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Structural Elucidation of Twelve Novel Esters Composed of Five Fatty Acids and Three New Branched Alcohols Together with Four Monoterpenoids from *Sancassania shanghaiensis* (Acari: Acaridae)†

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A total of 12 novel esters and four monoterpenoids (rosefuran, (2*R*,3*R*)-epoxyneral, and α- and β-acaridials) were detected by GC/MS analyses as the opisthonotal gland components of *Sancassania shanghaiensis*. The acidic fraction after hydrolysis was composed of five common fatty acids (palmitic, stearic, oleic, linoleic and arachidic acid), while the alcoholic fraction consisted of two major components (C_6 and C_8 alcohols with branched methyls), together with a trace amount of C_9 alcohol. The two major alcohols were identified as new alcohols [(*S*)-2-methylpentanol and (2*S*,4*S*)-2,4-dimethylhexanol] by comparing the physico-chemical data of their 3,5-dinitrobenzoates with those of regio-selectively synthesized alcohols. The C_9 alcohol was suggested as (2*S*,4*S*)-2,4-dimethylheptanol, based on a structural and biogenetic analogy to the C_6 and C_8 alcohols. Five of the compounds were each identified by GC to be (*S*)-2-methylpentyl esters from five fatty acids, and the other five components likewise as (2*S*,4*S*)-2,4-dimethylhexyl esters. The remaining two were suggested as (2*S*,4*S*)-2,4-dimethylheptyl stearate and linolate.

Key words: astigmatid mite; *Sancassania shanghaiensis*; (*S*)-2-methylpentanol; (2*S*,4*S*)-2,4-dimethylhexanol; fatty acid ester of (*S*)-2-methylpentanol and (2*S*,4*S*)-2,4-dimethylhexanol

Astigmatid mites generally possess a pair of glands on both sides of the lateral opisthosoma (opisthonotal glands) which contain volatiles specific to a species. It is interesting to investigate the gland exudates not only from the aspect of natural product chemistry but also from chemical ecology. Ten compounds among a total of 50 have so far been identified as new natural products[1,2] from 31 species of 7 families; 6 monoterpenoids, three salicylaldehyde analogs and one of polyketide origin composed of propionates. Twelve compounds function as the alarm pheromone for 16 species,[3] one as the aggregation pheromone for one species,[4] and five compounds as the female sex pheromone for 6 species.[5] In addition, strong antifungal activities have been demonstrated in three new compounds together with citral which may act as defense substances.[6-8]

We collected the astigmatid mite, *Sancassania shanghaiensis* Zen et Wang, from organic soil in Kyoto, Japan and found the presence of 12 new esters composed of three novel alcohols and five common fatty acids in the gland, together with 6 components as volatiles. In the present paper, we report the identification of two of the new alcohols by stereoselective syntheses, the structural elucidation of the third alcohol, and the resulting identification and elucidation of the 12 new esters.

Material and Methods

Mites. Sancassania shanghaiensis obtained from organic soil at Kyoto University in 1994 were reared at 25°C under high humidity by feeding with dried yeast in a petri dish (9 cm i.d. × 2 cm in height) in which a sheet of filter paper had been placed as a foothold for the mites.

Analytical methods. GC was conducted with an HP 5890 series II Plus instrument equipped with FID and an HP-1 capillary column (0.20 mm i.d. × 25 m, split-less mode, abbreviated as HP-1), and an HP-5 column (0.25 mm i.d. × 30 m, split-less mode, abbreviated as HP-5) under two temperature programs: (1) from 60°C to 290°C at 10°C/min with an initial 2-min hold, and (2) from 180°C to 290°C at

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10°C/min with an initial 1-min hold. No indication of columns and conditions in the text implies HP-1(1). GC/MS data were measured by a Hitachi M-80 gas chromatograph-mass spectrometer in the low resolution mode of 70 eV with the HP-1 column under condition (1). HRMS data were obtained by a Jeol JMS HX/HX 110A spectrometer. ^{1}H- and ^{13}C-NMR studies were carried out with a Bruker AC300 instrument (^{1}H, 300 MHz; ^{13}C, 75.5 MHz) in $CDCl_3$, and IR spectra were recorded by a Shimadzu IR-400 instrument. HPLC was operated by an Altex 110A pump and detected by a UV-spectrophotometer (Jasco Uvidec-100-II) at 254 nm, using (1) a YMC Pack A-024 S-5 60A SIL column [10 mm i.d × 300 mm, eluted with 2% EtOAc in hexane at 7 ml/min, column and conditions abbreviated as HPLC(Sil)] and (2) Shiseido Ceramospher chiral RU-1 S-5 column [4.6 mm i.d. × 250 mm, eluted with a MeOH /CH_3CN (1/1) mixture or with CH_3CN alone, both at 1.0 ml/min, abbreviated as Chiral HPLC(1) and (2)]. Optical rotation data were measured with a JASCO DIP-370 digital polarimeter. The stereochemistry of epoxyneral was determined by GC at 100°C with a Chrompack chiral capillary column (CP-cyclodex B-236M, 0.25 mm i.d. × 25 m in the split mode). All solvents except $CDCl_3$ that were used for analyses, extraction and syntheses were dried and freshly distilled.

Extraction and collection of the ester fraction. To monitor the mites' components by GC and GC/MS analyses, one mite at a time (male, female or nymphal stage) was picked up from the stock culture by a needle tip and dipped into hexane (3 μl) for 3 min. The resulting hexane layer was then subjected to the analyses.

In order to isolate the ester fraction, the extract (8 mg) prepared from medium-free mite bodies (3.9 g) by dipping in hexane for 3 min was loaded into a SiO_2 (Wako Gel C-200, 300 mg) column and eluted stepwise with 3 ml each of ether (1, 3, 5, 10, 20 and 50%) in hexane. The esters (5 mg) were recovered in the 1% ether-hexane fraction.

Preparation of 3,5-dinitrobenzoates (DNBs) from the ester fraction, and isolation of C_6-DNB and C_8-DNB. $LiAlH_4$ (5 mg) was added to the esters (5 mg) in ether (0.8 ml) at 0°C under N_2, and the solution kept for 5 min. After quenching the mixture with water (0.5 ml), the product was extracted with ether, washed with brine, and dried over Na_2SO_4.

To the extract after its concentration to ca. 1 ml, 3,5-dinitrobenzoyl chloride (10 mg) in pyridine (1 ml) was added, and the mixture was stirred for 20 h at r.t. Pyridine was then azeotropically removed *in vacuo* with toluene. The resulting product was chromatographed over SiO_2 (50 g; hexane:ether = 10:1) to give a mixture of DNBs, among which C_6-DNB and C_8-DNB were isolated by HPLC(Sil). C_6-DNB: t_R [HPLC(Sil)] 18.4 min, t_R [chiral HPLC(1)] 4.4 min, t_R [GC, HP-1(2)] 5.926 min, t_R [GC, HP-5(1)] 18.776 min. C_8-DNB: t_R [HPLC(Sil)] 15.7 min, t_R [chiral HPLC(2)] 4.5 min, t_R [GC, HP-1(2)] 8.179 min.

Synthesis of 2-Methylpentyl DNB (2). 3,5-Dinitrobenzoyl chloride (355 mg, 1.4 mmol) was added to 2-methylpentanol (**1**; 143 mg, 1.4 mmol) in pyridine (5 ml), and the mixture was stirred overnight. The product, after azeotropically removing pyridine with toluene *in vacuo*, was chromatographed over SiO_2 (30 g, hexane:ether = 10:1) to give **2** [393 mg, 95%, HP-5(1) t_R: 18.787 min] as a colorless oil. Chiral HPLC(1) gave two peaks (t_R 4.4 min and 5.0 min). The ^{1}H-NMR, IR and MS data were the same as those for (*S*)-2-methylpentyl DNB [(*S*)-**2**] shown later.

Synthesis of 2,4-dimethylhexyl DNB (9). Hydroxy ester (5). *n*-Butyllithium (1.6 M in hexane, 6.9 ml, 11 mmol) was added dropwise to diisopropylamine (1.55 ml, 11.8 mmol) in THF (11 ml) at 0°C under N_2, and the mixture was stirred for 30 min at 0°C to prepare lithium diisopropylamide (LDA) in THF. To this LDA solution, methyl propionate (**3**; 1.1 ml, 11.4 mmol) in THF (2 ml) was added at −78°C, the mixture was stirred for 30 min, and then 2-methylbutanal (**4**) (1 g, 11.4 mmol) in THF (2 ml) was added. After being stirred for 30 min, the mixture was quenched by satd. aq. NH_4Cl (5 ml) and then warmed to r.t. The product extracted with ether (70 ml × 3) was washed with brine (20 ml), dried over Na_2SO_4, and concentrated *in vacuo*. The residual oil was chromatographed over SiO_2 (100 g, hexane:ether = 10:2) to give a diastereomeric mixture of (**5**; 1.55 g, 78%) as a colorless oil. ^{1}H-NMR δ_H ($CDCl_3$): 3.71–3.67 (3H, apparently multiplet, $-OCH_3$), 3.76–3.60 (1H, m), 2.80–2.60 (1H, m), 1.55–1.10 (3H, m), 1.25–1.14 (3H, d), 0.97–0.82 [(3H, d), (3H, t)]. EIMS *m/z* (%): 174 (M^+, 8), 159 (85), 156 (44), 143 (65), 117 (100), 97 (92), 88 (99).

Mesylate (6). Triethylamine (0.60 ml, 4.3 mmol) and methanesulfonyl chloride (0.5 ml, 6.5 mmol) were added to **5** (324 mg, 1.86 mmol) in CH_2Cl_2 (8 ml) at 0°C under N_2, and the mixture was stirred for 2 h. After being quenched by H_2O (5 ml) and warmed to r.t., the product was extracted with $CHCl_3$ (30 × 3 ml), washed with brine (10 ml), dried over Na_2SO_4, and concentrated *in vacuo*. The residual oil was chromatographed over SiO_2 (15 g, hexane:ether = 3:2) to give a diastereomeric mixture of **6** (410 mg, 87%) as a colorless oil. ^{1}H-NMR δ_H ($CDCl_3$): 4.97–4.93 (1H, dd), 3.73–3.72 (3H, apparently multiplet), 3.04–2.99 (3H, apparently multiplet), 2.93–2.81 (1H, m), 1.90–1.15 (3H, m),

1.29–1.18 (3H, d), 1.02–0.91 (6H, d & t).

Ethyl 2,4-dimethyl-2-hexenoate (7). Sodium ethoxide (100 mg, 1.42 mmol) was added to **6** (200 mg, 0.79 mmol) in ethanol (5 ml) under N_2. After being stirred at r.t. for 16 h, the mixture was cooled to 0°C and then by H_2O (5 ml). The mixture was warmed to r.t. and extracted with ether (30 ml × 3). The extract was successively washed with 2 N HCl (10 ml), 1 N $NaHCO_3$ (10 ml) and brine (15 ml), and dried over Na_2SO_4. After its concentration, the residual oil was chromatographed over SiO_2 (18 g, hexane:ether = 10:1) to give **7** (94 mg, 70%) as a colorless oil. IR ν cm^{-1} ($CHCl_3$): 1690. ^{1}H-NMR δ_H ($CDCl_3$): 6.53 (1H, dq, J = 10.1, 1.4), 4.19 (2H, q, J = 7.1), 2.41 (1H, m), 1.84 (3H, d, J = 1.4), 1.50–1.23 (2H, m), 1.30 (3H, t, J = 7.1), 1.00 (3H, d, J = 6.7), 0.86 (3H, dd, J = 7.4, 7.4). EIMS m/z (%): 170 (M^+, 87), 155 (7), 41 (15), 125 (75), 113 (88), 95 (83), 81 (31), 67 (56), 55 (100).

Diastereomers of 2,4-dimethylhexyl DNB (9). $LiAlH_4$ (84 mg, 2.2 mmol) was added to **7** (94 mg, 0.55 mmol) in ether (10 ml) at 0°C. After being warmed to r.t. and stirred for 30 min, the mixture was quenched by H_2O (1 ml) at 0°C and extracted with ether (15 ml × 3). The extract was washed with brine (3 ml), dried over Na_2SO_4 and concentrated to about 1 ml. To prevent loss by evaporation, the product, 2,4-dimethyl-2-hexenol, was used for the next step without purification. The structure of the product was confirmed by GC/MS and ^{1}H-NMR in a pilot synthesis. ^{1}H-NMR δ_H ($CDCl_3$): 5.17 (1H, dd, J = 1.2, 9.5), 4.00 (2H, br s), 2.29 (1H, m), 1.67 (3H, d, J = 1.2), 1.42–1.18 (2H, m), 1.30 (3H, t, J = 7.1), 0.94 (3H, d, J = 6.7), 0.84 (3H, dd, J = 7.4, 7.4). EIMS m/z (%): 128 (M^+, 21), 110 (10), 97 (89), 95 (57), 73 (92), 71 (91), 57 (92), 43 (100). PtO_2 (10 mg) suspended in ethanol (5 ml) was stirred under H_2 for 30 min. 2,4-Dimethyl-2-hexenol in ether (1 ml) and ethanol (5 ml) was then added, and the mixture was stirred under H_2 for 2 h. The catalyst was removed by filtration. The resulting filtrate, after careful concentration *in vacuo*, gave a mixture of four stereoisomers of **8** which showed two peaks by a GC/MS analysis. Peak 1 of **8**: EIMS m/z (%): 112 (M^+-18, 2), 97 (7), 83 (77), 70 (82), 57 (95), 55 (90), 43 (84), 41 (100). Peak 2 of **8**; EIMS m/z (%): 112 (M^+-18, 2), 97 (8), 83 (86), 70 (84), 57 (92), 55 (91), 43 (83), 41 (100). The diasteromeric mixture of **8** was dissolved in pyridine (8 ml), and 3,5-dinitrobenzoyl chloride (318 mg, 1.38 mmol) was added. The mixture was stirred overnight and then pyridine was azeotropically removed with toluene *in vacuo*. The residue was chromatographed over SiO_2 (5 g, hexane:ether = 10:1) to give a mixture of four stereoisomers of **9** (82 mg, 49%, 3 steps from **7**) as a colorless oil which was further separated by HPLC(Sil) to fractions 1 (t_R: 15.7 min) and 2 (t_R: 16.2 min). Fraction 1 [a mixture of (2*S*,4*S*)- and (2*R*,4*R*)-2,4-dimethylhexyl DNB]: chiral HPLC(2): t_R 4.5 and 5.4 min. The ^{1}H-NMR, IR and GC/MS data were the same as those of natural C_8-DNB and regioselectively synthesized (2*S*,4*S*)-2,4-dimethylhexyl DNB[(2*S*,4*S*)-**9**]. Fraction 2 [a mixture of (2*R*,4*S*)- and (2*S*,4*R*)-2,4-dimethylhexyl DNB]: IR ν cm^{-1} ($CHCl_3$): 1720 [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 9.23 (1H, dd, J = 2.2, 2.2), 9.16 (2H, d, J = 2.2), 4.32 (1H, dd, J = 5.8, 10.7), 4.22 (1H, dd, J = 7.1, 10.7), 2.10 (1H, ddddq, J = 5.8, 7.1, 6.7, 6.7, 6.7), 1.55–1.15 (5H, m), 1.03 (3H, d, J = 6.7), 0.87 (3H, t, J = 7.0) and 0.86 (3H, d, J = 6.3); ^{13}C-NMR δ_C ($CDCl_3$): 162.6, 148.8, 134.24, 129.3, 122.3, 72.4, 40.5, 31.6, 30.3, 30.2, 18.9, 16.9 and 11.3. EIMS m/z (%): 267 (M^+-57, 7), 195 (35), 179 (4), 149 (22), 129 (2), 112 (15), 103 (9), 83 (48), 70 (97) and 57 (100).

Synthesis of (S)-2-methylpentyl DNB [(S)-2]. Aldol (12). Di-*n*-butylboron triflate (1.0 M in CH_2Cl_2, 6.7 ml, 6.7 mmol) and triethylamine (1.2 ml, 8.6 mmol) were successively added to (*S*)-(+)-4-isopropyl-3-propionyl-2-oxazolidinone (**11**)[9] (1.15 g, 6.22 mmol) in CH_2Cl_2 (11 ml) at −78°C while stirring. After 30 min, the temperature was raised to 0°C and held for 50 min. Propanal (**10**) (301 mg, 5.18 mmol) in CH_2Cl_2 (5 ml) was then added at −78°C, and the mixture was stirred for 30 min. After the temperature had been raised again to 0°C and held for 1 h, the mixture was successively quenched by a phosphate buffer (Wako standard buffer solution, pH 6.86, 5 ml), methanol (5 ml) and 30% aq. H_2O_2 (3 ml), and kept for an additional 1 h at 0°C. After removing the solvent *in vacuo*, H_2O (10 ml) was added, and the product was extracted with CH_2Cl_2 (50 ml × 3). The extract was washed with brine (10 ml), dried over Na_2SO_4, and concentrated. The residual oil was chromatographed over SiO_2 (80 g, hexane:ether = 6:4) to give **12** (806 mg, 64%) as a colorless oil. $[\alpha]_{20}^{D}$; 60.0 (*c* 1.07, $CHCl_3$). IR ν cm^{-1} ($CHCl_3$): 1775 [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 4.48 (1H, ddd, J = 3.2, 3.7, 8.2), 4.29 (1H, dd, J = 8.2, 9.1), 4.22 (1H, dd, J = 2.6, 7.0), 3.86 (1H, dddd, J = 2.6, 2.7, 5.3, 8.0), 3.80 (1H, dq, J = 2.6, 7.0), 2.98 (1H, d, J = 2.7), 2.35 (1H, dqq, J = 4.0, 7.1, 6.9), 1.50 (1H, ddq, J = 8.0, 13.8, 7.5), 1.56–1.38 (1H, m), 1.25 (3H, d, J = 7.0), 0.97 (3H, dd, J = 7.5), 0.93 (3H, d, J = 7.1), 0.89 (3H, d, J = 6.9). EIMS m/z (%): 225 (M^+-18, 6), 214 (4), 196 (9), 185 (68), 156 (11), 142 (15), 130 (100), 115 (18), 96 (22), 86 (65), 85 (58), 57 (54).

Hydroxy ester (14). $LiOH \cdot H_2O$ (412 mg, 9.84 mmol) and 30% aq. H_2O_2 (3.4 ml) were successively added to **12** (778 mg, 3.28 mmol) in aq. THF (80%, 34 ml) at 0°C while stirring. After 1.5 h, the mixture was quenched by $Na_2S_2O_6 \cdot 5H_2O$ (1.5 g) while stirring for 20 min more. The product was extracted with

$CHCl_3$ (50 ml × 3) under acidified conditions (pH 1) with 2 N HCl, and subsequently washed with brine, dried over Na_2SO_4 and concentrated to 10 ml. After methylation with diazomethane in ether by the conventional method and evaporation of the solvent, the resulting oil was chromatographed over SiO_2 (40 g, hexane:ether = 3:1) to give a hydroxy ester (**14**, 244 mg, 51%) as a colorless oil. $[\alpha]_{20}^{D}$: −0.37 (*c* 0.53, $CHCl_3$). IR ν cm^{-1} ($CHCl_3$): 3600–3300 (-OH) and 1725 cm^{-1} [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 3.82 (1H, dddd, J=3.6, 4.7, 4.9, 8.5), 3.71 (3H, s), 2.57 (1H, dq, J=3.6, 7.2), 2.43 (1H, d, J=4.7), 1.58–1.38 (2H, m), 1.18 (3H, d, 7.2), 0.97 (3H, dd, J=7.4, 7.4). EIMS *m/z* (%): 131 (M^+-15, 3), 128 (5), 117 (18), 115 (12), 97 (5), 88 (100), 85 (26) and 57 (54).

Thioimidazolide (16). 1,1′-Thiocarbonyldiimidazole (1.2 g, 6.7 mmol) was added to a stirred solution of **14** (244 mg, 1.67 mmol) in THF (6 ml), and the mixture was refluxed for 10 h. After concentrating the mixture *in vacuo*, the residue was chromatographed over SiO_2 (30 g, hexane:ether = 3:1) to give **16** (306 mg, 71%) as a yellow oil. $[\alpha]_{20}^{D}$: 12.5 (*c* 3.13, CH_2Cl_2). IR ν cm^{-1} ($CHCl_3$): 1740 [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 8.33 (1H, dd, J=0.9, 0.8), 7.62 (1H, dd, J=1.6, 1.4), 7.04 (1H, dd, J=0.9, 1.6), 6.01 (1H, dd, J=4.7, 7.0), 3.70 (3H, s), 3.03 (1H, dq, J=7.0, 7.0), 1.84 (1H, dddq, J=4.7, 5.7, 9.4, 6.8), 1.48 (1H, ddq, J=12.9, 5.7, 7.4), 1.34–1.19 (1H, m), 1.25 (3H, d, J=7.0), 1.01 (3H, d, J=6.8), 0.96 (3H, d, J=7.4). EIMS *m/z* (%): 256 (M^+, 79), 241 (1), 225 (11), 197 (6), 169 (10), 129 (90), 128 (68), 113 (24), 97 (32), 69 (100).

(S)-2-methylpentyl DNB [(S)-2]. Tri-*n*-butyltinhydride (0.17 ml, 0.63 mmol) was added to **16** (160 mg, 0.63 mmol) in hexane (30 ml) while stirring. After 5 h, the mixture was concentrated *in vacuo* to ca. 5 ml, and the product was chromatographed over SiO_2 (20 g, hexane:ether = 95:5) to give a crude methyl ester (**18**) along with a hydroxy ester (**14**) as a by-product (**18**:**14** = 1:1). Methyl ester **18** [EIMS *m/z* (%): 115 (M^+-15, 5), 101 (36), 88 (100), 71 (21), 59 (17), 43 (44)] without further purification was reduced by $LiAlH_4$ (19 mg, 0.5 mmol) in ether (20 ml) at 0°C while stirring. After 30 min, the mixture was quenched by satd. aq. NH_4Cl (5 ml), and the product (**20**) was extracted with ether (30 ml × 3), washed with brine (15 ml), dried over Na_2SO_4 and concentrated to ca. 5 ml. Pyridine (5 ml) was added to the concentrate, and the mixture stirred overnight with 3,5-dinitrobenzoyl chloride (71 mg, 0.31 mmol). After the solvent had been azeotropically distilled with toluene, the residue was chromatographed over SiO_2 (6 g, hexane:ether = 10:1) to give (*S*)-2-methylpentyl DNB [(*S*)-**2**, 10 mg, 5%, 3 steps from **16**] as a colorless oil. Chiral HPLC(1): one peak (100% ee) at t_R 4.4 min, $[\alpha]_{30}^{D}$ −0.53 (*c* 0.38, CH_2Cl_2). IR ν cm^{-1} ($CHCl_3$): 1720 [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 9.23 (1H, dd, J=2.2), 9.16 (2H, d, J=2.2), 4.35 (1H, dd, J=5.8, 10.7), 4.25 (1H, dd, J=7.0, 10.7), 2.09–2.00 (1H, m), 1.49–1.20 (4H, m), 1.06 (3H, d, J=6.8), 0.95 (3H, d, J=6.8). EIMS *m/z* (%): 296 (M^+, 4), 195 (28), 182 (16), 165 (5), 149 (26), 84 (76), 57 (84), 43 (100). HRMS (DICI): calcd. for $C_{13}H_{17}O_6N_2$ [$(M+H)^+$], 297.1087; found, 297.1082.

(2S,4S)-2,4-dimethylhexyl DNB ((2S,4S)-9). Aldol (**13**). Di-*n*-butylboron triflate (1 M in CH_2Cl_2, 10.3 ml, 10.3 mmol) and triethylamine (1.7 ml, 12.2 mmol) were reacted with (*S*)-(+)-4-isopropyl-3-propionyl-2-oxazolidinone (**3**) (1.91 g, 10.3 mmol) in CH_2Cl_2 (20 ml). (*S*)-**4** in pentane (ca. 20 ml) which had been prepared from (*S*)-2-methylbutanol (1.2 ml, 11 mmol) by Swern oxidation,[10] was reacted and successively quenched by a phosphate buffer (Wako standard buffer solution pH 6.86, 20 ml), methanol (20 ml) and 30% aq. H_2O_2 (9 ml) as described in **12**. The product was chromatographed over SiO_2 (80 g, hexane:ether = 7:3) to give an aldol (**13**; 2.38 g, 78%, 2 steps from (*S*)-2-methylbutanol) as colorless crystals. Recrystallization from chloroform-hexane gave colorless needles, mp 79–80°C; $[\alpha]_{20}^{D}$ 60.5 (*c* 0.8, CH_2Cl_2). IR ν cm^{-1} ($CHCl_3$): 1775 [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 4.47 (1H, ddd, J=3.5, 4.0, 8.1), 4.28 (1H, dd, J=8.1, 9.1), 4.21 (1H, dd, J=3.5, 9.1), 3.99 (1H, dq, J=3.6, 7.0), 3.67 (1H, ddd, J=3.6, 3.6, 7.0), 2.75 (1H, d, J=3.6), 2.35 (1H, dqq, J=4.0, 7.0, 7.0), 1.56–1.40 (2H, m), 1.26 (3H, d, J=7.0), 1.24–1.04 (1H, m), 0.98 (3H, d, J=7.0), 0.92 (3H, d, J=7.0), 0.90 (3H, t, J=7.4), 0.88 (3H, d, J=7.0). EIMS *m/z* (%): 253 (M^+-18, 1), 214 (15), 185 (35), 142 (8), 130 (100), 86 (31), 85 (32), 57 (41).

Hydroxy ester (15). $LiOH \cdot H_2O$ (162 mg, 3.9 mmol) and 30% aq. H_2O_2 (1.4 ml) were added to **13** (349 mg, 1.29 mmol). The reaction was quenched by $Na_2S_2O_6 \cdot 5H_2O$ (730 mg), and the product was extracted as described for **14**. After methylation with diazomethane, the product was chromatographed over SiO_2 (10 g, hexane:ether = 7:3) to give **15** (179 mg, 80%) as a colorless oil. $[\alpha]_{20}^{D}$ −3.7 (*c* 1.467, CH_2Cl_2). IR ν cm^{-1} ($CHCl_3$): 3700–3300 (-OH) and 1730 [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 3.70 (3H, s), 3.68 (1H, ddd, J=5.1, 5.1, 4.8), 2.68 (1H, dq, J=5.1, 7.1), 2.20 (1H, d, J=4.8), 1.46–1.34 (2H, m), 1.23–1.04 (1H, m), 1.21 (3H, d, J=7.1), 0.96 (3H, d, J=6.6), 0.90 (3H, t, J=7.1). EIMS *m/z* (%): 159 (M^+-15, 2), 143 (2), 117 (44), 97 (8), 88 (100), 85 (4), 57 (61).

Thioimidazolide (17). 1,1′-Thiocarbonyldiimidazole (614 mg, 3.45 mmol) was reacted with **15** (187 mg, 1.07 mmol) in THF (2.6 ml) as described for **16**. The product was chromatographed over SiO_2 (24 g, hexane:ether = 4:1) to give **17** (278 mg, 91%) as

a yellow oil. $[\alpha]_{20}^{D}$ −7.07 (*c* 0.93, CH_2Cl_2). IR ν cm^{-1} ($CHCl_2$): 1735 [-O-(C=O)-]. ^{1}H-NMR δ_H ($CDCl_3$): 8.33 (1H, dd, J=0.9, 0.8), 7.62 (1H, dd, J=1.6, 1.4), 7.04 (1H, dd, J=0.9, 1.6), 6.01 (1H, dd, J=4.7, 7.0), 3.70 (3H, s), 3.03 (1H, dq, J=7.0, 7.0), 1.84 (1H, dddq, J=4.7, 5.7, 9.4, 6.8), 1.48 (1H, ddq, J=12.9, 5.7, 7.4), 1.34-1.19 (1H, m), 1.25 (3H, d, J=7.0), 1.01 (3H, d, J=6.8), 0.96 (3H, d, 7.4). EIMS *m/z* (%): 284 (M^+, 1), 157 (23), 125 (31), 97 (100), 69 (46), 54 (47).

(2S,4S)-2,4-dimethylhexyl DNB ((2S,4S)-9). Tri-*n*-butyltinhydride (0.28 ml, 1.04 mmol) was reacted with **17** (293 mg, 1.03 mmol) to give **19** as described for **18**. The product was chromatographed over SiO_2 (24 g, hexane:ether=97:3) to give a crude methyl ester (**19**) together with **15** as a by-product (**19**:**15**=4:1). Methyl ester (**19**): ^{1}H-NMR δ_H ($CDCl_3$): 3.66 (3H, s), 2.56 (1H, m), 1.71 (1H, m), 1.35-1.05 (4H, m), 1.14 (3H, d, J=7.0), 0.87 (3H, d, J=6.8), 0.86 (3H, t, J=7.4). EIMS *m/z* (%): 143 (M^+-15, 5), 129 (36), 127 (27), 115 (31), 101 (92), 88 (100), 87 (76), 73 (53), 69 (85), 57 (92).

This methyl ester (**19**) without further purification was reduced by $LiAlH_4$ (39 mg, 1.03 mmol) to **21**, which was, without purification, reacted with 3,5-dinitrobenzoyl chloride (300 mg, 1.3 mmol) in pyridine (8 ml), as described for (*S*)-**2**. The product was chromatographed over SiO_2 (30 g, hexane:ether=10:1) to give (2*S*,4*S*)-**9** [90 mg, 27%, 3 steps from thioimidazolide **18**, 100% ee by chiral HPLC(2), t_R 4.3 min] as a colorless oil. $[\alpha]_{20}^{D}$ +5.71 (*c* 2.1, CH_2Cl_2). IR ν cm^{-1} ($CHCl_3$): 1730 [-O-(C=O)-], 1550, 1350 and 1280. ^{1}H-NMR δ_H ($CDCl_3$): 9.23 (1H, dd, J=2.1, 2.1), 9.15 (2H, d, J=2.1), 4.35 (1H, dd, J=5.4, 10.7), 4.21 (1H, dd, J=7.1, 10.7), 2.11 (1H, ddddq, J=5.4, 7.1, 6.8, 6.8, 6.8), 1.56-1.33 (3H, m), 1.28-1.02 (2H, m), 1.06 (3H, d, J=6.8), 0.93 (3H, d, J=6.4) and 0.89 (3H, t, J=7.3). ^{13}C-NMR δ_C ($CDCl_3$): 162.6, 148.7, 134.2, 129.3, 122.3, 71.8, 40.7, 31.6, 30.2, 29.1, 19.6, 17.8 and 11.1. EIMS *m/z* (%): 267 (M^+-57, 2), 195 (36), 182 (23), 165 (14), 149 (19), 129 (5), 112 (13), 97 (11), 83 (41), 70 (69) and 57 (100). HRMS (DICI): calcd. for $C_{15}H_{21}O_6N_2$ [$(M+H)^+$], 325.1400; found, 325.1405.

Synthesis of the esters. 2-Methylpentanol (0.10 ml, 0.81 mmol) and stearic acid (0.1 g, 0.35 mmol) were heated at 70°C for 17 h with *p*-TsOH.H_2O (20 mg, 0.11 mmol) as a catalyst. After cooling to r.t., the residual oil was chromatographed over SiO_2 (4 g, hexane:ethyl acetate=100:3) to give an ester (96 mg, 74%). Esters between 2-methylpentanol with palmitic, linolenic, oleic and arachidic acid were synthesized in the same way. The palmitate (GC t_R 18.67 min), linolate (20.00 min), oleate (20.06 min), stearate (20.29 min) and arachidate (21.81 min) each corresponded to peak A, C, D, E and J, respectively. The ester of (2*S*,4*S*)-2,4-dimethylhexanol was synthesized by reacting (2*S*,4*S*)-2,4-dimethylhexyl DNB [(2*S*,4*S*)-**9**, 15 mg, 0.046 mmol] with $LiAlH_4$ (2 mg) at 0°C to give a mixture of alcohols which was further chromatographed over SiO_2 (0.5 g, hexane:ether=8:1) to give (2*S*,4*S*)-2,4-dimethylhexanol. (2*S*,4*S*)-2,4-dimethylhexanol was reacted with stearic acid (10 mg, 0.035 mmol) in the same way as with 2-methylpentanol to give the corresponding ester [5 mg, 28% from (2*S*,4*S*)-**9**]. The palmitate (GC t_R 19.89 min), linolate (21.17 min), oleate (21.22 min), stearate (21.45 min) and arachidate (22.89 min) each corresponded to peak B, F, G, H and L, respectively.

Results and Discussion

The most prominent feature in the GC profile of the species was the presence of a total of 12 peaks (peaks A-L) in a less volatile range (or cuticular component range, t_R 18.0-25.3 min by GC/MS) together with 6 compounds (peaks 1-6) in a volatile range (semiochemical range,[8] t_R 4-12 min by GC/MS) (Fig. 1). Squalene was a contaminant from the rearing diet. No differences were apparent among the components in the GC profiles due to sex or development stage, except for the deutonymph (phoretic stage) whose major components consisted of heptadecadiene and heptadecene.

GC and GC/MS analyses of peaks 1-6 by the co-injection method with authentic samples revealed that peak 1 (t_R 4.48 min, M^+ *m/z* 150), 2 (t_R 6.20 min, M^+-18 *m/z* 150), 4 (t_R 6.82 min, M^+ *m/z* 166) and 5 (t_R 7.85 min, M^+ *m/z* 166) respectively corresponded to rosefuran,[11] (2*R*,3*R*)-epoxyneral,[12] α-acaridial[13] and β-acaridial.[14] The structures of the other two minor peaks, 3 (t_R 6.52 min, M^+ *m/z* 156) and 6 (t_R 8.12 min, M^+ *m/z* 152) remained obscure.

Peaks A-L in the less-volatile range were recovered

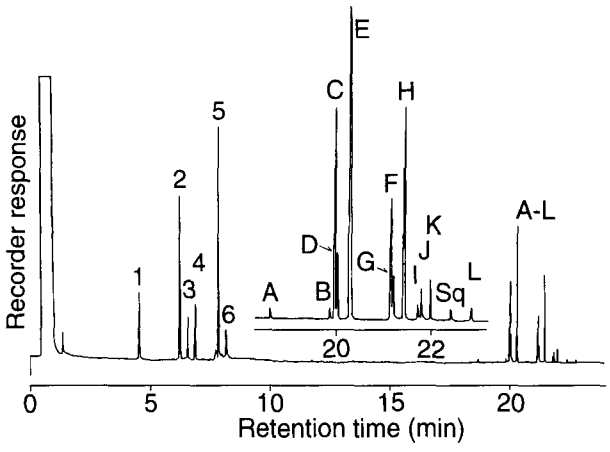


Fig. 1. Typical GLC Profiles of a Hexane Extract from *Sancassania shanghaiensis*.

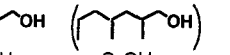
HP-1, 60°C (2 min), 10°C/min, 290°C (5 min). See the text for details of the compounds in each peak 1-6 and A-L.

in the 1% ether-hexane fraction by an SiO_2 column, indicative of esters. Each GC/MS analysis gave the following sets of M^+ ion (base ion) and diagnostic fragment ions: peak A (t_R 18.67 min): M^+ not detectable (base ion at m/z 84); peak B (t_R 19.87 min): M^+ not detectable (m/z 57); peak C (t_R 19.99 min): M^+ m/z 364 (m/z 85), m/z 279 and 263; peak D (t_R 20.04 min): M^+ not detectable (m/z 85), m/z 281, 279, 265 and 264; peak E (t_R 20.30 min): M^+ m/z 368 (m/z 84), m/z 285, 284 and 267; peak F (t_R 21.16 min): M^+ m/z 392 (m/z 57), m/z 280, 279 and 263; peak G (t_R 21.21 min): M^+ not detectable (m/z 57), m/z 281, 279, 265 and 264; peak H (t_R 21.44 min): M^+ m/z 396 (m/z 57), m/z 285, 284 and 267; peak I (t_R 21.75 min): M^+ not detectable (m/z 127); peak J (t_R 21.80 min): M^+ not detectable (m/z 84), m/z 312 and 295; peak K (t_R 22.00 min): M^+ not detectable (m/z 126), m/z 285, 284 and 267; peak L (t_R 22.88 min): M^+ not detectable (m/z 57), m/z 312, 311 and 295. Peaks A–L were all typical of those from esters between aliphatic acids and alcohols,[15] and classifiable into the following three groups by their base ions: (1) with m/z 84 or 85 (derived from a C_6-alcohol moiety), peaks A, C, D, E and J; (2) with m/z 57 (derived from a C_8-alcohol moiety), peaks B, F, G, H and L; and (3) with 126 or 127 (derived from a C_9-alcohol moiety), peaks I and K. The diagnostic fragment ions enabled the peaks were to be further classified into four groups: (I) with m/z 279 and 263 (suggesting a linoleic acid moiety), peaks C and F; (II) with m/z 281, 279, 265 and 264 (indicative of an oleic acid moiety), peaks D and G; (III) with m/z 285, 284 and 267 (suggestive of a stearic acid moiety), peaks E, H and K; and (IV) with m/z 312, (311) and 295 (indicative of an arachidic acid moiety), peaks J and L. Although M^+ ions were not detected with peaks A, B and I due to their low abundance, those peaks were respectively suggested as esters between the C_6-alcohol and palmitic acid, C_8-alcohol and palmitic acid, and C_9-alcohol and linoleic acid from their diagnostic ions and GC t_R values (Fig. 1).

The GC/MS analysis of the $LiAlH_4$ reduction product from the ester fraction indicated three alcohols: diagnostic ions at m/z 84 (C_6-alcohol-m/z 18), 112 (C_8-alcohol-m/z 18) and 126 (C_9-alcohol-m/z 18), together with saturated and unsaturated fatty alcohols (C_{16}–C_{20} alcohols). These results suggested that all peaks (A–L) were esters derived from commonly-occurring fatty acids and a C_6-, C_8- or C_9-alcohol, as summarized in Table 1. Each t_R of peaks A, E, B, H and K was, however, shorter than that of the corresponding ester synthesized between fatty acids (palmitic and stearic) and an *n*-alcohol (0.42, 0.37, 0.74, 0.67 and 0.80 min, respectively). These facts imply that the alcoholic components of the natural esters were branched.

In order to elucidate the structure of the alcoholic moiety, $LiAlH_4$ reduction products were converted to

Table 1. Combination of Both Acid and Alcohol Components of the Esters

	 C_6OH m/z 84 and (or) 85*	 C_8OH m/z 57*	 C_9OH m/z 126 and (or) 127*
Palmitic acid (not found)**	A	B	
Linoleic acid (m/z 263)**	C	F	I
Oleic acid (m/z 265)**	D	G	
Stearic acid (m/z 267)**	E	H	K
Arachidic acid (m/z 295)**	J	L	

* Base ion.
** Representative diagnostic ion of the acyl moiety.

DNBs. C_6-DNB and C_8-DNB were both successfully isolated from the reaction mixture by preparative HPLC(Sil). Purification of C_9-DNB was, however, unsuccessful, because of the minute amount available. ^{1}H-NMR data for both C_6-DNB and C_8-DNB are summarized in Table 2.

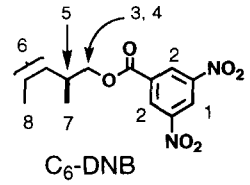
Two characteristic sets of coupling patterns (dd, geminal and vicinal coupling) of each methylene proton (partial structures 3 and 4 in Table 2) adjacent to ethereal oxygen of the benzoates were observed at δ_H 4.2–4.4 in both ^{1}H-NMR spectra of the isolated C_6- and C_8-DNBs, suggesting the presence of a methylene group next to chiral methine. Two methyls in C_6-DNB appeared at δ_H 1.06 (3H, d, J=6.8 Hz) and 0.95 (3H, t, J=7.0 Hz) ppm, and three methyls in C_8-DNB at δ 1.06 (3H, d, J=6.8 Hz), 0.93 (3H, d, J=6.4 Hz) and 0.89 (3H, t, J=7.3 Hz) ppm. As a result, the structure of C_6-DNB was elucidated to be 2-methylpentyl DNBs and that of C_8-DNB to be either 2,4-dimethylhexyl DNB or 2,3-dimethylhexyl DNB (Table 2).

The ^{1}H-NMR spectrum of C_6-DNB was identical to that of 2-methylpentyl DNB (**2**) prepared from 2-methylpentanol (**1**) that is available commercially. GC t_R HP-5(1) of C_6-DNB (18.776 min) was also identical to that of **2** (18.787 min).

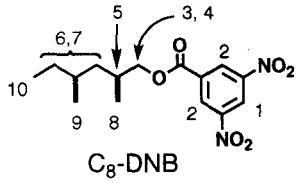
Diastereomeric-mixtures of 2,4-dimethylhexyl DNB (**9**) were prepared, starting from an aldol condensation between methyl propionate (**3**) and 2-methylbutanal (**4**) as summarized in Fig. 2. Dehydration of the product (**5**) *via* a mesylate (**6**) gave ethyl 2,4-dimethyl-2-hexenoate (**7**) which was then converted to 2,4-dimethylhexanol (**8**) by two-step reduction. DNB ester **9** of alcohol **8** consisted of four stereoisomers which were separated by HPLC(Sil) to give **9**-fraction 1 (t_R 15.7 min) and **9**-fraction 2 (t_R 16.2 min). GC [HP-1(2)] of **9**-fraction 1 gave t_R 8.177 min. ^{1}H- and ^{13}C-NMR of **9**-fraction 1 were both

Table 2. NMR Data for C_6-DNB and C_8-DNB and Their Structures

Chemical shift (ppm)	Proton	Multiplicity	Coupling constant (Hz)	Partial structure
C_6-DNB				
9.23	1H	dd	2.2	1
9.16	2H	d	2.2	2
4.35	1H	dd	5,8, 10.7	3
4.25	1H	dd	7.0, 10.7	4
2.09-2.00	1H	m		5
1.49-1.20	4H	m		6
1.06	3H	d	6.8	7
0.95	3H	t	7.0	8
C_8-DNB				
9.23	1H	dd	2.1	1
9.15	2H	d	2.1	2
4.35	1H	dd	5.4, 10.7	3
4.21	1H	dd	7.1, 10.7	4
2.11	1H	ddddq	5.4, 7.1, 6.8, 6.8, 6.8	5
1.56-1.33	3H	m		6
1.28-1.02	2H	m		7
1.06	3H	d	6.8	8
0.93	3H	d	6.4	9
0.89	3H	t	7.3	10



C_6-DNB



C_8-DNB

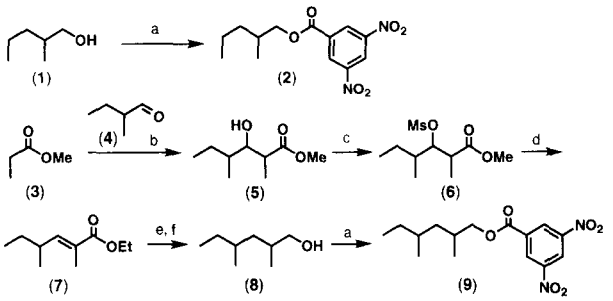


Fig. 2. Synthetic Procedure for 2-Methylpentyl DNB (**2**) and 2,4-Dimethylhexyl DNB (**9**).

(a) 3,5-dinitrobenzoyl chloride, pyridine; (b) LDA, THF, −78°C; (c) MsCl, Et_3N, CH_2Cl_2, 0°C; (d) NaOEt, EtOH; (e) $LiAlH_4$, ether, 0°C; (f) H_2, PtO_2, EtOH.

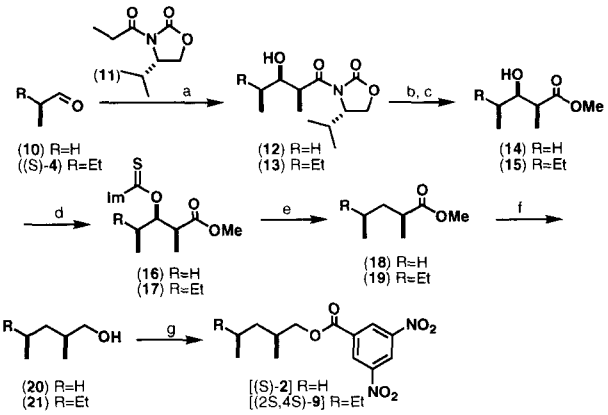


Fig. 3. Synthetic Procedure for (*S*)-2-Methylpentyl DNB [(*S*)-**2**] and (2*S*,4*S*)-2,4-Dimethylhexyl DNB [(2*S*,4*S*)-**9**].

(a) Bu_2BOTf, Et_3N, CH_2Cl_2, −78°C; (b) LiOH, H_2O_2, THF, H_2O, 0°C; (c) CH_2N_2, ether; (d) Im_2CS, THF, reflux; (e) Bu_3SnH, toluene, reflux; (f) $LiAlH_4$, ether; (g) 3,5-dinitrobenzoyl chloride, pyridine.

identical to those obtained from isolated C_8-DNB. HPLC t_R (Sil) and GC t_R [HP-1(2)] of the C_8-DNB were also identical to those of **9**-fraction 1; therefore, the possibility of 2,3-dimethylhexyl DNB was eliminated for C_8-DNB.

In order to determine each absolute configuration of C_6- and C_8-DNB, (*S*)-2-methylpentyl DNB [(*S*)-**2**] and (2*S*,4*S*)-2,4-dimethylhexyl DNB [(2*S*,4*S*)-**9**] were regioselectively synthesized (Fig. 3).[16] Evans aldol condensation[17] of imide **11** with propanal (**10**) or (*S*)-**4** afforded each corresponding aldol (**12** and **13**) with a trace amount of diasteromers. After removing the chiral auxiliary to give hydroxy esters (**14** and **15**), a Barton deoxygenation reaction[9] of derived thioimidazolides (**16** and **17**) gave methyl esters (**18** and **19**). Reduction to alcohols (**20** and **21**), and subsequent esterification with 3,5-dinitrobenzoyl chloride gave desired products (*S*)-**2** and (2*S*,4*S*)-**9**. The ^{1}H-NMR spectra of (*S*)-**2** and (2*S*,4*S*)-**9** were identical to those of isolated C_6-DNB and C_8-DNB.

Both racemic 2-methylpentyl DNB (**2**) and 2,4-dimethylhexyl DNB [(2*S*,4*S*)-**9** and (2*R*,4*R*)-**9**] each showed two peaks by chiral HPLC: t_R(1) 4.4 min and 5.0 min, and t_R(2) 4.5 min and 5.4 min. The peaks of (*S*)-2-methylpentyl DNB [(*S*)-**2**, t_R(1) 4.4 min] and (2*S*,4*S*)-2,4-dimethylhexyl DNB [(2*S*,4*S*)-**9**, t_R(2) 4.5 min] corresponded to antipods with shorter t_Rs, and also gave identical t_Rs to those of natural C_6-[t_R(1) 4.5 min] and C_8-DNBs [t_R(2) 4.5 min], respectively. All identities were confirmed by the co-injection method. Consequently, the absolute configuration of each natural C_6- and C_8-alcohol was concluded to be *S* and 2*S*, 4*S*, respectively.

A total of ten (*S*)-2-methylpentyl and (2*S*,4*S*)-2,4-dimethylhexyl esters were prepared from palmitic, stearic, oleic, linoleic and arachidic acids. Their GC t_Rs were compared to those of the natural esters. As a result, peaks A, C, D, E and J were identified as (*S*)-2-methylpentanol (**20**) esters of palmitic acid (peak A), linoleic acid (peak C), oleic acid (peak D), stearic acid (peak E) and arachidic acid (peak J), and likewise, peaks B, F, G, H and L as (2*S*,4*S*)-2,4-dimethylhexanol (**21**) esters of palmitic acid (peak B), linoleic acid (peak F), oleic acid (peak G), stearic acid (peak H) and arachidic acid (peak L), respectively (Table 1). Alcohols **20** and **21** were both novel, and their fatty acid esters were also new as natural products.

Many natural products such as serricornin,[18] stegobinone[19] and the sap beetle pheromones[20] are conceived to be biosynthesized from polyketide precursors mainly composed of propionate units, instead of acetates. Lardolure [(1*R*,3*R*,5*R*,7*R*)-1,3,5,7-tetramethyldecyl formate],[4] the aggregation pheromone of the acarid mite, *Lardoglyphus konoi*, is also conceivably derived from propionate polyketide and is the first example found as a mite product. The structures of **20** and **21** imply that these alcohols are built from propionate units, and they are the second and the third examples among astigmatid mites. Based on these biogenetic considerations, and the hypothesis that a C_9-alcohol is also a product from the three propionate polyketides by the same biogenetic mechanisms as those for **20** and **21**, it seems reasonable to postulate that the structure of the C_9-alcohol is (2*S*,4*S*)-2,4-dimethylheptanol. The structures of peaks I and K were then elucidated as those of an ester with linoleic acid (peak I) and with stearic acid (peak K), respectively.

Astigmatid mites can be categorized into the following four groups by the opisthonotal gland components putatively functioning to protect their bodies from desiccation: 1) mites covered only with *n*-hydrocarbons (C_{13}–C_{19}, satd. and unsatd.), 2) mites with both hydrocarbons and fatty acid esters, 3) mites possessing only fatty acid esters other than semiochemicals, and 4) mites possessing none of the peculiar waxy compounds or detectable esters. The present species is the first example of group 3, and no hydrocarbon could be detected in the gland, while *n*-hydrocarbons (C_{13}–C_{19}, satd. and unsatd.) are common components among a wide variety of Astigmata,[21] this species belonging to group 1. Two fatty acid esters, hexyl linolate[22] and neryl myristate,[23] have so far been identified from the *Tyrophagus* species and *Aleuroglyphus ovatus* together respective with hydrocarbons, and these species belong to group 2.

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