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Cuticular lipids of orthopterous insects analyzed with GC-MS method

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Abstracts

The cuticular lipids of 8 orthopterous insects were analyzed by GC-MS method, and 13 esters were identified in addition to hydrocarbons and fatty acids. These esters can be useful for distinction of the characteristic, for example a classification of orthopterous insects.

Introduction

The cuticular lipids of insects are produced in the dermis cell and secreted. The functions of the cuticular lipids of insects are prevention of water evaporation and prevention of microbe infection. The function developed further, the lipid of the termite resembles closely that of the rove beetle which has a symbiotic relation (Howard *et al*., 1980). The compositions of cuticular hydrocarbons of Japanese ant from different colonies were the same, but the relative proportions of some compounds were colony-specific (Liu, *et al*., 1998).

Alkanes have been reported in the cuticular lipids of cockroaches (Jackson, 1970, Jackson, 1972), big stoneflies (Armold *et al*., 1969), and common house crickets (Hutchins and Martin, 1968). Since hydrocarbons from *n*-decane to *n*-pentatriacontane (HC10 to HC35) are easily analyzed with gas chromatography-mass spectrometry (GC-MS) method, the studied lipid compounds are hydrocarbon.

In orthopterous insects, the principal alkanes in both the cuticular lipids of grasshoppers *Melanoplus sanguinipes* and *M. packardii* are *n*-nonacosane (HC29) and *n*-heptacosane (HC27) with a range from heneicosane to tritriacontane (HC21 to HC33) (Soliday *et al*., 1974). The alkanes in the cuticular lipids of grasshopper *Anabrus simplex* are hydrocarbons range from *n*-octacosane to *n*-nonatriacontane (HC28 to HC39) (Jackson and Blomquist, 1976). Anderssohn and Hadley (1987) identified the alkenes in the cuticular lipids of cricket *Acheta domesticus*.

We studied whether any other compounds, in addition to hydrocarbon, would be detected from the cuticular lipids. The lipids of eight orthopterous insects were analyzed with GC-MS method.

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Analytical methods and Results

Collection of insects

Patanga japonica BOLIVAR (**A**), *Stethophyma magister* REHN (**B**), and *Parapodisma mikado* BOLIVAR (**C**) were collected in Himi-shi, Toyama prefecture (Aug. 20 ~ Sep. 3, 1993). *Atractomorpha lata* LINNE (**D**) and *Teleogryllus emma* OHMACHI et MATSUURA (**E**) were collected in Hitachi-oomiya-shi (old Ogawa-mura), Ibaraki prefecture (Sep. 11, 1993). *Gastrimargus marmoratus* HAAN (**F**) and *Locusta migratoria* LINNE (**G**) were collected in Daigo-machi, Ibaraki prefecture (Aug. 21, 1997). *Oxya yezoensis* SHIRAKI (**H**) was collected in Mito-shi, Ibaraki prefecture (Aug. 21, 1997).

The sampling process of the cuticular lipids

The bodies of **A~E** were extracted in *n*-hexane for 1 week at room temperature. The extracted bodies were further extracted in *n*-hexane for 5 months. The bodies of **F~H** were extracted in *n*-hexane for 45 min at room temperature. The extracted bodies were further extracted in *n*-hexane for 1 week. Evaporation of the solvent by the rotary evaporator left sticky colorless extracts. The amount of these extracts entered to Table 1 (weight per 1 body, mg).

Analysis of GC-MS and a mass spectrum

Gas chromatography-mass spectrometry (GC-MS) was performed using an OV-17 capillary column : 0.37mm i.d.×15m column, oven temperature 100~250 °C (16 °C/min), injector temperature 250°C; carrier gas He 40ml/min.

Mass spectrum and retention time of n-alkane

The following alkanes were identified from fragment ions (m/e 57, 71, 85, 99, 113, 127, 141, 155) and clear parent ion (M^+ 366 (HC26), 380 (HC27)). Alkanes: *n*-pentacosane (HC25) (Rt: 10’45”), *n*-hexacosane (HC26) (Rt: 11’43”), *n*-heptacosane (HC27) (Rt: 12’57”), *n*-octacosane (HC28) (Rt: 14’34”), *n*-nonacosane (HC29) (Rt: 16’44”), *n*-triacontane (HC30) (Rt: 19’15”), *n*-hentriacontane (HC31) (Rt: 22’43”).

Mass spectrum and retention time of methyl-alkane

The following methyl-alkanes were identified from fragment ions of alkane (m/e 57, 71, 85, 99, 113, 127, 141, and 155), the fragment ions of a branch portion, the weak molecule ion, and the retention time in mass-chromatogram. Methyl-alkanes: 3-methylheptacosane (3C-HC27) (Rt: 13’58”), 3-methylnonacosane (3C-HC29) (Rt: 18’28”), 3-methyltriacontane (3C-HC30) (Rt: 20’42”), 13,14-dimethyltriacontane (13C14C-HC30) (Rt: 23’36”), 3-methylhentriacontane (3C-HC31) (Rt: 25’13”).

Mass spectrum and retention time of alkene

The following alkanes were identified from the retention time in mass-chromatogram and the molecular ion. Alkenes: pentadecene ($HC15_{-1}$) (Rt: 5’03”), heptadecadiene ($HC17_{-2}$) (Rt: 5’33”), heptadecatriene ($HC17_{-3}$) (Rt: 5’39”), octadecene ($HC19_{-1}$) (Rt: 6’56”), heneicosadiene ($HC21_{-2}$) (Rt: 8’13”).

Mass spectrum and retention time of carboxylic acids

The following carboxylic acids were identified from the fragment ion (m/e 57 (C_4H_9), 60 (CH_3COOH), 73 (C_2H_4COOH), 129 ($C_6H_{12}COOH$), 185 ($C_{10}H_{20}COOH$), M^+-43 (-C_3H_7), M^+) which shows the feature of the carboxylic acid. Saturated carboxylic acids: hexadecanoic acid: palmitic acid

(CA16) (Rt: 8’00”), octadecanoic acid: stearic acid (CA18) (Rt: 9’14”), eicosanoic acid: arachidic acid (CA20) (Rt: 10’34”). Unsaturated carboxylic acids: octadecadienoic acid: linoleic acid ($CA18_{-2}$) (Rt: 9’ 19”), octadecatrienoic acid: linolenic acid ($CA18_{-3}$) (Rt: 9’36”).

Mass spectrum and retention time of esters.

The following methyl esters were identified from the fragment ion which shows the feature of methyl ester (m/e 74 (CH_3O-CO-CH_2-H), 87 (CH_3O-CO-CH_2-CH_2), 143 (CH_3O-CO-C_6H_{12}), M^+-31(-OCH_3), M^+). The following ethyl esters were identified from the characteristic fragment ion of ethyl ester (m/e 88 (C_2H_5O-CO-CH_2-H), 101(C_2H_5O-CO-CH_2CH_2), 157 (C_2H_5O-CO-C_6H_{12}), M^+-45 (-O C_2H_5), M^+). The following butyl esters were identified from the characteristic fragment ion of butyl ester (m/e 57 (C_4H_9), 129 (C_4H_9O-CO-CH_2CH_2), 185 (C_4H_9O-CO-C_6H_{12}), M^+-75 (-OC_4H_9), M^+-55 (- C_4H_9), M^+). The following pentyl esters were identified from the characteristic fragment ion of pentyl ester (m/e 70 (C_5H_{10}), 129 (C_4H_9O-CO-CH_2-CH_2), 185 (C_4H_9O-CO-C_6H_{12}), M^+-87(-OC_5H_{11}), M^+-70 (- C_5H_{10}), M^+). Moreover, The synthesis esters from various alcohols and higher fatty acids were measured with GC-MS, and the structures of ester were examined by the mass spectra and retention times.

Esters: methyl hexadecanolate (Es16-O-1) (Rt: 7’40”), ethyl hexadecanolate (Es16-O-2) (Rt: 8’04”), butyl hexadecanolate (Es16-O-4) (Rt: 8’54”), pentyl hexadecanolate (Es16-O-5) (Rt: 9’32”), methyl octadecanolate (Es18-O-1) (Rt: 8’54”), ethyl octadecanolate (Es18-O-2) (Rt: 9’ 16”), butyl octadecanolate (Es18-O-4) (Rt: 10’08”), pentyl octadecanolate (Es18-O-5) (Rt: 11’01”), ethyl octadecenoate: ethyl oleate ($Es18_{-1}$-O-2) (Rt: 9’17”), methyl octadecadienoate: methyl linolate ($Es18_{-2}$-O-1) (Rt: 8’58”), ethyl octadecadienoate : ethyl linolate ($Es18_{-2}$-O-2) (Rt: 9’20”), methyl octadecatrienoate: methyl linolenate ($Es18_{-3}$-O-1) (Rt: 9’08”), ethyl octadecatrienoate: ethyl linolenate ($Es18_{-3}$-O-2) (Rt: 9’28”)

The amounts of the relative compounds were calculated in the area of each narrow peak of chromatogram, and the quantity of each compound was expressed as percentage in Table 1. The horizontal axis of Table 1 shows retention time of compounds, and the vertical axis shows biologically classification.

Discussion

The carboxylic acids CA16 and CA18 were identified in all lipid samples. Unsaturated carboxylic acid CA 18_{-2} and CA 18_{-3} were identified in many samples (**B, C, F, G,** and **H**). Therefore, it is difficult to distinguish the lipid samples based on carboxylic acid.

The hydrocarbons, HC 27 and HC29, were identified in many samples (**A, B, C, D, F, G,** and **H**). Methyl-hydrocarbon 3-C-HC27 and 3-C-HC29 were identified in **D** and **B**. Unsaturated hydrocarbons, $HC15_{-1}$, $HC17_{-2}$, $HC17_{-3}$, $HC19_{-1}$ and $HC21_{-2}$, were identified only in the cricket **E**. These unsaturated hydrocarbons are one of the characteristic compounds of cricket **E**.

Thirteen esters were identified in the lipid samples. They are methyl, ethyl, butyl and pentyl esters of carboxylic acid C16, methyl, ethyl, butyl and pentyl esters of carboxylic acid C18, ethyl ester of unsaturated carboxylic acid $C18_{-1}$, methyl and ethyl esters of $C18_{-2}$, and methyl and ethyl esters of $C18_{-3}$.

Pentyl and butyl esters were identified only in **D**, and these esters are the characteristic compounds of **D**. The ester of **B** was simple structure with two ethyl esters of carboxylic acid C16 and C18. As for the ester of **C**, there are the same ethyl esters identified in **B**, and additional of 3 different esters with unsaturated carboxylic acids.

F and **G** in the Oedipodinae had common hydrocarbon and carboxylic acid, and had common methyl esters of C16 and C18. **F** and **G** belong to same sub-family, Oedipodinae, and the composition of their cuticular lipids resembles each other.

Catantopidae **H** and **A** have almost the same hydrocarbons and carboxylic acids, however they have different esters. Although **H** had methyl esters of C16 and C18-1, **A** had ethyl esters of C18-1, C18-2, and C18-3. It is the difference between methyl ester and ethyl ester within the same family; Catantopidae. The esters of **E** cricket resembled **H** locust.

These esters have a possibility of becoming an index showing distinction of the characteristic like a classification of orthopterous insects.

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Table 1. Composition of the cuticular lipids compounds of orthopterous insects (percentage by the area of each peak of chromatogram in each fraction).

Classification		Orthoptera バッタ目															
		Caelifera バッタ亜目														Ensifera キリギリス亜目	
		Pygomorphidae オンブバッタ科		Acrididae バッタ科								Catantopidae イナゴ科				Gryllidae コオロギ科	
				Acridinae ショウリョウバッタ亜科		Melanoplinae フキバッタ亜科		Oedipodinae トノサマバッタ亜科				Oxyinae イナゴ亜科		Cyrtacanthacridina ツチイナゴ亜科			
Retention Time,Compound		**D.**.*Atractomorpha lata* オンブバッタ		**B.**.*Stethophyma magister* ツマグロバッタ		**C.**.*Parapodisma mikado* ミカドフキバッタ		**F.**.*Gastrimargus marmoratus* クルマバッタ		**G.**.*Locusta migratoria* トノサマバッタ		**H.**.*Oxya yezoensis* コバネイナゴ		**A.**.*Patanga japonica* ツチイナゴ		**E.**.*Teleogryllus emma* エンマコウロギ	
Extract time by hexane		1week	5man	1week	5man	1week	5mon	45min	1week	45min	1week	45min	1week	1week	5mon	1week	5mon
Weight per 1 body		1.2mg	1.7mg	2.6mg	2.5mg	7.3mg	8.6mg	1.3mg	9.2mg	1.4mg	8.0mg	0.6mg	3.2mg	8.0mg	14 mg	3.9mg	5.2mg
4’03”	HC15$_{-1}$															3.2	
5’33”	HC17$_{-2}$															5.1	
5’39”	HC17$_{-3}$															3.7	
6’56”	HC19$_{-1}$															12.4	
7’16”	CA15																
7’40”	Es16-O-1								1.2		1.0		10.2			12.4	
8’00”	CA16		39.1	5.9	32.5		24.2	4.3	13.3		9.3		16.2	3.5	22.1		43.5
8’04”	Es16-O-2	2.9		7.0		7.7								7.5		5.7	
8’13”	HC21$_{-2}$															5.3	
8’34”	CA17		3.5		5.2		4.4						1.7		2.0		2.9
8’54”	Es18-O-1								2.9		2.0		13.6			12.9	
8’54”	Es16-O-4	3.4															
8’58”	Es18$_{2}$-O-1												2.6			6.0	
9’08”	Es18$_{3}$-O-1												7.6			2.8	
9’14”	CA18		59.4	10.0	52.7		59.8	2.1	20.3		23.1		19.1		64.3		46.2
9’16”	Es18-O-2	2.4		10.2		30.3	2.5							31.7	2.0	4.8	
9’17”	Es18$_{1}$-O-2					5.8											

9’19”	$CA18_2$				3.0		4.9		20.3		20.6		4.6				
9’20”	$Es18_2$-O-2					10.3								12.9			
9’27”	$CA18_3$				2.7		2.3		42.3		45.8						
9’28”	$Es18_3$-O-2					6.5								6.9			
9’32”	Es16-O-5	13.6															
9’36”	CA19																
9’58”	HC24																
10’08”	Es18-O-4	3.6															
10’34”	CA20																2.6
10’45”	HC25	2.9		5.0		4.5								6.3			
11’01”	Es18-O-5	8.5															
11’43”	HC26			2.0													
12’57”	HC27	4.1		4.3		4.5		22.0		24.0		27.0		2.7			
13’58”	3-C-HC27	3.9		2.0													
14’34”	HC28			3.2				5.0		7.8		12.7					
16’44”	HC29	8.4		11.5		3.9		34.8		39.0		60.4		12.5			
18’28”	3-C-HC29	2.2		12.1													
19’15”	HC30			2.2				17.8		21.6							
20’42”	3-C-HC30															3.7	
22’43”	HC31	9.2				2.6		14.2		7.8				2.2			
23’36”	13C14C-HC30			6.7													
25’13”	3-C-HC31	2.4															

Alkanes: HC25 pentacosane, HC26 hexacosane, HC27 heptacosane, HC28 octacosane, HC29 nonacosane, HC30 triacontane, HC31 hentriacontane.
Methyl-alkanes: 3-C-HC27 3-methylheptacosane, 3-C-HC29 3-methylnonacosane, 3-C-HC30 3-methyltriacontane, 13C14C-HC30 13,14-dimethyltriacontane, 3-C-HC31 3-methylhentriacontane.

Alkenes: $HC15_{-1}$ pentadecene, $HC17_{-2}$ heptadecadiene, $HC17_{-3}$ heptadecatriene, $HC19_{-1}$ octadecene, $HC21_{-2}$ heneicosadiene.

Saturated carboxylic acids: CA16 hexadecanoic acid: palmitic acid, CA18 octadecanoic acid: stearic acid, CA20 eicosanoic acid: arachidic acid.
Unsaturated carboxylic acids: $CA18_{-2}$ octadecadienoic acid: linoleic acid, $CA18_{-3}$ octadecatrienoic acid: linolenic acid.

Methyl esters: Es16-O-1 methyl hexadecanolate, Es18-O-1 methyl octadecanolate, $Es18_{-1}$-O-1 methyl octadecenoate: methyl oleate, $Es18_{-2}$-O-1 methyl octadecadienoate: methyl linolate, $Es18_{-3}$-O-1 methyl octadecatrienoate: methyl linolenate.

Ethyl esters: Es16-O-2 ethyl hexadecanolate, Es18-O-2 ethyl octadecanolate, $Es18_{-2}$-O-2 ethyl octadecadienoate: ethyl linolate, $Es18_{-3}$-O-2 ethyl octadecatrienoate: ethyl linolenate.

Butyl esters: Es16-O-4 butyl hexadecanolate, Es18-O-4 butyl octadecanolate.