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112. Mieko Suzuki (née Saito),*1 Nobuo Ikekawa,*2 Eiko Akaike,*1 and Kyosuke Tsuda*3 : Studies on Hydrocarbons of *Bombyx mori* L.

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The present paper is an extention of our previous report[1] on unknown substances in the unsaponifiable fraction from the silkworm, and described the chemical structure of hydrocarbons in the unknown substance comparing with hydrocarbons from mulberry leaf, using gas chromatographic technique and mass spectrometry.

Hydrocarbons in insects are an important group of lipids as a component of cuticulin waxes which have been recognized as a factor in the mechanism for the control of their water balance,[2] and which have been correlated with the problems of insecticide penetration.

In addition to these significances, hydrocarbons in the silkworm are a component of waxes contained in the raw silk as the second products. The waxes have been recognized as a factor involved in both the imhibition to water and protection of raw silk from environmental factors.[3]

It has been reported by two workers[4,5] that a hydrocarbon in waxes from both plants and insects consisted of one or more odd-numbered carbons from C_{25} to C_{37} and in cuticulin from the silkworm consisted of odd-numbered carbons from C_{27}–C_{31} as the probable hydrocarbon. It has later been shown by gas chromatographic technique and mass spectrometry that hydrocarbons from mormon cricket were both odd- and even-numbered from C_{12} to C_{32}[6] and those from adult house fly were both odd- and even-numbered from C_{16} to C_{35}.[7]

Experimental

Material——The 5th instar larvae and moths of the silkworm of F_1 hybrid between two races, Zuiko and Ginpaku, were used as experimental animals. Mulberry leaves from Ichinose race were collected as the plant material. Prior to the experiment, 2,122 larvae without the inclusion of digestive tract, in order to eliminate the contamination of mulberry leaves, the blood from 4,380 larvae, and wing from 18,900 moths were prepared.

The standard mixture of both odd- and even-numbered straight-chain hydrocarbons from C_{20} to C_{36}, obtained from the shale oil in Thailand, and synthesized standard saturated hydrocarbons of C_{28}, C_{32}, and C_{36} were kindly given by Dr. Iida, University of Toyama.

Extraction of Hydrocarbons——Each of homogenates obtained from wings, blood and larvae was extracted with methanol at room temperature. After removal of methanol, each residue was extracted with ether and the ether extracts were saponified with 10% KOH in ethanol. The unsaponifiable fraction

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obtained from each extract was separated into several fractions by silica gel column. The infrared spectra of the first fractions eluted with petroleum ether exhibited the typical hydrocarbon absorption, as shown in Fig. 1.

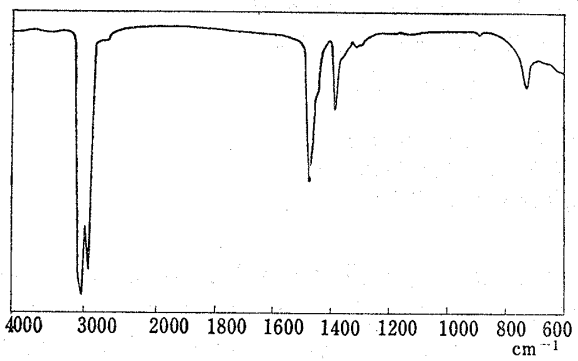

Fig. 1. Infrared Spectrum of the Silkworm Hydrocarbons

Mulberry leaves were dried at room temperature for 2 days and then dried at 60° for 3 hr. The dried leaves were ground in a mortar. The leaf powder was soaked in methanol for one day and refluxed for 3 hr. After removal of methanol, the residue was saponified with 10% KOH in ethanol. The hydrocarbon fraction was obtained from unsaponifiable fraction by silica gel chromatography as above.

Analysis of Hydrocarbons——Hydrocarbons were analyzed by Shimadzu gas chromatograph Model GC-1B with hydrogen flame ionization detector. U-Shaped stainless steel column (150 cm., ×4 mm., i. d.) was used. The column packing was 1.5% JXR (dimethyl polysiloxane) on Chromosorb W (80～100 mesh, acid washed, and siliconized). The sample heater and detector temperatures were 280° and 270°, respectively, in both isothermal and programmed temperature chromatographies. In isothermal condition, the column temperature was maintained at 264°(Fig. 4), and in the programmed condition, the column temperature was programmed from 120° to 250° on the rate of 4°/min. (Figs. 2 and 3). The flow rate of carrier gas, N_2, was 93 ml./min. in former case and 120 ml./min. at 120° in the latter.

The mass spectrometry of hydrocarbon fraction was carried out with Hitachi model RMU-6D mass spectrometer.

Hydrogenation of hydrocarbons was carried out as follows : About 10 mg. of each hydrocarbon in 5 ml. hexane was shaken with 50 mg. platinum oxide catalyst in hydrogen stream for 2 hr. After removal of both catalyst and solvent, each residue was analyzed by gas chromatography.

Result and Discussion

Temperature programming gas chromatograms of wings, blood, larva, mulberry leaf, and standard mixture are presented in Fig. 2. Each hydrocarbon composition differed in each sample, showing that hydrocarbon of wings consisted of larger carbon number than that of other samples and standard mixture. Retention times of both odd- and even-numbered straight-chain hydrocarbons from C_{16} to C_{29} in wing, C_{23} to C_{27} in blood, C_{16} to C_{31} in both larva and mulberry leaf were completely coincident with those of standard ones. Many hydrocarbons but not straight-chain ones were seen in each sample as shown in Fig. 2. Mulberry leaf hydrocarbons consisted of C_{17} to C_{33} having predominantly odd-numbered carbons, and also amount of both C_{29}- and C_{31}-hydrocarbons was larger than that of other hydrocarbons. These results demonstrated that hydrocarbons from both the silkworm and mulberry leaf consisted of a mixture of odd- and even-numbered carbons, showing the same results as four species of fly and mormon cricket.[6,7]

Table I. Molecular Size Percentage of Total Hydrocarbons in the Silkworm and Mulberry Leaf

Hydrocarbons from	Percentage of				
	C_{16} to C_{20}	C_{21} to C_{27}	C_{28} to C_{33}	C_{34} to C_{36}	C_{37} to C_{42}
Wings of moth	4.80	15.53	16.04	53.30	10.33
5th instar larva-blood	—	64.23	31.81	3.96	—
5th instar larva without intestine	18.81	72.73	8.46	—	—
Mulberry leaf	57.55	17.33	25.12	—	—

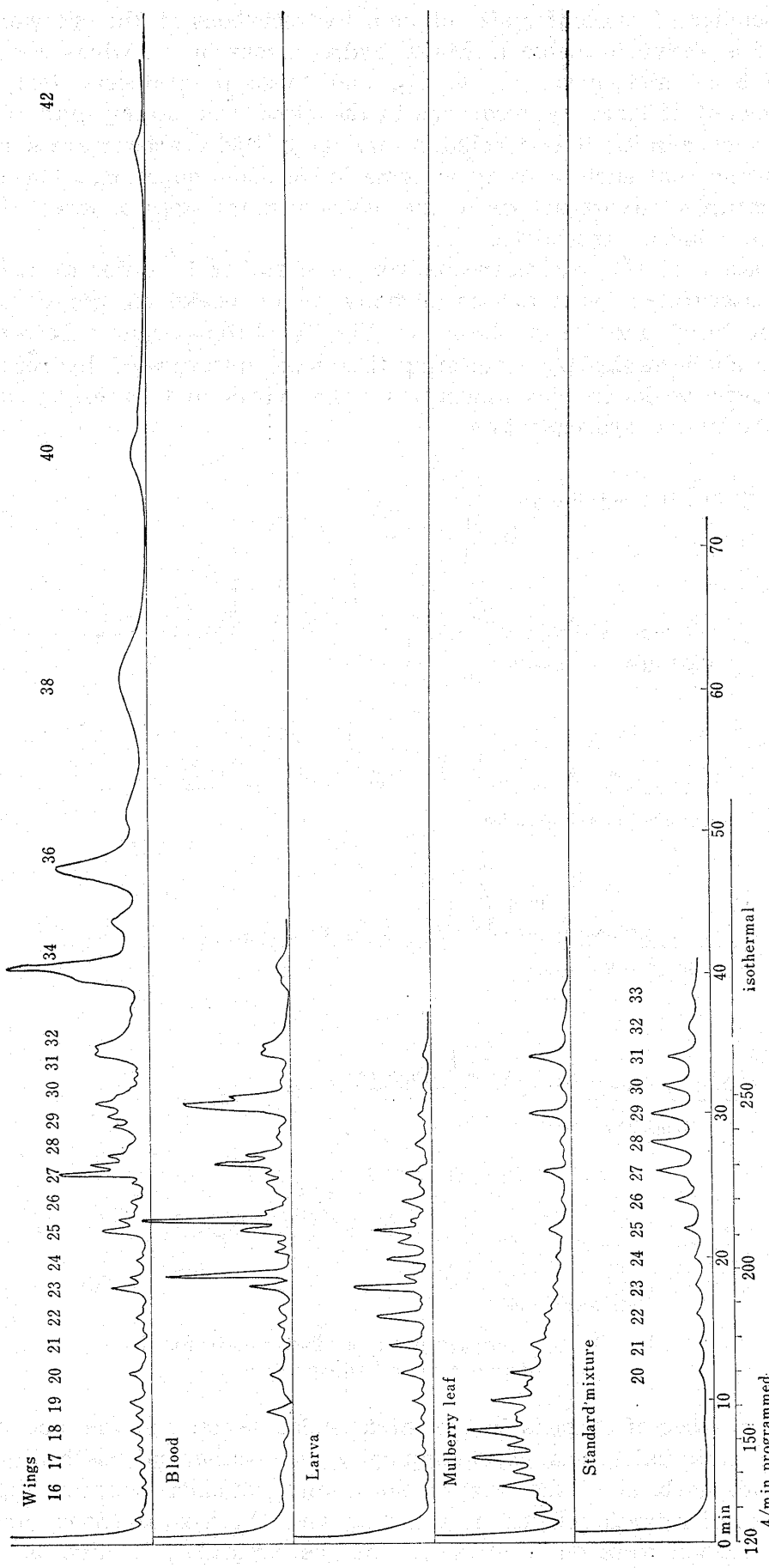

Fig. 2. Gas Chromatograms of Hydrocarbons in the Silkworm and Mulberry Leaf

The percentage of molecular size of total hydrocarbons in the silkworm and the mulberry leaf is shown in Table I. Major hydrocarbons in the wings were C_{34} to C_{36}, those in both blood and larva, C_{21} to C_{27}, and those in mulberry leaf, C_{16} to C_{20}. Moreover, amount of large hydrocarbons in the blood was larger than that in larval body. From such results, it is concluded that large hydrocarbons are synthesized in the silkworm and that such activity is remarkable after pupation. Therefore, difference in the nature of hydrocarbons in the developmental steps of insect must be considered, before using an insecticide.

Hydrogenation of the hydrocarbons was undertaken in order to investigate the presence of unsaturated hydrocarbons in many minor peaks on the chromatograms. In the case of blood and larva shown in Fig. 3, chromatograms before and after hydrogenation changed slightly, suggesting that some unsaturated hydrocarbons were present. However, peaks on chromatograms from wings and mulberry leaf did not show any shift by the hydrogenation.

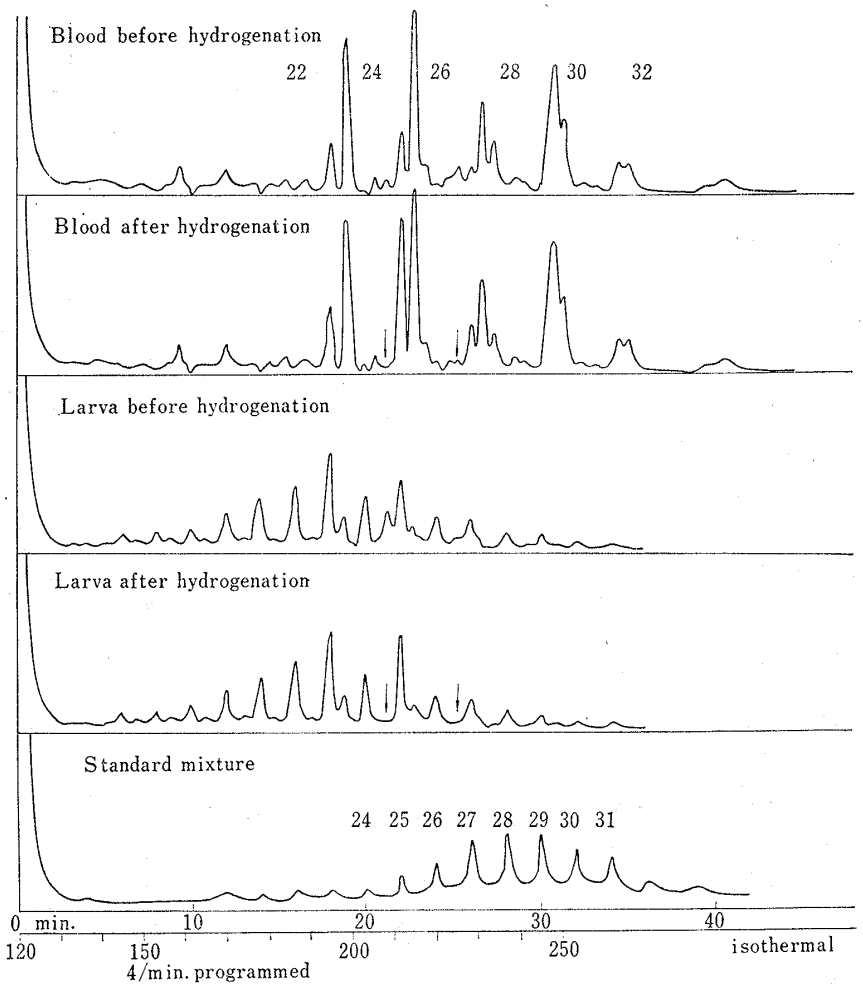

Fig. 3. Gas Chromatograms of Silkworm Hydrocarbons before and after Hydrogenation

Since the number of carbons in the high molecule region was not revealed by temperature programming gas chromatograghy, an isothermal method was carried out on the hydrocarbons of the wings, blood, and standard mixture (Fig. 4). The logarithmic plot of retention times of C_{28}, C_{32}, and C_{36} hydrocarbons, and standard mixture hydrocarbon were on a straight line, as presented in Fig. 5. Therefore, carbon number of a normal hydrocarbon was tentatively determined by the apparent

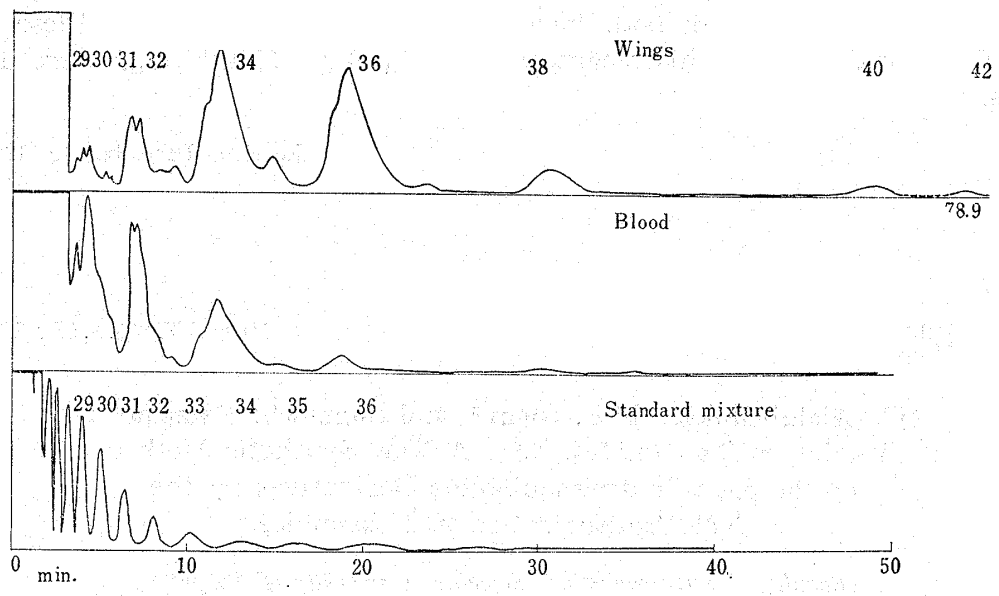

Fig. 4. Gas Chromatograms of Silkworm Hydrocarbons

retention time. The logarithmic plot of retention time of the peaks on chromatogram of wing-hydrocarbon was added to the straight line, as shown in Fig. 5. Therefore, it is assumed from Fig. 5 that carbon numbers of large hydrocarbons from wings are C_{32}, C_{34}, C_{36}, C_{38}, C_{40} and C_{41}, although retention times of hydrocarbon from wings did not agree with those expected from Fig. 5. This discrepancy may depend on the type of carbon chain.

According to mass spectrometry, however, carbon number more than C_{38} was not detected in the wings. This may be due to a low concentration of a large hydrocarbon in the mixture. Up to the present, the presence of C_{38}-, C_{40}-, C_{41}-hydrocarbons as found in wings has not been reported in insects.

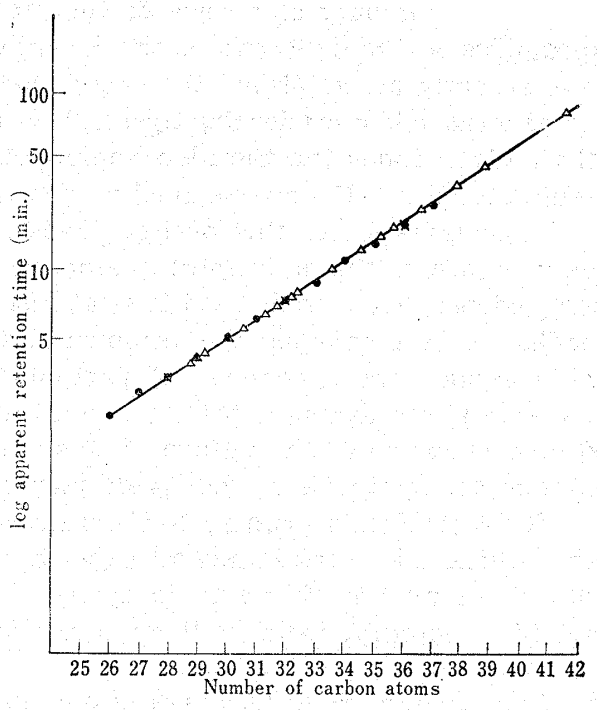

Fig. 5. Relationship between Chain Length and log of Apparent Retention Time of Standard and Wing Hydrocarbons

△ Wing hydrocarbon
× $C_{28}H_{58}$, $C_{32}H_{66}$, $C_{36}H_{74}$.
● Standard hydrocarbon mixture

We are deeply indebted to Drs. M. Shimizu and M. Kobayashi, The Sericultural Experiment Station, for their encouragements and valuable suggestions. Also, the authors express their deep gratitude to Prof. T. Iida, University of Toyama, for a gift of standard samples of hydrocarbons.

Summary

The chemical structure of hydrocarbons in the silkworm was shown as comparing with that in the mulberry leaf by means of gas chromatographic technique and mass spectrometry. The hydrocarbons from both the silkworm and mulberry leaf consisted of a mixture of odd- and even-numbered carbons and major hydrocarbons in the

wings were C_{34} to C_{36}, those in both blood and larva, C_{21} to C_{27}, and those in mulberry leaf, C_{16} to C_{20}. Large hydrocarbons, C_{32}, C_{34}, C_{38}, C_{40} and C_{41}, were found in the wings.

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113. Abdul-Mohsen M.E. Omar*1 and Shun-ichi Yamada*2 : Studies on Thioamides. III.*3 A New Synthetic Method of the 3,4-Dihydroisoquinoline Derivatives by the Cyclodesulfurization of Thioamides.

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In a preliminary step towards the realization of our exploratory project on using thioamides in the synthesis of the 3,4-dihydroisoquinoline nucleus, the present authors have recently accomplished the cyclization of several N-(2-arylethyl) thioamides with the general acidic condensing agents,[1] as reported for the cyclodehydration of N-phenethylamides under the Bischler-Napieralski reaction conditions,[2] and got the required 1-substituted-3,4-dihydroisoquinoline derivatives in good yield.

In continuation of this project, investigations were directed to take advantage of the presence of the active sulfur atom in these thioamides and attempt their cyclization, independently from the classical Bischler-Napieralski reaction and under milder conditions, by employing the common desulfurizing agents which can act as Lewis acids on such active centers. A part of the successful results of this investigation has been published, in a brief communication,[3] in advance to the present full detailed demonstration of the nature of this new type of ring closure as well as its establishment in the synthesis of the 3,4-dihydroisoquinoline derivatives.

Choosing N-homoveratrylthiobenzamide (Ia) as a model compound of the N-(2-arylethyl) thioamides series, several experiments were carried out to effect its cyclization with silver nitrate,[4~6] mercuric cyanide,[7] mercuric acetate,[8~10] mercuric oxide,[11~17] metallic mercury, iodine,[18,19] triethyl phosphite,[20~25] calomel, ethylmercuric chloride,

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