Chemical Ecology of Astigmatid Mites XLIV
Identification of (Z,Z,Z)-5,9,12-Octadecatrienoic Acid and (Z,Z)-5,9-Octadecadienoic Acid as Possible Biosynthetic Precursors of New Hydrocarbons (Z,Z,Z)-4,8,11-Heptadecatriene and (Z,Z)-4,8-Heptadecadiene Found in the Astigmatid Mite, *Tortonia* sp.¹

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(Z,Z,Z)-5,9,12-Octadecatrienoic acid and (Z,Z)-5,9-octadecadienoic acid were isolated as components of body lipid from an unidentified species of the genus *Tortonia* [Astigmata: Suidasiidae]. The chemical structures were elucidated either by partial hydrogenation and subsequent GC/MS analysis of their dimethyldisulfide (DMS) derivatives, or by GC/MS analysis of their DMS derivatives. Both are new fatty acids constituting natural products obtained from mites and are possible biosynthetic precursors of the new hydrocarbons (Z,Z,Z)-4,8,11-heptadecatriene and (Z,Z)-4,8-heptadecadiene found in the same species.

Key words: *Tortonia* sp., (Z,Z,Z)-5,9,12-octadecatrienoic acid, (Z,Z)-5,9-octadecadienoic acid

INTRODUCTION

An unidentified species of the genus *Tortonia* [Astigmata: Suidasiidae] (provisionally named Kenaga-tsutuhana-chibikonadani in Japanese by KUROSA) is commonly found in the nests of the megachilid bee, *Osmia cornifrons* (RADOSZKOWSKI) (in Japanese, Mamekobachi) with *Chaetodactylus nipponicus* KUROSA (Astigmata: Chaetodactylidae) in central and northern Japan, feeding on pollen mass stored in the bee's nest and destroying bee larva in the cell. Although the bee is univoltine in nature, the species is a beneficial pollinator for apple-growers in Japan, and therefore, these nest mites are noxious pests.

(Z,Z)-6,9-Heptadecadiene from the astigmatid mite *Tortonia* sp., has been identified as the alarm pheromone of this species, and two new hydrocarbons (Z,Z)-4,8-heptadecadiene and (Z,Z,Z)-4,8,11-heptadecatriene have also been found (KUWAHARA et al., 1995). Hydro-

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carbons are derived from the corresponding fatty acids via decarboxylation (MAJOR and BLOMQUIST, 1978). Linoleic acid, a common lipid component, might be the precursor for the alarm pheromone. However, presumable precursors such as (Z,Z)-5,9-octadecadienoic acid and (Z,Z,Z)-5,9,12-octadecatrienoic acid (= pinoleic acid) for these new hydrocarbons are presently unknown from mites.

The present paper deals with isolation and identification of the rare and new fatty acids in the astigmatid mite *Tortonia* sp., corresponding to the newly identified hydrocarbons (Z,Z)-4,8-heptadecadiene and (Z,Z,Z)-4,8,11-heptadecatriene of the species.

MATERIALS AND METHODS

The unidentified species of the genus *Tortonia* sp. [Astigmata: Suidasiidae] (provisionally named Kenaga-tsutsuhana-chibikonadani in Japanese by KUROSA) was originally collected from the nest of the megachilid bee *Osmia cornifrons* (RADOSZKOWSKI) (in Japanese, Mamekobachi). The species was raised by feeding dried yeast at 70% RH and 25°C.

Analytical methods. Purities and compositions of methylated fatty acids were monitored by following three gas liquid chromatograph (GLC) systems; a Hitachi model 263-30 with a split mode using a fused silica CP-Sil 19CB capillary column (0.22 mm × 25 m, Chrompack) at 135–250°C (4°C/min), a Yanaco G-180 with a split mode using a FFAP capillary column (0.22 mm × 25 m, Quadrex) at 200°C, and a Hewlett Packard model 5790 with a split-less mode using a Carbowax 20M column (0.2 mm × 50 m, HP) at 160–220°C (2°C/min). GC/MS were measured either by a Hitachi M-80B high-resolution mass spectrometer at 70 eV, using an Al-clad fused silica capillary column ('400' methyl silicone, 0.25 mm i.d. × 25 m, d.f. = 0.1 µm, Quadrex) with a split mode at 110–250°C (4°C/min) or by a M-80 model with a split-less mode using an HP 20M capillary column (0.2 mm × 50 m) at 160–220°C (2°C/min) and an HP-1 column (0.2 mm × 25 m) at 250–330°C (4°C/min). ¹H-NMR was measured by JEOL FX-100 and Bruker AM-500 spectrometers in CDCl₃.

Preparation of methyl esters from *Tortonia* sp. Mites (2.22 g) separated from culture media by the saline flotation method were extracted by dipping in hexane for 22 h to obtain body lipids (170 mg). After evaporation of the solvent, the extract was hydrolyzed by 3% alcoholic KOH at r.t. overnight, and fatty acids were extracted from the reaction product by ether using conventional methods. After concentration under reduced pressure, the acids was changed to the corresponding methyl esters by refluxing in MeOH with a catalytic amount of conc. H₂SO₄. After examining the composition by the GLC, the resulting methyl esters (70 mg), was applied to a 5% AgNO₃/SiO₂ column (1.5 g, 7 mm i.d. × 7.8 cm), and each was eluted with 15 ml of hexane, mixtures of ether in hexane (1%, 2%, 5%, 10%, 20% and 50%), and ether successively. Fatty acid compositions of each eluate were monitored by GLC and GC/MS. The separation method and results are summarized in Fig. 1.

Determination of double bond positions in unsaturated fatty acid methyl esters. Positions of double bonds in methyl alkenoates and alkadienoates were determined by GC/MS of dimethyl-disulfide (DMDS) derivatives, following the method reported (VINCENTI et al., 1987; HOWARD et al., 1988; KUWAHARA et al., 1988, 1992). Positions of double bonds in methyl alkatrienoates were analyzed by a combination of partial hydrogenation and the DMDS method, as reported (KUWAHARA et al., 1995). About 200 µg of methyl alkatrienoate was mixed with 100 µl of each hydrazine hydrate solution and H₂O₂ solution, prepared as reported previously (YAMAOKA et al., 1976). The mixture was kept at 60°C and the progress of the reaction was intermittently monitored by GLC. It took about 4 h to reach an

Fig. 1. Separation procedure of compounds in the hexane extract from *Tortonia* sp. Results of GLC and GC/MS analysis are also indicated. Letters of peaks indicate the following compound names: peak A, methyl tetradecanoate; peak B, methyl 9-tetradecenoate; peak C, methyl hexadecenoate; peak D, methyl 9-hexadecenoate; peak E, methyl heptadecanoate; peak F, methyl octadecanoate; peak G, methyl 9-octadecenoate; peak H, methyl 5,9-octadecadienoate; peak I, methyl 9,12-octadecadienoate; peak J, methyl 5,9,12-octadecatrienoate; peak K, methyl 9,12,15-octadecatrienoate. Hx, hexane; Et, ether.

appropriate mixture of partially hydrogenated products. After quenching the reaction by addition of 2 N HCl, the resulting mixtures containing methyl alkenoates and alkadienoates along with starting methyl alkatrienoate and per-hydrogenated methyl alkanoate were extracted with CS₂. After evaporation of the solvent, the CS₂ extract was dissolved in dimethyldisulfide (200 μ l) with a catalytic amount of I₂, and was changed to a mixture of each corresponding DMDS derivative. The double bond positions in the starting methyl alkatrienoate were elucidated from a combination of double bond positions determined for each of these resulting methyl alkenoates and methyl alkadienoates.

Large scale purification of methyl 5,9-octadecadienoate and methyl 5,9,12-octadecadienoate. Methyl esters (5.4 g) from the hexane extract (6.0 g) obtained from 53 g of mites were applied to a 5% AgNO₃/SiO₂ column (50 g, 23 mm i.d. $\times$ 27 cm) and eluted with a mixture of ether and hexane. Methyl octadecadienoate was recovered in the combined hexane and 1–5% ether/hexane eluate (4.3 g) along with methyl alkanoates and methyl alkenoates. Methyl octadecatrienoate was found in the ether eluate (0.27 g). Both dienoate and trienoates were further purified by repeated column chromatography using SiO₂ and AgNO₃–SiO₂ columns to give methyl 5,9-octadecadienoate (53 mg, m/z 294) and methyl 5,9,12-octadecatrienoate (3 mg, m/z 292), respectively. Both esters were analyzed by GLC and ¹H-NMR.

Preparation of methyl (Z,Z,Z)-5,9,12-octadecatrienoate. Pinoleic acid, [(Z,Z,Z)-5,9,12-octadecatrienoic acid], a commonly distributed component in pine seed oil (HANSEN and MEIKLEN, 1969), was prepared from roasted pine seeds (400 g) for use as the standard, as summarized in Fig. 2.

Roasted seeds (400 g), edible and available at a Chinese food market, were ground to

Fig. 2. Purification procedure of methyl pinolate. Hx, hexane; Et, ether.

Table 1. NMR data of methyl (*Z,Z,Z*)-5,9,12-octadecatrienoate

Chemical shift (ppm)	Proton	Multiplicity	Coupling constant (Hz)	Partial structure
5.44–5.32	6H	m		1
3.67	3H	s		2
2.78	2H	t	6.1	3
2.32	2H	t	7.5	4
2.14–2.01	6H	m		5
1.69	2H	tt	7.5, 7.5	6
1.37–1.28	6H	m		7
0.89	3H	t		8

a flour and extracted with a mixture of chloroform and MeOH (1 : 1) for 12 h. Fatty acids (174 g) were separated from the extract (210 g) by conventional alkaline hydrolysis. A portion (42 g) of the fatty acids (174 g) was treated with urea (200 g) in hot MeOH, and the crystalline adduct was separated by filtration. The recovered fatty acids (27 g) from the

Fig. 3. A typical gas liquid chromatogram of methylated fatty acids from *Tortonia* sp. on a Carbowax-20M fused silica capillary column at temperatures programmed from 160°C to 220°C at 2°C/min. See legend to Fig. 1 for names of compounds indicated by letters.

adduct were changed to the corresponding methyl esters (25 g). A portion of the methyl esters (150 mg) was subjected to a 5% $\text{AgNO}_3/\text{SiO}_2$ column (4 g, 10 mm i.d. $\times$ 10 cm), and eluted with a mixture of ether and hexane. GLC and GC/MS analysis revealed that the 50% ether/hexane eluate from a 5% $\text{AgNO}_3/\text{SiO}_2$ column was methyl octadecatrienoate (11 mg, 1.9% based on fatty acids; m/z 292). The IR spectrum of the ester gave no absorption band around 970 cm^{-1} , suggesting the existence of *Z*-geometries at the double bonds. As summarized in Table 1, the indicated structure by the NMR spectrum was methyl (*Z,Z,Z*)-5,9,12-octadecatrienoate.

RESULTS AND DISCUSSION

The methyl esters (70 mg) gave at least 11 peaks (A–K) by GLC (Fig. 3), which were then subjected to a 5% $\text{AgNO}_3/\text{SiO}_2$ column (1.5 g, 7 mm i.d. $\times$ 7.8 cm) and separated into five fractions. The composition of each fraction was monitored by GLC. The separation method and composition of the separated fractions are summarized in Fig. 1. The combined hexane and 1–2% ether/hexane eluates (41 mg) consisted of four methyl alkanoates [peaks A (M^+ ; m/z 242), C (m/z 270), E (m/z 284) and F (m/z 298)], three methyl alkenoates [peaks B (m/z 240), D (m/z 268) and G (m/z 296)] and methyl octadecadienoate [peak I (m/z 294)] by GLC and GC/MS. The 5% ether/hexane eluate (9 mg) was composed of a mixture of methyl alkenoate [peak G (m/z 296)] and alkadienoates [peaks H and I (m/z 294)]. The 10% ether/hexane eluate (6 mg) contained two isomers of methyl octadecadienoate (peak H and I). Both eluates by 20–50% ether/hexane (4 mg) and by ether (4 mg) consisted of two isomers of methyl octadecatrienoate [peaks J and K (m/z 292)].

GC/MS of DMDS derivatives derived from the combined hexane and 1–2% ether/hexane fraction gave M^+ ions at m/z 334, 362 and 390, each of which corresponded to DMDS-adducts of methyl tetradecenoate (peak B), methyl hexadecenoate (peak D) and methyl octadecenoate (peak G), respectively. Based on the following three sets of diagnostic ions (set of α and β fragments); 1-thiomethyl-9-carbomethoxynonyl (α , m/z 217, 36%) and 1-thiomethylpentyl (β , m/z 117, 42%) (see Fig. 4a), α (m/z 217, 37%) and 1-thiomethylheptyl (β , m/z 145, 31%), and α (m/z 217, 100%) and 1-thiomethylnonyl (β , m/z 173, 13%), the three original methyl alkenoates were concluded to be methyl 9-tetradecenoate (peak B),

Fig. 4. Mass fragmentation DMDS derivatives derived from (a) methyl 9-tetradecenoate and (b) methyl 5,9-octadecadienoate. $\rightarrow$, fragmentation.

methyl 9-hexadecenoate (peak D) and methyl 9-octadecenoate (peak G). The results are summarized in Table 2.

DMDS derivatives from the 10% ether/hexane fraction (mixture of peaks H and I), which theoretically consisted of a mixture of 32 stereoisomers, gave a broad GLC peak (t_R : 8.0 min) by the Al-clad fused silica capillary column. Mass spectra of the peak gave M^+ ion at m/z 420 which corresponded to cyclic derivatives, e.g. two molar addition of DMDS (m/z 94 $\times$ 2) to the diene (m/z 294) minus MeSMe (m/z 62) (Fig. 4b). The following two sets of diagnostic ions ($\alpha 1$ and $\beta 1$, and $\alpha 2$ and $\beta 2$); 1-thiomethyl-4-carbomethoxybutyl ($\alpha 1$, m/z 161, 7%) and the ring moiety ($\beta 1$, m/z 259, 21%), and the ring moiety of the other cyclyzation product ($\alpha 2$, m/z 247, 11%) and 1-thiomethylnonyl [$\beta 2$, m/z 173 (not detected) minus MeSH at m/z 125 (10%)], disclosed one of the original dienoates (peak H) as methyl 5,9-octadecadienoate. Likewise, methyl 9,12-octadecadienoate (peak I) was concluded from the following two sets of diagnostic ions; $\alpha 1$ (m/z 217, 26%) and $\beta 1$ (m/z 203, 20%), and $\alpha 2$ [m/z 289 (not detected) minus MeSH at m/z 241 (25%)] and $\beta 2$ (m/z 131, 25%) (Table 2). Co-injection of commercially available methyl linoleate [methyl (Z,Z)-9,12-octadecadienoate] with the present fraction by GLC revealed that peak I was methyl linoleate.

The mixture of 20% and 50% ether/hexane eluate containing methyl octadecatrienoates (peaks J and K) was partially hydrogenated to monoenes and dienes. After reaction with DMDS, the products were analyzed by GC/MS. The mass chromatogram indicated two M^+ ions at m/z 390 and 420, each of which corresponded to DMDS-adducts of monoenes and to two molar DMDS adducts to dienes along with subsequent

Table 2. Determination of double bond position of unsaturated fatty acid methyl ester found in *Tortonia* sp. by GC/MS of its DMDS derivative

GC-peak ^a	M ⁺ (<i>m/z</i>)	DMDS-derivative			Elucidated structure of fatty acid methyl ester
		M ⁺ (<i>m/z</i>)	Diagnostic ions (<i>m/z</i>)		
			α 1 fragment α 2 fragment	β 1 fragment β 2 fragment	
Peak A	242				C ₁₄
Peak C	270				C ₁₆
Peak E	284				C ₁₇
Peak F	298				C ₁₈
Peak B	240	334	217→157, 185	117→57	C ₁₄ -9-ene
Peak D	268	362	217→157, 185	145→85	C ₁₆ -9-ene
Peak G	296	390	217→157, 185	173→113	C ₁₈ -9-ene
Peak H	294	420	161→113, 129→81 247→199→167	259→211 (173)→125	C ₁₈ -5,9-diene
Peak I	294	420	217→169, 185→137 (289)→241→209	203→155 131→83	C ₁₈ -9,12-diene
Peak J	292				C ₁₈ -5,9,12-triene
Peak K	292				C ₁₈ -9,12,15-triene
After partial reduction					
Monocenes		390	161→113, 129→81	229→181	C ₁₈ -5-ene
			217→185→137	173→(125)	C ₁₈ -9-ene
			259→227	131→83	C ₁₈ -12-ene
			301→269	89→41	C ₁₈ -15-ene
Dienes		420	161→113, 129→81	259→211	C ₁₈ -5,9-diene
			247→199→167	(173)→125	
			217→169, 185→137	203→155	C ₁₈ -9,12-diene
			(289)→241→209	131→83	
			259→227	161→113	C ₁₈ -12,15-diene
			(331)→283→251	89→41	

^a See Fig. 1 for explanation of peaks.

dimethylsulfide elimination to form cyclic derivatives. The monoene fraction with M⁺ at *m/z* 390 consisted of methyl 5-octadecenoate (α , *m/z* 161, 26% and β , *m/z* 229, 20%), methyl 9-octadecenoate (α , *m/z* 217, 100% and β , *m/z* 173, 83%), methyl 12-octadecenoate (α , *m/z* 259, 36% and β , *m/z* 131, 42%) and methyl 15-octadecenoate (α , *m/z* 301, 72% and β , *m/z* 89, 54%). The diene fraction with M⁺ at *m/z* 420 consisted of methyl 5,9-octadecadienoate [α 1, *m/z* 161, 6%; β 1, *m/z* 259, 20%; α 2, *m/z* 247, 10%; and β 2 (not detected)-MeSH, *m/z* 125, 8%], methyl 9,12-octadecadienoate [α 1, *m/z* 217, 24%; β 1, *m/z* 203, 19%; α 2 (not detected)-MeSH, *m/z* 241, 24%; and β 2, *m/z* 131, 24%] and methyl 12,15-octadecadienoate [α 1, *m/z* 259, 38%; β 1, *m/z* 161, 24%; α 2 (not detected)-MeSH, *m/z* 283, 30%; and β 2, *m/z* 89, 16%]. The above results are summarized in Table 2. The original trienes (peaks J and K) were concluded to be methyl 5,9,12-octadecatrienoate and methyl 9,12,15-octadecatrienoate. Co-injection of this fraction with commercially available methyl linolenate [methyl (*Z,Z,Z*)-9,12,15-octadecatrienoate] by GLC indicated that peak K was identical with methyl linolenate.

Table 3. Methyl esters of fatty acids from *Tortonia* sp., and their relative abundances

GLC peak ^a	M ⁺ (m/z)	Identified compound	Relative abundance (%)
A	242	Methyl tetradecanoate	6.9
B	240	Methyl (Z)-9-tetradecenoate	4.5
C	270	Methyl hexadecanoate	4.8
D	268	Methyl (Z)-9-hexadecenoate	11.3
E	284	Methyl heptadecanoate	0.9
F	298	Methyl octadecanoate	11.7
G	296	Methyl (Z)-9-octadecenoate	28.9
H	294	Methyl (Z,Z)-5,9-octadecadienoate	1.6
I	294	Methyl (Z,Z)-9,12-octadecadienoate	25.6
J	292	Methyl (Z,Z,Z)-5,9,12-octadecatrienoate	0.3
K	292	Methyl (Z,Z,Z)-9,12,15-octadecatrienoate	1.0

^a See Fig. 1 for explanation of peaks.

As described above, the methylated acid fraction contained unusual fatty acids, that is, methyl 5,9-octadecadienoate (peak H) and methyl 5,9,12-octadecatrienoate (peak J). In order to characterize these acids, large scale purification from 53 g of mites was carried out to obtain a sufficient amount of methyl 5,9-octadecadienoate (53 mg, peak H) and methyl 5,9,12-octadecatrienoate (3 mg, peak J).

The NMR spectrum for methyl 5,9,12-octadecatrienoate (3 mg, peak J) was identical to that from the standard methyl (Z,Z,Z)-5,9,12-octadecatrienoate, as summarized in Table 1. The retention time of the standard methyl pinolate was also identical by GLC to that of peak J (methyl 5,9,12-octadecatrienoate) from *Tortonia* sp. As a result, peak J was identified as methyl (Z,Z,Z)-5,9,12-octadecatrienoate.

A component of the partially hydrogenated products from methyl (Z,Z,Z)-5,9,12-octadecatrienoate gave the same retention time to that of peak H from *Tortonia* sp. Thus, it was suggested that the geometry of both double bonds in methyl 5,9-octadecadienoate was that of Z. Four olefine protons of the isolated methyl 5,9-octadecadienoate indicated chemical shifts at δ 5.34 ppm and 5.37 ppm with the coupling constant of 10.8 Hz by NMR spectrum, so that geometries of both double bonds were also concluded to be Z. As a result, compound H was identified as methyl (Z,Z)-5,9-octadecadienoate.

A total of 11 fatty acids were then identified as components of the *Tortonia* sp., as summarized in Table 3. Although (Z,Z)-5,9-octadecadienoic acid has been found in sponge (GILLAN et al., 1988) and (Z,Z,Z)-5,9,12-octadecatrienoic acid in seed oil of the Pinus family, both acids were newly identified as compounds in the mite, and might be precursors of the rare hydrocarbons (Z,Z,Z)-4,8,11-heptadecatriene and (Z,Z)-4,8-heptadecadiene present in the species. (Z)-9-Hexadecenoic acid (= palmitoleic acid), (Z)-9-octadecenoic acid (oleic acid) and (Z,Z)-9,12-octadecadienoic acid (linoleic acid) might also be precursors corresponding to (Z)-7-pentadecene, (Z)-8-heptadecene and (Z,Z)-6,9-heptadecadiene, found in mites.

This is the first time that precursor fatty acids of rare hydrocarbons in the mite have been directly identified among lipid components of the species.

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