrahedron Lett.*, 30, 2911–2914 (1989).