Fatty Acid Composition of the Rice Stem Borer *Chilo suppressalis* Walker. Yasumasa Kuwahawa and Shoziro Ishii (Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto) Received March 18, 1968. *Botyu-Kagaku*, 33, 42, 1968.

7. ニカメイガ幼虫および蛹の脂肪酸組成. 桑原保正・石井象二郎 (京都大学農学部農薬研究施設, 京都市左京区) 43. 3. 18 受理

ニカメイガ幼虫および蛹の油脂構成脂肪酸をガスクロマトグラフで分析した. その結果 lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic acids が検出された. 不飽和脂肪酸は全脂肪酸の約 85% を占め, 最も含量の多い脂肪酸は palmitoleic acid で全体の 54～57% を占めており, 他のりん翅目幼虫の脂肪酸組成と異なっていた. 無脂肪の人工飼料で無菌飼育した幼虫の脂肪酸組成は植物を寄主とした幼虫の脂肪酸組成と若干変わっていた.

Introduction

Unsaturated fatty acids have been found to be essential as a dietary source for the growth and development of some lepidopterous insects.[1~4] It was found, however, unsaturated fatty acids of C_{18} series have neither growth promoting activity for the growth of the rice stem borer larvae *Chilo suppressalis* Walker nor any improving effects on the pupation and the adult emergence as a dietary source[5].

It is of interest to know the composition of fatty acids in the rice stem borer lipid in comparison with that of other insects.

The present paper deals with results of gas chromatographic analysis of fatty acids of the rice stem borer larvae and pupae reared under various conditions.

Materials and Methods

The rice stem borer larvae:

The following two different sources of larvae were used for extraction of fat;

i) Hibernating larvae collected at Okayama, Shiga and Kagawa prefectures.
ii) Larvae reared aseptically on a synthetic food medium given in Table 1.

This medium was not suitable for the growth and development of larvae, but it was sufficient to obtain samples for the following lipid extraction.

Preparation of Samples:

Total lipids were extracted with chloroform-methanol (1:1) from homogenized insects. Procedures for the extraction of lipid and methylation of fatty acids are given in Fig. 1.

Table 1. Composition of a synthetic food medium for rearing the rice stem borer larvae.

Water	10m*l*	Vitamin B mixture	
Agar	0.1g	Thiamine HCl	10μg
Cellulose	0.3g	Riboflavine	5 〃
Glucose	0.5g	Ca-pantothenate	10 〃
Casein	0.5g	Nicotinic acid	10 〃
Cholesterol	0.006g	Pyridoxine HCl	5 〃
Wesson's salt mixture	0.05g	Folic acid	1 〃
		Biotin	1 〃
		Choline chloride	200 〃
		Inositol	100 〃
		p-Aminobenzoic acid	10 〃

Micro analysis of Fatty Acid Methyl Esters:

All analyses were performed by gas liquid chromatography. The instrument used was a linear programmed gas chromatograph equipped with a flame ionization detector (Hitachi-Perkin Elmer's Model F-6) employing isothermal run (190°C) to separate the methyl esters. Nitrogen gas (37m*l*/min) was run as the moving phase. Separation of the methyl esters was carried out in a 3m stainless steal column (i.d. 3mm) packed with chromosorb W (80～100 mesh) 10% impregnated with 1, 3-butandiol succinate polyester.

All peaks were identified by co-chromatography with authentic samples of C_{12} to C_{18} saturated and unsaturated fatty acid methyl esters. Only one peak, possibly a C_{14} unsaturated acid, was unable to identify because of lacking its authentic sample. The relative amounts of the fatty acid esters were calculated from the relative peak area by the triangulation method.

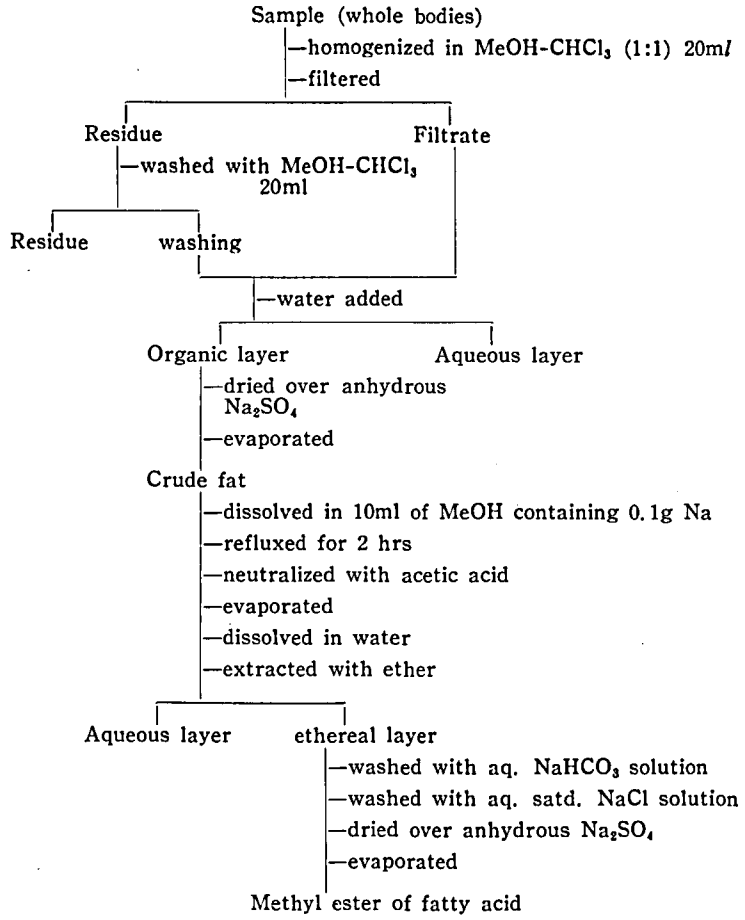

Fig. 1. Extraction of fat, and preparation of fatty acid methyl esters for analyses

Table 2. Lipids of the rice stem borer larvae and pupae used for analyses

Insects used		Fresh wt. (mg)	Crude fat. (mg)	Fat content (%)
No. of insects	Source			
15 (L)	Okayama, Hibernating	—	—	—
65 (L)	Okayama, Synthetic Food	1155	142.1	12.3
20 (L)	Shiga, Hibernating	917	158.7	17.3
20 (L)	Shiga, Hibernating	949.8	140.7	14.8
20 (L)	Shiga, Hibernating	798.8	109.9	13.8
10 (L)	Kagawa, Hibernating	855.5	157.2	18.4
10 (L)	Kagawa, Hibernating	657.0	125.5	19.1
10 (L)	Kagawa, Hibernating	705.0	—	—
8 (P♀)	Kagawa, Hibernating	501.2	68.9	13.8
8 (P♀)	Kagawa, Hibernating	541.4	71.0	13.1
7 (P♀)	Kagawa, Hibernating	524.0	86.0	16.4
9 (P♂)	Kagawa, Hibernating	511.0	77.7	15.2
10 (P♂)	Kagawa, Hibernating	503.5	78.0	15.5
10 (P♂)	Kagawa, Hibernating	505.0	65.5	13.0

L: larvae, P: pupae

Results

Samples to be analyzed were listed in Table 2, and a typical gas chromatogram of fatty acid esters was given in Fig. 2. As shown in Table 2, the content of crude fat in the living bodies was varied among samples even in the same source. The analysis for fatty acids was replicated more than three times for each sample. Results of these analyses were given in Table 3.

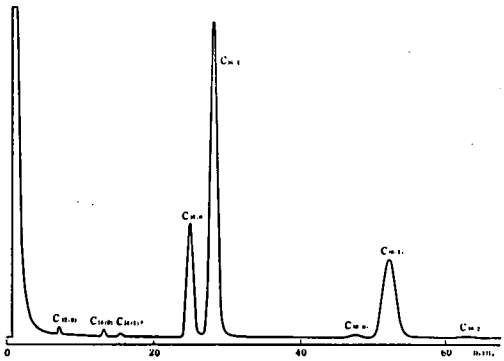

Fig. 2. A typical chromatogram of fatty acid methyl esters of lipid in the rice stem borer. Number in parentheses indicates number of double bond.

Table 3 showed that palmitoleic acid was dominant among fatty acids in all samples analysed. The composition of these seven acids in all samples revealed a fairly consistent picture except the sample of larvae fed on the artificial diet.

There was no remarkable difference in the fatty acid composition between male and female pupae, and among hibernating larvae collected from different localities. About 83% (82~86%) of the fatty acids was unsaturated in the lipids of the hibernating larvae and pupae, whereas about 75% was unsaturated in the lipid of larvae fed on the artificial diet. The present analytical results indicated that linolenic acid and higher fatty acids were not contained in the larval and pupal bodies, if any, in a small quantity.

Discussion

The composition of fatty acids is largely different in lipids of insect species. A high proportioneof linolenic acid ($C_{18(3)}$) seems to be one of characteristic nature of lipids in lepidopterous insects[6]. In the silkworm, linolenic acid has also been confirmed as the dominant acid[7] They suggested that this acid seems to be derived from mulberry leaves as a dietary source.

In available data published, palmitoleic acid was dominant mainly in lipids of dipterous insects[8~11], and rear in lepidopterous insects[12].

It has been known that the rice stem borer larvae have an ability to synthesize fat and deposit in their bodies[13]. Moreover, oleic and linoleic acids added to synthetic media have neither growth promoting effects on the larval growth nor improving effect on the pupation and the adult emergence[5]. When these acids were added

Table 3. Percentage of fatty acids in the rice stem borer lipids.

	C_{12}	C_{14}	C_{16}	$C_{16(1)}$	C_{18}	$C_{18(1)}$	$C_{18(2)}$
OHL[1]	0.3	0.9	16.4	56.9	0.7	24.8	trace
OSL[1]	0.2	0.5	22.8	47.5	1.5	27.5	trace
SHL[2]	0.2±0.03	0.7±0.09	12.9±0.44	54.5±1.03	0.7±0.09	18.8±0.7	71.1±0.49
KHL[2]	0.3±0.02	0.8±0.16	15.6±0.84	54.4±0.60	0.7±0.13	26.9±0.8	91.3±0.50
KFP[2]	0.3±0.07	0.4±0.08	16.3±0.61	56.4±1.69	0.7±0.11	24.7±1.0	11.2±0.39
KMP[2]	0.3±0.07	0.4±0.05	14.1±0.71	56.2±0.68	0.9±0.02	26.7±0.9	71.3±0.35

OHL : Okayama, hibernating larvae
OSL : Okayama, larvae fed on Synthetic food
SHL : Shiga, hibernating larvae
KHL : Kagaya, hibernating larvae
KFP : Kagawa, female, lst generation pupae
KMP : Kagawa, male, lst generation pupae
1) Average of 3 replicates
2) Agerage of 9 replicates

into a synthetic medium at higher doses the growth of larvae was inhibited. Linolenic acid has no effect on the larval growth in the early larval stages, but shows toxic effect when the larvae attained the 4th or 5th instar[5]

The present results obtained by gas chromatographic analyses indicated that palmitoleic acid is the dominant fatty acid in the rice stem borer lipid. This is the first case in lepidopterous insects. No remarkable difference was found in fatty acid composition between hibernating larvae collected from different fields, and male and female pupae. In the larvae fed on the synthetic food medium without fatty materials, palmitoleic acid was also contained as a dominant acid, but its proportion was smaller than that fed on natural food plants. On the other hand, content of palmitic acid in the larvae fed onthe synthetic food was higher than that fed on the natural food as shown in Table 3.

Linolenic acid, known to be an essential nutrient for some lepidopterous insects, could not be detected in the rice stem borer lipid.

It seems that the role of C_{18} unsaturated fatty acids in the nutrition of the rice stem borer larvae may be different from that in other lepidopterous insects.

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Summary

Fatty acid composition of lipid in the rice stem borer *Chilo suppressalis* Walker was analyzed by gas chromatography. Major seven acids, lauric, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acid, were identified. The dominant fatty acid was palmitoleic acid accounting for 54~57% in total fatty acids. About 85% of the fatty acids were unsaturated acids and the remaining 15% were saturated. Some differences were observed in the fatty acid composition between the larvae fed on natural food plants and these fed on a artifial medium.

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