Lipid of the Rice Stem Borer, *Chilo suppressalis Walker* (Lepidoptera: Pyralidae) II. Polar Lipid and Neutral Lipid of the Larvae from Different Colonies. Haruka Oouchi (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya Japan) Seisuke Ito (Department of Agricultural Chemistry, Obihiro Zootechnical University, Obihiro, Hokkaido, Japan) Received August 6, 1970 *Botyu-Kagaku* 35, 144, 1970.

21. ニカメイガの脂質に関する研究 II. 産地を異にするニカメイガ幼虫の極性脂質と非極性脂質. 大内 晴 (名古屋大学農学部害虫学教室, 名古屋市千種区不老町), 伊藤精亮 (帯広畜産大学農産化学科, 北海道帯広市稲田町) 45. 8. 6 受理.

産地を異にするニカメイガ幼虫の脂質を極性と非極性の二群の脂質に分画し, 両画分の構成脂質と脂質組成を調べ, 特に, 極性脂質の脂肪酸組成をも調べた. 1) 非極性脂質から多量のトリグリセリド, その他, ジ, モノの両グリセリド, 遊離脂肪酸, コレステロール及び炭化水素を検出した. ステロールエステルは認められなかった. 2) 極性脂質ではホスファチジルコリン (PC) とホスファチジルエタノールアミン (PE) が大半を占めていた. その他少量のスフィンゴミエリン (SPM) 及びリゾ型の PC と PE を検出した. 3) 総脂質の脂肪酸組成は C_{12} から C_{18} までの9種であった. そのうち $C_{16:1}$ が最も多くついで $C_{18:1}$, $C_{16:0}$, $C_{18:0}$ などの順であった. 極性脂質全体では C_{18} から C_{26} までの長鎖脂肪酸が大半を占め, そのうち $C_{26:0}$ が最も多く, $C_{26:1}$ は少量しか検出されなかった. 4) PC, PE の主要構成脂肪酸は $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ などであった. また比較的多量の $C_{20:5}$ も検出した. SPM のそれらは $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, $C_{20:5}$ などであり, $C_{16:1}$ が最も多く, ついで $C_{20:5}$ が多量に検出された. 5) 上の諸結果には地域による質的差は認められなかったが量的な差が認められた.

Introduction

The rice stem borer, *Chilo suppressalis* Walker, contains 12 to 18 per cent lipid on fresh weight basis[1] but the amount of polar lipid was very small[2]. It is known that many insects contain phosphatidylcholine and phosphatidylethanolamine as major components of phospholipid[3,4], however, there are no informations on them in the rice stem borer lipid. In the previous report[2], the authors did not refer to the analysis of polar lipid of this insect. But it is of interest to investigate the component phospholipid of the rice stem borer and also to compare the lipid compositions of the insect collected from different localities. In this paper, the authors report on polar lipids with special reference to the component fatty acid and neutral lipid in the rice stem borer, and the differences in their composition. As a preliminary report, it would be contributed to know the physiological differences between Saigoku- and Shonai- ecotypes.

Materials and Methods

Insect used

Egg masses of the rice stem borer, *Chilo suppressalis* Walker were collected from paddy fields at Kurashiki in Okayama prefecture, and Shonai district in Yamagata on August, 1969 and Okazaki in Aichi prefectures on June, 1969. Newly hatched larvae were reared on the rice seedlings successively at 27°C, 85% R.H. under a constant illumination for 16 hrs per day. The full grown larvae of the first generation of Aichi strain and that of the second generation of Okayama and Yamagata were used in the present experiment.

Extraction of total lipid (TL)

After 6 hrs starvation, the larvae were anaethetized by CO_2 and weighed. Total lipids were extracted with chloroform-methanol (2 : 1, v/v) according to the method previously reported[2]. The lipids obtained were weighed and stocked as chloroform solution at 5°C untill further use.

Column chromatography

a) Silisic acid chromatography

Total lipids obtained were applied to a silicic acid column chromatography and separated into "neutral" lipid (NL) and "polar" lipid (PL) by the method of CARTER and HIRSCHBERG[5]. Column preparations were carried out as follows: Silicic acid (100 mesh, Malinckrodt) was washed with water 4 to 5 times and successively with methanol 2 times. After air drying at room temperature, it was activated at 110°C for 6 hrs and then it was suspended in methanol. The suspension was packed in a column, 1.2 cm in diameter ×40 cm in length. The column beds were washed with excess amount of chloroform. Scheme of column elution based on each lipid samples was shown in Table 1.

Table 1. Scheme of silicic acid column chromatography of *C. suppressalis*

Lipid applied (mg)	Silicic acid (g)	Solvent system $CHCl_3$:MeOH (ml)	Lipid obtained NL:PL (mg)	Ratio of NL/PL
A* 114	10	400:350	101:14	7/1
B 230	15	600:400	212:20	11/1
C 140	10	400:350	116:24	5/1

* Colony: A-Aichi, B-Okayama and C-Yamagata

b) *Florisil column chromatography*

Neutral lipid eluted from the column supported by silicic acid was separated into each component with a Florisil column chromatogrphy following the method of CARROLL[6]: Florisil (60 to 100 mesh, Floridin Co.) was deactivated by adding 25 ml of water per 100g of Florisil and, after shaking, it was allowed to stand overnight. It was then packed into the column, 1.2cm×40cm, pre-filled with hexane. The column beds were washed with excess amount of hexane. The neutral lipids applied on the column were eluted successively with hexane, and 5, 15, 25% ether and 2% methanol in hexane and 4% acetic acid in ether. The eluents from the column were collected each 10 ml fraction, and the distribution of the lipids was determined gravimetrically. And they were monitored by thin-layer chromatography as described below. Results are shown in Table 2 and Fig. 3.

Thin-layer chromatography (TLC)

Neutral and polar lipids were determined by TLC prepared as follows: Samples were applied in spots or bands on a plate coated with 250 mμ thick Wakogel B-5 (5% binder, Wako Pure Chemical Industries Co.) and activated for 4 hrs at 110° C; triglycerides (TG) eluted from the column supported by Florisil were separated on a plate loaded with 300mμ thick Wakogel B-5 containing 12.5% silver nitrate. The developing solvent systems were petroleum ether/ether/acetic acid (80:30:1 or 90:10:1)[7,8] for NL, chloroform/methanol/water (65:25:4)[9] for PL and benzene/ether (8:2)[10] for TG fraction, respectively. Iodine vapor and 50% H_2SO_4 were mainly used for detection of spots. Other detection reagents for PL were shown in Table 3.

Analysis of fatty acid from phospholipids (PLS)

Each spot of PLS on thin-layer chromatogram (Fig.5) was scraped and extracted with chloroform-methanol (2:1, v/v). After filtration, the solvent was evaporated under N_2 flow, and no further purification was carried out. The lipids were converted into methyl esters by transesterification method using 5% HCl in methanol under reflux at 100°C for 2 hrs[11]. The methylated compounds were extracted with hexane and purified by washing

Table 2. Scheme of Florisil column chromatography of NL of *C. suppressalis* eluted from the silicic acid column

	NL applied (mg)	Florisil (g)	Solvent system (ml) H	5%-E	15%-E	25%-E	50%-E	2%-MeOH	4%-Ac
A*	101	12	20	50	70	60	60	75	75
B	210	22	40	100	150	120	120	150	150
C	116	12	20	50	75	60	60	75	75

* Colony: See Table 1.
Solvent: H-hexane, E-diethyl ether and Ac-acetic acid.

Table 3. Color reaction of polar lipid by the various reagents

Spot* no	Dragendorff reagent	Ninhydrin reagent	Anthrone reagent	Phosphomolybdate reagnt
1.2	−	−	−	−
3	−	−	±	−
4	−	+	−	+
5	+	−	−	+
6	−	+	−	+
7	+	−	−	+
8	±	−	−	+

+: positive, −: negative * see Fig. 5

4 times with distilled water and the extracts were dried over with anhydrous sodium sulfate. Fatty acid methyl esters from TL and PL fractions were also obtained as described above. The composition of these methyl esters were determined by the gas-liquid chromatography (GLC), Yanagimoto's Model GCG-5DH, equipped with hydrogen flame ionization detector. Operative conditions were given in Table 6. Qualitative analysis of the fatty acid methyl esters were carried out by comparing their retention times to those of authentic standards and plotting the logarithm of their retention times against their carbon numbers.

Authentic standards

Triglyceride, cholesterol and cholesteryl acetate purchased from Wako Pure Chemical Industries Co., monostearin and palmitic acid from Katayama Chemical Industries Co., sphingomyelin prepared from the horse spinal cord by Fujino and Negishi[12] phosphatidylcholine from the yolk of hen by Mon'ma (unpublished) and phosphatidylethanolamine from the sea urchin by Umatani[13] were respectively used. Fatty acid methyl esters as follows; $C_{12:0}$ to $C_{20:0}$ were supplied by courtesy of Dr. Kaltayama, Department of Fisheries Science and Animal Husbandry, Hiroshima University, and $C_{18:3}$, $C_{20:4}$, $C_{20:5}$, $C_{22:4}$, were purchased from Sigma Chemical Co..

Results

Total lipid content on fresh weight basis of the rice stem borer from different colonies was shown in Table 4. The relative amount of TL varied from 3.8 to 6.3 among the different origins. The amounts of NL and PL eluted from the column

Table 4. Total lipid content of *C. suppressalis* from three colonies

Insect No.	Fresh W. t.	Extracted residue (dry)	Total lipid (mg)	% for fresh (dry)
A* 75	5.23	—	392	6.3(—)
B 89	5.84	0.23	230	3.8(50)
C 74	3.43	0.10	148	4.3(60)

* Colony: See Table 1.

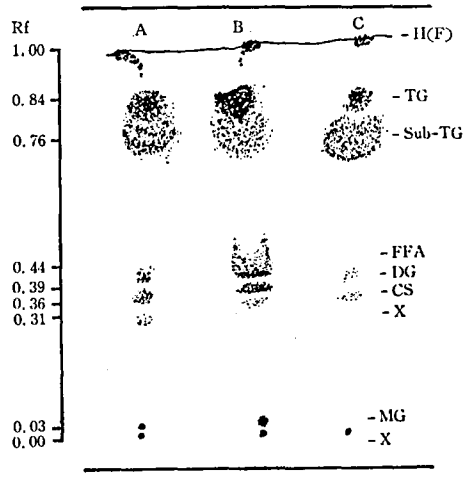

Fig. 1. TLC of the neutral lipid of *Chilo suppressalis* from three colonies.
Lipid samples: A-Aichi, B-Okayama and C-Yamagata.
Solvent system : petroleum ether/ diethylether/acetic acid (80:30:1, v/v).
Spots were detected by charring with 50% H_2SO_4. O-Origin, MG-monoglyceride, X-unknown, CS-cholesterol, DG-diglyceride, FFA-free fatty acid, TG-triglyceride, H-hydrocarbon and F-solvent front.

supported by silicic acid were listed in Table 1. It was found that the amount of PL of the larvae from Yamagata colony was the highest (20% of total) among three colonies.

Neutral lipid composition

a) Thin-layer chromatography of NL

Thin-layer chromatograms of NL eluted from silicic acid column were shown in Fig. 1. As shown in this figure, triglyceride (TG) was main component, and diglyceride (DG), free fatty acid (FFA), cholestrol (CS) and hydrocarbon (H) were minor components. Their relative amounts were determined by a photodensitometer as listed in Table 5. As seen in the chromatogram (Fig. 1), sub-triglyceride fraction in Yamagata colony was also relatively high. However, this spot on the TLC was photodensitometrically insensitive and was not separated by the TLC with the other

Table 5. Percentages of each lipid class of *C. suppressalis**

	NL						
	H	TG	FFA	DG	CS	X_1	MG
A	4.4	72.4	0.5	9.0	9.8	2.7	1.3
B	10.9	55.0	15.8	10.9	5.3	2.1	8.2
C	12.8	65.4	1.7	7.3	9.6	2.3	0.9

	PL					
	X_2	PE	PC	LPE(?)	SPM	LPC(?)
A	2.6	39.0	45.2	6.7	3.5	1.8
B	7.5	43.9	37.7	5.0	5.0	0.7
C	2.9	28.0	48.5	14.1	4.4	2.0

* Analyzed by photodensitometry

Abbreviations: described in Fig. 1, 5 and Table 1

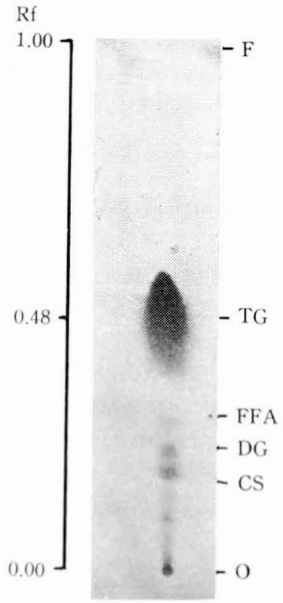

Fig. 2. A typical thin-layer chromatogram of the neutral lipid of *C. suppressalis*. Sample: from Okayama colony. Solvent system: Petroleum ether/diethyl ether/acetic acid (90:10:1, v/v). See Fig. 1 for detection and abbreviations.

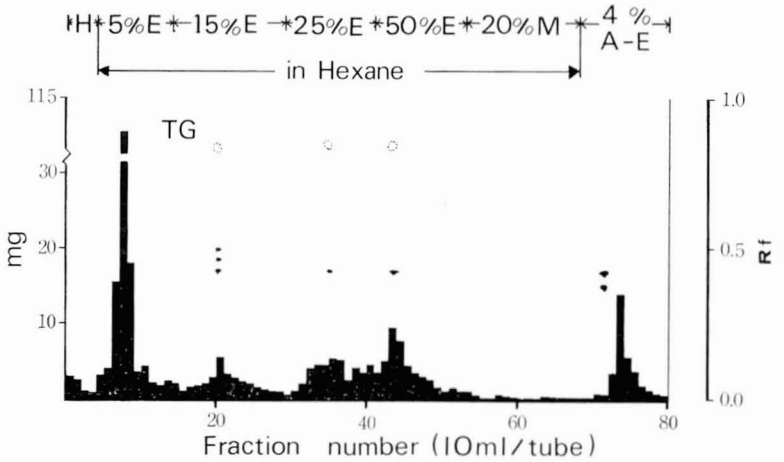

Fig. 3. Purification of triglyceride from the neutral lipid of *C. suppressalis* (Okayama) by Florisil column chromatography. The eluents from the column were monitored by TLC as described in Fig. 1. See text for further explanation. H-hexane, E-diethyl ether, M-methanol, A-acetic acid and TG triglyceride.

solvent system as seen in Fig. 2. From the chromatogram sub-triglyceride fraction on the TLC was suggested to be a triglyceride analogue.

Neutral lipid of the larvae was further fractionated into each component by Florisil column chromatography to obtain some informations on TG and sub-TG fraction on the TLC. The elution patterns from Florisil columns and rechromatography of the eluents were shown in Fig. 3. As shown in the figure two components of TG were separated from other components of neutral lipid, although the elution pattern in this experiment was different from the results of CARROLL[6].

b) Argentation TLC of TG and sub-TG fraction

The two components obtained as above mentioned were applied on a plate of silicic acid impregnated with 12.5% silver nitrate, and separated into six components as illustrated in Fig. 4, but in this case the spots could not be identified. It was not confirmed that the quantitative differences were apparent between TG and sub-TG which were observed in the chromatogram in Fig. 1.

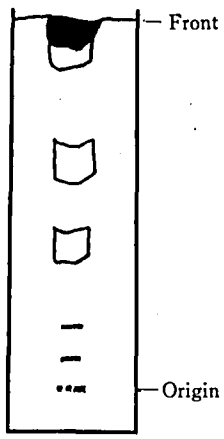

Fig. 4. A diagram of argentation TLC of triglyceride after purification by Florisil column chromatography given in Fig. 3
The plate was developed under dark condition. Solvent system: benzene/diethyl ether (8:2, v/v). Spots were detected by exposure to iodine vapor. See text for further explanation.

Phospholipid composition

Polar lipids fractionated by methanol as mentioned above were applied in bands or spots for photodensitometric determination and developed with WAGNER's solvent system. Figure 5 shows, at least, six components being phosphatidylcholine (PC) (39 to 49%), phosphatidylethanolamine (PE) (28 to 44%) as major components, sphingomyelin (SPM), lyso-phosphatidylcholine (LPC) and -phosphatidylethanolamine (LPE) as minor components, and relatively high amount of unidentified substance giving no phosphorous reaction by spraying with ammonium molybdate reagent, were found. Their color reaction against various spraying reagents were listed in Table 3. No remarkable differences were found in qualitative observation among the colonies of the rice stem borer.

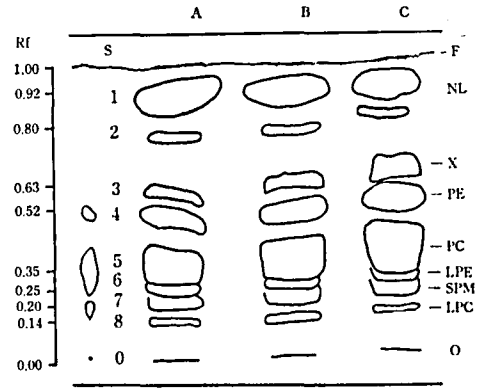

Fig. 5. TLC of the polar lipid of *C. suppressalis* from three colonies.
Lipid samples: S-Standards, A-Aichi, B-Yamagata and C-Okayama. Solvent system: Chloroform/methanol/water (65:25:4, v/v). Spots were detected by exposure to iodine vapor. Standards: Sphingomyeline[12] (SPM), Phosphatidylcholine (see text, PC) and Phosphatidylethanolamine[13] (PE). O-Origin, LPC-lysophosphatidylcholine, LPE-lysophosphatidylethanolamine, X-unknown, NL-neutral lipid and F-solvent front.

Fatty acid composition of TL and PL

The results of fatty acid analysis of TL, PL eluted from the column supported by silicic acid, and PLS re-extracted from the TLC (Fig. 5) applied on WAGNER's solvent system were presented in Tables 6 and 7. As listed in the tables, eleven or ten of fatty acids composed of C_{12} to C_{18} were detected in TL. Main components of these acids were $C_{16:0}$, $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$ in three colonies

Table 6. Fatty acid composition of TL and PL of *C. suppressalis* from three colonies (%)
Operative conditions of GLC: Column 3mm ×2m with 10% DEGS; temp, 180° C; N_2, 15 ml/min; H_2, 25ml/min.

Fatty acid	TL			PL		
	A	B	C	A	B	C*
$C_{11:0}$	—	—	—	—	Tr	Tr
UK	—	—	—	—	—	Tr
$C_{12:0}$	Tr	0.6	Tr	—	—	Tr
$C_{13:1}$(?)	0.5	—	—	0.6	0.6	Tr
$C_{14:0}$	1.0	1.3	1.2	Tr	Tr	Tr
$C_{14:1}$	Tr	0.7	0.4	Tr	—	Tr
$C_{15:0}$	—	—	—	—	Tr	Tr
$C_{15:1}$	Tr	—	—	Tr	0.5	Tr
$C_{16:0}$	17.1	13.1	24.1	7.2	12.5	9.3
$C_{16:1}$	33.4	25.5	29.9	4.6	5.2	5.0
$C_{17:0}$	—	—	—	—	0.6	0.5
$C_{17:1}$	—	—	—	—	Tr	Tr
$C_{18:0}$	2.1	2.4	2.0	6.5	13.1	9.5
$C_{18:1}$	24.3	24.4	21.4	13.2	25.0	15.5
$C_{18:2}$	15.9	23.5	17.0	15.9	29.4	20.7
$C_{18:3}$, $C_{20:0}$	4.0	3.3	3.9	2.6	4.9	4.0
$C_{20:1}$(?)	—	—	—	—	0.9	—
$C_{20:4}$, $C_{22:0}$	—	—	—	0.8	—	0.6
$C_{20:5}$	1.2	—	—	1.1	1.5	2.0
$C_{19:0}$	—	—	—	6.3	—	1.8
$C_{24:0}$	—	—	—	1.7	—	Tr
$C_{25:0}$, $C_{22:4}$	—	—	—	8.0	3.0	5.6
$C_{26:0}$	—	—	—	31.3	2.0	23.7

* two replications, UK; unknown, Tr; trace (less than 0.5%) —; non-detectable, See Table 1 for A, B and C.

in which $C_{16:1}$ was equally the highest component and $C_{18:2}$ was followed in Aichi and Yamagata colonies. In PL, longer chain fatty acid, more than C_{20} carbons, and odd numbered fatty acids were found. The number of fatty acids detected in PL was twenty three, C_{11} to C_{26}. Among them $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{26:0}$ were main component except Okayama colony where $C_{18:2}$ was the highest being 29.4% and C_{26} was only 2% of the total, while the long chain fatty acid was the highest one in the other colonies (23 to 31%).

In PC, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{20:5}$ were main components, and the highest one in this lipid was $C_{16:0}$ (23.4%) in Aichi and $C_{18:2}$ in Yamagata (26.4%) and in Okayama (22.5%). It is noticeable that $C_{20:5}$ was about 16.0% in Okayama colony. Oleic acid was the highest fatty acid in PE among three colonies. In SPM, $C_{20:5}$ was found generally, amounting 15 to 30% of the total.

Table 8 shows the ratio of saturated fatty acid to unsaturated one calculated from Table 6 and 7. It was clarified that the ratio in total lipid of Yamagata colony was higher than that of the other two colonies, and that the unsaturated fatty acid level was two times higher than that of the saturated one from polar lipid in Okayama colony. On the other hand the saturated level in Aichi and Yamagata colony was about 50% of polar lipid. In each component of PLS the ratio was

Table 7. Fatty acid composition of PE, PC and SPM of *C. suppressalis* from three colonies (%)

Fatty acid	PE			PC			SPM		
	A	B	C	A	B	C	A	B	C
$C_{11:0}$	—	—	—	—	Tr	—	Tr	Tr	—
UK	—	—	—	—	0.5	—	—	1.0	—
$C_{12:0}$	—	—	Tr	Tr	Tr	—	—	1.0	—
$C_{13:0}$	2.5	—	—	—	—	—	—	—	—
$C_{13:1}$	—	Tr	1.4	1.9	1.1	0.8	8.3	5.6	7.6
$C_{14:0}$	0.6	Tr	1.4	1.6	1.1	0.7	2.3	2.2	1.5
$C_{14:1}$	Tr	—	0.7	1.3	0.4	Tr	3.1	1.6	1.9
$C_{15:0}$	Tr	Tr	0.6	0.9	Tr	Tr	1.3	1.0	1.5
$C_{15:1}$	1.3	Tr	0.5	1.3	0.9	Tr	4.9	3.6	4.5
$C_{16:0}$	20.7	19.9	18.0	23.4	14.3	18.7	24.0	21.4	20.6
$C_{16:1}$	6.7	4.1	6.7	10.9	7.3	6.0	9.5	9.1	10.1
$C_{17:0}$	1.0	—	0.7	1.6	0.6	Tr	—	—	0.6
$C_{17:1}$	Tr	—	Tr	—	2.2	Tr	3.5	2.5	1.2
$C_{18:0}$	13.5	13.7	13.6	13.4	12.9	14.1	6.4	7.1	7.1
$C_{18:1}$	27.6	34.2	24.5	17.4	14.3	19.8	7.7	11.4	8.8
$C_{18:2}$	15.1	17.2	19.5	16.3	22.5	26.4	0.6	2.9	5.1
$C_{18:3}$, $C_{20:0}$	3.0	3.0	4.3	3.5	5.0	2.7	7.7	2.0	12.3
$C_{20:5}$	7.2	6.9	7.4	6.3	16.1	6.7	15.0	29.3	17.4

See Table 6 for abbreviation.

Table 8. Ratio of saturated to unsaturated fatty acid from the lipid classes of *C. suppressalis* from three colonies

Lipid class	Aichi		Okayama		Yamagata	
TL	20.2/78.1	1:3.0	17.4/76.4	1:4.4	27.3/72.6	1:2.7
PL	61.0/38.8	1:0.6	31.2/68.0	1:2.2	51.0/45.2	1:0.9
PC	40.8/58.9	1:1.4	28.9/70.3	1:2.4	35.5/62.3	1:1.9
PE	38.3/60.9	1:1.6	33.6/65.4	1:1.9	34.3/65.0	1:1.9
SPM	34.0/65.3	1:1.9	32.7/68.0	1:2.1	31.3/69.9	1:2.2

* The ratio was calculated from Tables 6 and 7.
Abbreviations: See text for lipid classes

almost constant among the larvae from three colonies.

Discussion

Lipid may be expected to be an energy source during starvation[14], but the present paper dose not refer to the changes of the lipid composition of *Chilo suppressalis* Walker during its starvation period.

The lipid content of the hibernating larvae of "Saigoku ecotype was higher than that of "Shonai" ecotype. However, in the present experiment it was difficult to find out the clear relationship between lipid content and the ecotypes, although considerable differences were found in the ratio of NL/PL, as shown in Table 1. High level of PL (about 17% of the total) was observed in Yamagata colony.

As shown in Fig. 1 and Table 5, with regard to NL composition, high amounts of FFA and MG, 16 and 11% respectively, were found in Okayama colony, and their composition was fairly constant both Aichi and Yamagata colonies. The high amount of FFA and MG may be due to the enzymatic degradation of TG, but their physiological significance is entirely obscure from this experiment. Triglyceride in Yamagata colony showed a remarkable pattern on the TLC as seen in Fig. 1. The spot of sub-TG on the TLC situated between TG and FFA was clearly different from that of other two strains. It is suggested that there are some differences in the chain length or the unsaturation of the acyl group of TG between Okayama colony and the other two colonies. But no informations were available in the experimental data shown in Figs. 2 to 4.

Although studies on PLS of insects have been accumulated, major work on this subject are restricted to some species of diptera[16~20], coleoptera[21], lepidoptera[22~26] and orthoptera[27]. There are no informations on PLS pattern of the rice stem borer, *C. suppressalis*. The present results demonstrated that main components of PLS in this insect larvae were PC and PE without difference to other lepidopterous insects[22~26]. Lyso types of PC and PE were also found in PLS of the larvae of *C. suppressalis* as reported on the fat body of *Sarcophaga bullata*[20]. It had been concluded that the absence of sphingomyelin in insect was a notable departure from the PLS pattern in vertebrates[23]. However, SPM found in the rice stem borer has been also detected in *Bombyx mori*[26], *Periplaneta americana*[27], *Tenebrio molitor*[21], *Hyalophora cecropia*[25], *Philosomia cynthia*[28] and *Torogoderma granarium*[29]. One unidentified substance on the TLC was detected in the PL fraction. Its Rf value was closely associated with PE on WAGNER's solvent system, and it was negative color reaction for phosphorous and choline. There was no obvious color reaction with anthrone reagent. Further analysis is under carring out.

In comparison with PLS among the larvae from different colonies, PL contents in the total lipid were calculated from Table 1 as 8 to 17%. The ratio of PE:PC was varied among colonies, that is, 1:1.2, 1:0.9 and 1:1.7 for Aichi, Okayama and Yamagata, colony, respectively. The predominance of PC for Yamagata seemed to be a character associating with high content of LPE comprised about 14% of PL.

There are few investigations on the fatty acid composition in PLS of insects. Fast and Brown[18] reported the distribution pattern of fatty acid in cephaline (phosphatidyl-ethanolamine and -serine)

of the mosquito with high amount of $C_{16:0}$, $C_{16:1}$ and $C_{18:1}$. The predominance of C_{18} series was found in *Bombyx mori*[24], the sarcosomes of *Hyalophola cecropia*[25] and in other lepidopterous insect species[3]. Characteristically high amount of $C_{16:1}$ was previously reported in the fatty acid composition of the hibernating rice stem borer lipid[30]. Small amount of $C_{16:1}$ in PL contrasting with that in TL[2], as confirmed in the present work, suggested that the different physiological significance exists between the fatty acid ($C_{16:1}$) of the rice stem borer and that of other dipterous insect which is characterized by the high amount of $C_{16:1}$ consisting about 10 to 40% in the PL (phosphatidyl-choline and -ethanolamine) and also in the NL[3]. Tables 6 and 7 shows that the significant difference in the amount of $C_{16:1}$ was found in PL and each PLS component of PC, PE and SPM. A high proportion of $C_{26:0}$ about 30% at highest was detected in the total PL with the polyunsaturated fatty acid and odd numbered fatty acid. It has been well known to present uniquely C_{24} to C_{26} fatty acid in vertebrates[31], but such no longer chain fatty acid has been demonstrated in the insect lipid except wax[4]. However, SPM of sarcosomes from *B. mori* may contain such long chain fatty acid, because unidentified fatty acids longer than C_{18} were approximately 30% of the total in the sarcosome SPM[25]. In the rice stem borer, the long chain fatty acid more than C_{20} was not detected in SPM and also in the other components of PL, though $C_{26:0}$ was detected in the total PL. The reason of the failure in detection of $C_{26:0}$ in SPM is entirely obscure.

As indicated in Table 8, from the ecological point of view, the ratio of saturated to unsaturated fatty acid of the total was 1:2.7 in Yamagata colony, which is mainly resulted from the high percentage of $C_{16:0}$. In Okayama colony, the ratio in PL was 1:2.2, and was different from the other colonies. It is mainly due to the less amount of $C_{26:0}$ only 2% of PL. On the contrary $C_{20:5}$ was predominant in Okayama colony for PC and SPM except for PE.

It is difficult to conclude that there are clear relationship between the so-called "ecotypes" and lipid composition. It is however, possible to expect the relationship between PLS and the ecotypes because of the shorter life cycle in Yamagata strain than the Saigoku strain[32], and the important role of PLS in tissue membrane[33]

Summary

Three ecotype colonies of the rice stem borer, *Chilo suppressalis* WALKER, from different localities were reared under identical conditions, and neutral and polar lipid compositions of the larvae were studied. Total and neutral lipids were determined by column chromatography and compositions of neutral and polar lipid were analized by thin- layer chromatography. The distribution pattern of fatty acids was also observed by gas-liquid chromatography.

1) Triglyceride, diglyceride, monoglyceride, free fatty acid, sterol and hydrocarbon were indentified from the neutral lipid, but no sterol ester was found.

2) Phosphatidyl-choline (PC) and -ethanolamine (PE) were major components of the polar lipid, and lyso types (?) of PC and PE in addition to sphingomyelin (SPM) were found as minor components. No qualitative difference was observed among the colonies.

3) Fatty acid composition in the total lipid was C_{12} to C_{18} with main component of $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$. Among them $C_{16:1}$ was equally dominant (about 30%) in the three colonies. In polar lipids, $C_{26:0}$, $C_{18:2}$, $C_{18:1}$ and $C_{16:1}$ were major components except for Okayama colony.

4) Polyunsaturated fatty acid of $C_{20:5}$ was detected as relatively high level in both PC and PE in addition to $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and small amount of $C_{16:1}$ (about 4%) was detected. Fatty acids of SPM were mainly $C_{16:0}$, $C_{16:1}$, $C_{18:1}$ and $C_{20:5}$, which was exceptionally high in Okayama colony (about 30%).

5) These fatty acids were qualitatively almost identical among three colonies, but quantitatively different.

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