い生理的特性をもつものと推察した.

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Résumé

L'auteur étudie la sensibilité comparée pour les pyréthrins, l'allethrin et le phthalthrin chez les mouches domestiques des colonies TaTara, Takatsuki, 213-ab et Lab-em-7-em à l'aide de la méthode de l'appareil du Type Boite 0.5 m³.

Les résultats montrent que pour tous les insecticides la vitesse du pouvoir Knock-down est la plus lente chez les mouches des colonies 213-ab, et qu'au point de vue de la mortalité la valeur est plus basse chez les mouches des colonies 213-ab et Lab-em-7-em que chez celles TaTara et Takatsuki. Ainsi, c'est un problème intéressant que les mouches des colonies européennes ont la résistibilité plus forte contre les insecticides que celles japonaises.

Fatty Acids, Alcohols and Hydrocarbons in the Body Lipid of *Ceroplastes pseudoceriferus* Green, *Ceroplastes japonicus* Green, and *Ceroplastes rubens* Maskell (Homoptera: Coccidae)

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8. ツノロウムシ，カメノコロウムシおよびルビーロウムシの体脂肪中の脂肪酸，アルコールおよび炭化水素の構成

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Ceroplastes 属のカイガラムシのワックス分泌と関連づけて，体脂肪中の脂肪酸，アルコールおよび炭化水素の構成をしらべた.

本邦産の *Ceroplastes* 属3種の体脂肪含量は生鮮重当り10.4から26.6%を示し，中性脂肪，遊離脂肪酸および燐脂質の含量はそれぞれ体脂肪の80～95，0.8～1.9，および2.7～18.8%を示した．中性脂肪中の脂肪酸は10種類（C_8～C_{20}）からなり，その主体は C_{10} と C_{12} (75～78%) であるが，遊離型脂肪酸は14種類（C_8～C_{20}）からなり，その主体は C_{18}，$C_{18:1}$ および $C_{18:2}$ (66～79%) であった．直鎖アルコールは C_{22} から C_{30} のもの5種が認められ，その主体は C_{26} または C_{28} であった．炭化水素は C_{15} から C_{47} にいたるもの約30種が認められ，その主要成分は C_{41}，C_{43} および C_{45} (59～69%) であった.

以上の結果にもとづいて，カイガラムシの体内脂肪酸の特徴，分泌ワックスの生合成の場，および *Ceroplastes* 属3種の分類学的相互関係について論議した.

Introduction

The insect body of *Ceroplastes pseudoceriferus* Green is covered with waxy secretion consisting of honeydew and wax material (Tamaki 1963). In a series of studies, compositions of amino acids and carbohydrates in the honeydew have been reported from a nutritional viewpoint of the scale insect (Tamaki, 1964a, b). The chemical composition of wax material in the waxy covering has also been published (Tamaki 1966).

Since principal consitituents of the wax material secreted by the insect are esters of higher fatty acids (C_{28}, C_{30} and C_{32}), it is of interest to elucidate the lipid composition of the insect body, which is considered to be a possible site of synthesis of these higher fatty acids. This paper deals with the fatty acid, alochol and hydrocarbon compositions in three species of adult scale insect, *Ceroplastes pseudoceriferus*, *Ceroplastes japonicus*, and *Ceroplastes rubens*.

Materials and Methods

Insects

The adult females of *C. pseudoceriferus* were collected from twigs of infested tea plants (*Thea*

sinensis L.), and *C. japonicus* and *C. rubens* were collected from twigs of *Ilex integra* Thunb., from October to November. All the insects were stored at−10°C until analyzed.

Extraction of total body lipid

The insects were shaken lightly with chloroform in an Erlenmeyer flask to remove the waxy covering from the bodies. The insect bodies were then washed successively with small portions of chloroform, water and acetone. Extraction of lipid from the insect bodies was accomplished according to Folch *et al.* (1957). The insect bodies were homogenized in an ice cold homogenizer with chloroform-methanol (2 : 1, v/v), and the debris removed by filtration. Water soluble impurities in the chloroform-methanol extract were washed off with approximate one-fifth volume of 0.2 M $MgCl_2$.

Fractionation of lipid

Total body lipid was dissolved in chloroform and poured onto 6~15 g of silicic acid (Kanto-Kagaku Co., Ltd., Tokyo) packed in a glass column, 12 mm diameter. Free fatty acids and neutral lipid were eluted with chloroform (150~300 m*l*) and then phospholipid with methanol (300~600 m*l*).

The chloroform eluent was concentrated *in vacuo*, and the resultant residue was dissolved in diethyl ether and shaked with 50% aqueous ethanol containing 2.5% KOH. The alkaline aqueous layer was acidified with sulfuric acid and the free acids extracted with diethyl ether. After the solvent was removed by evaporation with a stream of nitrogen, the free fatty acid fraction was analyzed by gas-liquid chromatography.

The ether layer was concentrated under a stream of nitrogen and the concentrate was dissolved in ethanol containing 5% KOH for saponification. After refluxing for 30 minutes at about 80°C, the reaction mixture was diluted with water and acidified with sulfuric acid. Then, both the saponifiable and unsaponifiable fractions were extracted with diethyl ether. Separation of saponified fatty acids from unsaponifiable matter was accomplished by shaking with ethanolic KOH as described above. The saponifiable matter (esterified fatty acids) was analyzed by gas-liquid chromatography.

The unsaponifiable matter dissoved in *n*-hexane was poured onto silicid acid column and eluted stepwise with (1) *n*-hexane, (2) *n*-hexane-diethyl ether (90 : 10), (3) *n*-hexane-diethyl ether (50 : 50), and (4) chloroform. It was previously assertained that hydrocarbons and alcohols were eluted with solvent (1) and (3) respectively.

The hydrocarbons and alcohols were treated with urea to separate straight chain compounds from cyclic and/or branched chain compounds. About ten volumes of urea were added to the hydrocarbons and alcohols dissolved in benzene-methanol (1 : 2), respectively. The mixture was refluxed for 15 minutes, cooled to room temperature with shaking, and then stored in a refrigerator overnight. The isolated urea adduct was treated with hot water to liberate the straight chain compounds.

Thin layer chromatography

Silica gel (Wakogel B-10, Wakō Pure Chemical Ind.) coated on glass plates (20 × 20 cm) was activated at 120~130°C for 1 hour. After development with a mixture of *n*-hexane-diethyl ether-glacial acetic acid (70 : 30 : 1), the plates were sprayed with 80% (w/v) H_2SO_4 saturated with $K_2Cr_2O_7$ and charred for 25 minutes in an oven at 180°C (Privett & Blank 1962), or exposed to iodine vapor.

Gas-liquid chromatography

A Shimazu GC-2C gas chromatograph equipped with a hydrogen flame ionization detector was used for analyses. The column was 2.25 meter U-shaped stainless steel tube packed with 10% diethyleneglycol adipate polyester (DEGA) coated on 60~80 mesh Neosorb NC (Nishio Ind., Tokyo), and an 0.75 meter U-shaped tube packed with 5% silicone SE-30 on 60~80 mesh Neosorb NC. The DEGA-column was held at 180°C and the SE-30 column at 225° or 255°C. The inlet pressure of helium, a carrier gas, was 0.8~1.0 kg/cm^2. The flow rates of air and hydrogen were 800 m*l*/min and 30~40 m*l*/min, respectively.

All the fatty acids were analyzed as their methyl esters which were prepared by treatment with diazomethane in diethyl ether-methanol (9 : 1) (Schlenk & Gellerman 1960). The straight chain alcohols were analyzed as their acetate

which were prepared with acetic anhydride-pyridine (Shriner *et al.* 1956). Identification of the fatty acid methyl esters, alcohol acetates and hydrocarbons was achieved by comparing their retention times with those of standard or by plotting the logarithm of the retention times versus carbon chain length.

Results

Fractionation of body lipid

The total body lipid contents in the female adults of *C. pseudoceriferus*, *C. japonicus*, and *C. rubens* were 26.6, 12.8 and 10.4%, respectively, on a wet weight basis. A large part of the total lipid, from 80 to 95%, was neutral lipid. Major component of the neutral lipid was found to be triglyceride, and small amounts of hydrocarbon, free sterol, diglyceride and monoglyceride were also detected by thin layer chromatography.

Fatty acids, particularly esterified acids, are the principal components of the total lipid ; i.e., saponifiable matter contributes 83.2, 72.2, and 60.7% of the total body lipid of *C. pseudoceriferus*, *C. japonicus* and *C. rubens*, respectively. Small amount of free fatty acids (0.8—1.9%) was also separated from the body lipid.

Unsaponifiable matter of the neutral lipid, around 2% of the body lipid of the scale insects, consists largely of hydrocarbons and alcohols. For example, hydrocarbons and alcohols of *C. pseudoceriferus* constituted 0.5% and 0.9% of the total body lipid, respectively. The results of urea treatment showed that straight chain compounds constitute 94.4% of the hydrocarbons and 41.3% of the alcohols.

The amount of methanol-eluted material, con-

Table 1. Quantity of body lipid fractions.

Fraction	Per cent in total body lipid		
	C. pseudoceriferus	*C. japonicus*	*C. rubens*
Neutral lipid	95.4%	89.6%	79.9%
Saponifiable matter	83.2	72.2	60.7
Unsaponifiable matter	1.6	2.1	1.7
Hydrocarbons	0.5(0.47)*	1.0	0.5
Alcohols	0.9(0.37)*	0.6	0.6
Free acid	1.9	0.8	1.3
Phospholipid	2.7	9.6	18.8

* The values in parenthesis are of straight chain compounds.

Table 2. Fatty acid composition.

Fatty acid	*C. pseudoceriferus*		*C. japonicus*		*C. rubens*	
	Free acid	Esterified acid	Free acid	Esterified acid	Free acid	Esterified acid
C_8	0.7%	4.0%	—%	1.3%	—%	3.0%
C_{10}	13.1	49.6	9.5	53.1	17.4	48.2
$C_{10:1}$	+	—	—	—	—	—
C_{12}	7.0	27.8	5.9	24.4	10.9	26.9
$C_{12:1}$	0.4	—	—	—	—	—
C_{14}	2.5	9.7	1.7	6.3	3.0	3.0
$C_{14:1}$	0.1	—	—	—	—	—
C_{16}	1.6	1.4	3.5	0.9	2.5	1.3
$C_{16:1}$	0.7	+	—	+	—	+
C_{18}	14.7	1.4	12.5	3.7	13.3	2.4
$C_{18:1}$	17.7	4.2	19.9	6.4	15.4	8.9
$C_{18:2}$	35.9	1.7	46.9	4.1	37.6	6.5
$C_{18:3}$	2.0	—	—	—	—	—
C_{20}	3.6	+	—	+	—	+

+ : trace, — : undetectable amount

sisting mostly of phospholipids, varied remarkably with species (2.7% in *C. pseudoceriferus*, 9.6% in *C. japonicus* and 18.8% in *C. rubens*).

These data are summarized in Table 1.

Fatty acids

Free fatty acids and esterified fatty acids were analyzed by gas-liquid chromatography (Table 2). The free fatty acid fraction of *C. pseudoceriferus* contained 14 fatty acids ranging from C_8 to C_{20}. Gas-liquid chromatographic patterns of free fatty acid fractions of *C. japonicus* and *C. rubens* resemble to *C. pseudoceriferus*. The major components were fatty acids of C_{18}-series. Stearic, octadecenoic, and octadecadienoic acids constituted about 68% of the free fatty acid fraction in *C. pseudoceriferus*, 79% in *C. japonicus*, and 66% in *C. rubens*. Capric acid was also found to be dominant in the free fatty acid fraction of these three species.

In the esterified fatty acid fractions, 10 fatty acids ranging from C_8 to C_{20} were detected in the three species. The patterns of the esterified acids in the three species closely resembled each other, but were quite different quantitatively from those of the free acids. Of 10 acids, capric and lauric acids were dominant and sum of the two acids accounted for over 75% in the esterified fatty acid fraction of the three species.

As 98~99% of the total fatty acids (free and esterified) consist of esterified acids, chemical nature of the esterified acids would strongly characterize the total fatty acids in the lipid of the three species. For instance, about 77% of the esterified fatty acids of *C. pseudoceriferus* consists of capric and lauric acids, which contribute over 75% of the total fatty acids in the body lipid of this insect. On the other hand, three acids of the 18-carbon series contributed about 70% of the free acid fraction, but these are only 8.7% of the total fatty acid.

Hydrocarbons

Urea treatment applied to the hydrocarbon fraction of *C. pseudoceriferus* revealed that urea non-adducts, cyclic and/or branched chain hydrocarbons, accounted for only 5.6% of this fraction. Gas chromatogram of this urea non-adduct showed only one peak, which was not identified but had a retention time corresponding to that of *n*-tricosane under the conditions of DEGA-column at 180°C.

According to gas chromatographic analyses, straight chain hydrocarbons of the three species are complex mixtures of 29~31 hydrocarbons of odd and even numbered carbon chain ranging from C_{15} to C_{47}. Of these, hydrocarbons of odd numbered carbon chain was predominant (Table 3). The major components of these hydrocarbon fractions are of C_{41}, C_{43} and C_{45}. Total of these three hydrocarbons accounted for 59.1, 66.9 and 69.0% of the straight chain hydrocarbons of *C. pseudoceriferus*, *C. japonicus* and *C. rubens*, respectively. Amount of C_{29}-hydrocarbon was also relatively large in *C. pseudoceriferus* and *C. japonicus*, but small in *C. rubens*. The high molecular hydrocarbons ranging from C_{33} to C_{47} of *C. pseudoceriferus* seemed to be saturated homologues, because the amount of these hydrocarbons was not changed after refluxing with $KMnO_4$ in acetone for one hour.

Alcohols

Alcohol fraction of *C. pseudoceriferus* was treated with urea. The urea non-adduct, accounting for 58.7% of the alcohol fraction and consisting possibly of sterols as major component, was not submitted for further analysis. Straight chain alcohols ranging from C_{22} to C_{30} were detected gas-chromatographically as shown in Table 4. Any homologue lower than C_{22} was not detected. The major components of the *n*-alcohols in *C. pseudoceriferus*, *C. japonicus*, and *C. rubens* were of C_{26}, C_{26}, and C_{28}, respectively.

Discussion

Recently Fast (1964) tabulated the lipid content of various insects in his review on insect lipids. There were data on Hemipterous insects showing a lipid content range of from 6.6 to 36.3% on a dry weight basis and from 5.2 to 20.0% on a wet weight basis. The body lipid content of *Ceroplastes pseudoceriferus* was 54.4% on a dry weight basis and 26.6% on a wet weight basis. This value is not only the highest so far obtained for Hemiptera, but also far larger than the mean lipid content of all adult insects, about 20% of dry weight (Fast 1964). Further, as about two-sevenths of the waxy covering of *C. pseudo-*

Table 3. Hydrocarbon composition

Hydrocarbon	*C. pseudoceriferus*	*C. japonicus*	*C. rubens*
C_{15}	+%	−%	−%
C_{16}	−	−	−
C_{17}	1.2	0.2	−
C_{18}	+	+	+
C_{19}	0.7	0.3	0.1
C_{20}	+	+	+
C_{21}	+	+	+
C_{22}	+	+	+
C_{23}	0.4	0.8	0.1
C_{24}	+	0.4	+
C_{25}	0.7	0.3	0.1
C_{26}	+	+	+
C_{27}	5.4	0.8	0.1
C_{28}	0.4	0.2	+
C_{29}	17.1	15.6	2.5
C_{30}	+	+	+
C_{31}	8.0	4.5	2.9
C_{32}	+	+	+
C_{33}	1.5	0.2	0.4
C_{34}	+	0.2	+
C_{35}	0.1	0.1	1.5
C_{36}	−	0.3	+
C_{37}	+	−	1.0
C_{38}	−	0.4	+
C_{39}	1.6	0.6	9.2
C_{40}	0.2	0.5	0.9
C_{41}	14.5	17.3	23.6
C_{42}	1.1	0.9	1.2
C_{43}	29.8	30.4	29.0
C_{44}	0.7	0.7	2.0
C_{45}	14.8	19.2	16.4
C_{46}	−	+	+
C_{47}	2.1	6.0	8.9

+ : trace, − : undetectable amount

Table 4. *n*-Alcohol composition.

n-Alcohol	*C. pseudoceriferus*	*C. japonicus*	*C. rubens*
C_{22}	+%	+%	+%
C_{24}	2.2	+	+
C_{26}	90.2	64.9	26.7
C_{28}	7.6	35.1	73.3
C_{30}	+	+	−

+ : trace, − : undetectable amount

ceriferus consist of wax (Tamaki 1963), the total lipid content of the whole scale insect accounts for 74% of its dry weight. *C. japonicus* and *C. rubens* contained lower level of lipid than *C. pseudoceriferus*, but classified to a group of high lipid content among Hemipterous insects.

Among the body lipids of the three scale insects, fatty acids are the most important component, and their contents exceed 60% of the total body lipid. Recently, some workers reported the fatty acid composition of insects using gas-liquid chromatography. Strong (1963), working with 21 species of aphids, found a high proportion of myristic acid as compared with the 18-carbon fatty acids. In *Macrosiphum barri* Essig., 56% of the free acids consisted of oleic and linoleic acid, but 78% of the fatty acids derived from triglycerides were lauric and myristic acid. On the other hand, the fatty acids of the boll weevil, *Anthonomus grandis* Boheman, was a complex mixture of 23 fatty acids and their principal components were palmitic, oleic, linoleic and linolenic acids. These four acids constituted about 86% of the total fatty acids. (Lambremont and Blum 1963). Major fatty acids in the body lipids of the Virginia pine sawfly, *Neodiprion pratti* Dyar, and the silkworm, *Bombyx mori* L., were also of the C_{16} and C_{18} series (Schaefer 1965, Sugiyama 1963, and Sridhara & Bhat. 1965). Barlow (1964) analyzed the fats of 30 species of insects and showed that the dominant fatty acids are generally of the C_{16} and C_{18} series, with the exception of aphids, in which the main fatty acid is myristic acid.

From the present study the fatty acid composition of the body lipid of *Ceroplastes* scale insects is similar to that of aphids; both the scale insects and aphids show high content of fatty acids from C_{10} to C_{14} as contrasted with C_{16} and C_{18} acids in other insects. The principal fatty acids in the body of *Ceroplastes* species are capric and lauric acids, instead of myristic acid in aphids.

A homologous series higher than arachidic acid could not be detected in the body lipid of *Ceroplastes* species. Since the principal fatty acid in the wax of their waxy covering is a 30-carbon fatty acid (Kono 1938, Tamaki 1966), it is reasonable to assume that synthesis of the 30-carbon fatty acid and subsequent synthesis of the wax in the waxy covering take place at a tissue, probably wax gland or related tissue of epidermal origin, closely connected with the exterior. Then the synthesized wax would be secreted on the dorsal surface of the scale insect.

Hydrocarbons and alcohols accounting for 1.1 ~1.6% of the body lipid of *Ceroplastes* species are considered not to be contaminants from external wax. Because, if contamination from external wax occurred, small amount of C_{30} acid, main component of the external wax (Tamaki 1966), would be detected in the fatty acid fraction of body lipid.

Though occurrance of hydrocarbons and *n*-alcohols in cuticular wax and waxy secretion has been reported on various insects (for instances, Chibnall *et al.* 1934, Kono 1938, Shikata 1960, Gilby & Cox 1963, Bower & Thompson 1965, and Tamaki 1966), there is little work on hydrocarbns in body lipid of insects (Baker *et al.* 1963, Acree *et al.* 1965, Louloudes *et al.* 1962). Louloudes *et al.* (1962) analyzed hydrocarbons of whole insect body of housefly, *Musca domestica*, and showed *n*-alkanes ranging from C_{16} to C_{35} and *n*-alkenes ranging from C_{20} to C_{33}. Of these, the major components are *n*-alkanes of C_{23}, C_{25} and C_{27} and *n*-alkenes of C_{27}, and C_{29}. In the cuticular wax of the housefly, on the other hand, the amount of shorter chain homologues (C_{17}~C_{21}) was relatively large.

Physiological role of the long chain hydrocarbons of from C_{43} to C_{47} in the body lipid of *Ceroplastes* species, and relations, if any, between these compounds of long chain skeleton in the body and higher fatty acids or higher alcohols in the external wax are of interest, but remain obscure. Extremely long chain hydrocarbons, tetrapentacontane, pentapentacontane and heptapentacontane, were isolated from whole insect body of *Leptinotarsa decemlineata* Say (Ardenne *et al.* 1965). As these hydrocarbons could not be detected in leaves of potato, host plant of the beetle, they concluded that the long chain hydrocarbons are products of biosynthesis by the beetle.

There are few reports on occurrance of *n*-

alcohol in body lipid of insects. Though a physiological role of the *n*-alcohols (C_{22}~C_{30}) detected in the body lipids of the three scale insects remains obscure, it is of interest to note that hexacosanol, a major *n*-alcohol in the body lipid was also confirmed as a major alcohol in the wax of waxy secretion of *C. pseudoceriferus*. (Tamaki 1966).

It is interesting to discuss the taxonomic relationships among the three species of *Ceroplastes* from the view point of the lipid composition. Some indications in this direction could be obtained from the data in Table 1 (neutral lipid, esterified fatty acid and phospholipid) and Table 4 (*n*-alcohols), suggesting that *C. japonicus* takes intermediate position between *C. pseudoceriferus* and *C. rubens*.

Acknowledgement: The authors wish to express their sincere thanks to Dr. Shoziro Ishii of Kyoto University, Dr. Chisato Hirano of the Sericultural Experiment Station, Tokyo and Dr. Takeshi Yushima of the National Institute of Agricultural Sciences, Tokyo for their kind advices and encouragements during this work.

Summary

The body lipids in the female adults of three species of scale insects (*Ceroplastes pseudoceriferus*, *C. japonicus* and *C. rubens*) were analyzed with special interest to straight chain compounds. The total body lipid contents ranged from 10.4 to 26.6% on a wet weight basis. The body lipid consisted of 80~95% of neutral lipid, 0.8~1.9% of free fatty acid and 2.7~18.8% of phospholipid. Among 14 free fatty acids (C_8~C_{20}), major components were C_{18}, $C_{18:1}$, and $C_{18:2}$ (66~79%); but C_{10} and C_{12} were dominant (75~78%) among 10 esterified fatty acid (C_8~C_{20}). From unsaponifiable fraction (1.6~2.1% of body lipid), about 30 straight chain hydrocarbons (C_{15}~C_{47}) and *n*-alcohols (C_{22}~C_{30}) were detected. Major hydrocarbons were C_{41}, C_{43} and C_{45} (59~69%).

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