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Summary

Oxidation of O-benzoylthiamine disulfide (I) with hydrogen peroxide in acetic acid yielded O-benzoylthiaminic acid (IIa) and its stereoisomerical compound (IIIa). It was confirmed that these two compounds were in the relation of geometrical isomers which have not yet been found in thiol-type thiamine derivatives: the compound obtained in this experiment was *cis*-2-(2-methyl-4-amino-5-pyrimidyl)methylformamido-5-benzoyloxy-2-pentene-3-sulfonic acid (IIIa) of which olefin-CH_3 and SO_3^- groups were in *cis*-configuration. Alkali decomposition of IIIa afforded *cis*-thiaminic acid (IIIb) corresponding to geometrical isomer of IIb.

The configuration of these compounds was confirmed by elemental analyses, ultraviolet, infrared, nuclear magnetic resonance spectra and dissociation constants.

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110. Mieko Suzuki (née Saito), Eiko Akaike,*1 Kyosuke Tsuda,*2 and Nobuo Ikekawa*3 : Studies on the Sterol of *Bombyx mori* L. II.*4 Quantitative Analysis of Total Sterol in the Silkworm.

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The sterol nature in insects was investigated by many workers,[1] but only a few workers[2,3] have reported the quantitative analysis of sterols in insects. On the other hand, as it has been demonstrated that cholesterol was one of active ingredients of the brain hormone in the silkworm[4,5] and was also a precursor of ecdysone,[6] the quantity of sterol in the silkworm through all its developmental stages became important.

In the previous report,*4 it was shown by gas chromatographic analysis that silkworm sterols consisted of three sterols; cholesterol, β-sitosterol, and campesterol, and their compositions became different with the progress of age. The present paper

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deals with quantitative analysis of sterols in the whole body, fat body, blood, and wings of silkworm using gas chromatography. Both weight and lipid percentage of whole body, fat body, blood and wings were presented in Table I and those of whole body were shown as the sum of each amount of fat body, blood, wings, and remainder.

Table I. Silkworms used as Starting Material

Stages of silkworm	Number of test insects	Whole body Weight (g.)	Whole body Lipid (%)	Fat body Weight (g.)	Fat body Lipid (%)	Blood Weight (g.)	Blood Lipid (%)	Wings Weight (g.)	Wings Lipid (%)
Newly hatched larva	—	231.2	3.94	—	—	—	—	—	—
3-Day-old 4th instar larva	312	162.3	1.51	—	—	16.2	0.11	—	—
3-Day-old 5th instar larva	200	266.2	0.64	36.5	0.78	12.3	0.03	—	—
6-Day-old 5th instar larva	120	412.9	1.01	71.4	2.23	24.2	0.21	—	—
1-Day-old pupa	163	340.8	1.03	99.0	0.73	36.7	0.22	—	—
5-Day-old pupa	200	342.9	1.38	83.6	1.31	40.2	1.37	—	—
Moth just after emergence	365	269.2	3.24	—	—	—	—	40.0	0.19

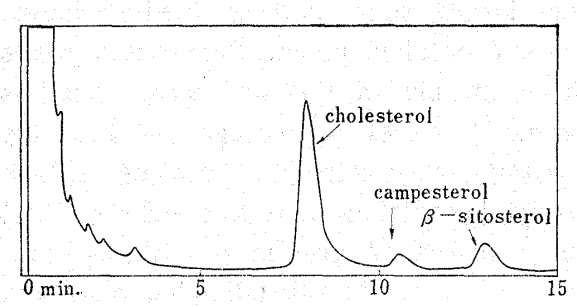

Fig. 1. Chromatogram of Unsaponifiable Fraction from Fat Body of Silkworm Pupa

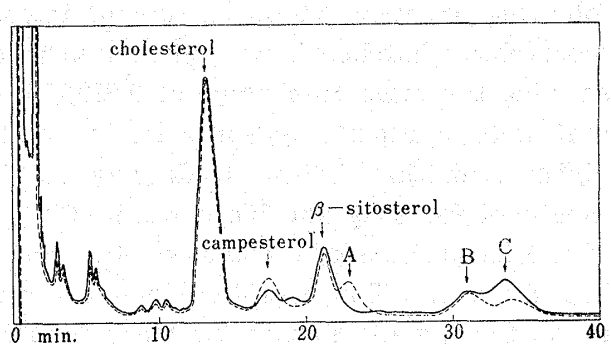

Fig. 2. Gas Chromatogram of Unsaponifiable Fraction from Wings of Moth and Blood on the 5th Instar Larva of the Silkworm
——— Wings
---------- Blood

A typical chromatogram of unsaponifiable fraction from fat body of silkworm pupa is presented in Fig. 1, and similar chromatograms were obtained for fat body in larvae. As shown in Fig. 2, the chromatogram from blood of the 5th instar larvae indicated three peaks for sterols, cholesterol, campesterol, and β-sitosterol, and three peaks of unknown substances, A, B, and C. That of wings gave three peaks of sterols, same peaks as in the blood, and two peaks of the unknown substances, B and C. The substance A was present in the 3-day-old 4 th instar, and 3-day-old and 6-day-old 5 th instar larvae, showing the largest amount in 6-day-old 5 th instar larva. As it will be published in the forthcoming paper, the substance B and C were hydrocarbons and the substance A was identified as triterpene, lupeol.

Both the total weight of sterols in the whole body and its percentage in the whole body, fat body, blood, and wings are shown in Table II. The amount and percentage of total sterols in the whole body were calculated from the sum of each amount in fat body, blood, wings and remainder.

Table II. Quantitative Analysis of Sterols in the Silkworm

Stages of silkworm	Whole body (mg.)	Total sterol (%)	Fat body (%)	Blood (%)	Wings (%)
Newly hatched larva	—	0.218	—	—	—
3-Day-old 4th instar larva	0.11	0.024	0.008	0.003	—
3-Day-old 5th instar larva	0.23	0.017	0.011	0.007	—
6-Day-old 5th instar larva	0.68	0.020	—	—	—
1-Day-old pupa	1.08	0.049	0.020	0.042	—
5-Day-old pupa	0.83	0.048	0.049	0.044	—
Moth just after emergence	0.62	0.083	—	—	0.016
Average	0.59	0.066	0.022	0.024	0.023

The amount of sterols in the whole body of a silkworm was 0.62 mg. and sterol percentage of live weight was 0.066% at an average. Sterol content in a silkworm increased gradually with lapse of time from 0.11 mg. in 3-day-old 4th instar larva to 0.62 mg. in the moth, showing the maximum value of 1.08 mg. in pupa immediately following pupation. Sterol percentage to the live weight of whole body in unfeeding insects (newly hatched larva pupa, and moth) was larger than that in feeding larva, showing the maximum value of 0.218% in the newly hatched larva. This fact suggests that sterol synthesis and/or selective retention of sterols in the unfeeding stage is higher than that in the feeding one. Furthermore, sterol percentages of the live weight of fat body and blood were 0.022% and 0.024%, respectively. Sterol percentage of the blood (0.024%) in the silkworm was markedly lower than that of a blood (0.21%) in mammals.[7] The sterol content in the fat body increased gradually with the progress of age, while that in the blood suddenly increased immediately following pupation.

From the cholesterol percentage of total sterols in pupa (67.1%) and in the 5th instar larva (64.4%) reported in the previous paper,*4 the amount of cholesterol was calculated as being about 0.56 mg. in a pupa and about 0.15 mg. in a larva of the 5th instar. These calculated amounts were larger than 0.02μg.[8,9] of cholesterol having the brain hormone activity in the dauer-pupa which did not show any sign of imaginal differentiation for more than 40 days after the extirpation of its brain immediately following pupation of the silkworm.[8,9]

The imaginal differentiation of the dauer-pupa is not induced without injection of 0.02 μg. of cholesterol or implantation of brain, even though the pupa has prothoracic gland as a secretory organ of ecdysone and contains a large amount of cholesterol. Therefore, it seems that cholesterol present in the body might be different from cholesterol isolated from the brain. On the other hand, it is interesting that the critical period of the brain hormone for the imaginal development is coincident with sudden increase of blood sterol.

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Experimental

Material——Newly hatched larvae, 3-day-old 4th instar larvae, 3-day-old and 6-day-old 5th instar larvae, 1-day-old and 6-day-old pupae, and moths of F_1 hydrid between two races, J. 124 and C. 124, were used as specimens. Each of fat body, blood, and remainder without digestive tract was collected from both larvae and pupae, and wings and remainder without rectum were collected from moths following dissection. Also, newly hatched larvae were used without dissection.

Method——Each of the materials mentioned above was homogenized and extracted with methanol. After evaporation of methanol, each residue was extracted with ether. Ether extracts were saponified with potassium hydroxide in ethanol. The unsaponifiable fractions were analyzed by gas chromatography using cholestane as the internal standard. The ratio of detector response of cholestane and cholesterol was 1 : 0.71 and detector responses of cholesterol, campesterol and β-sitosterol were regarded as the same. Gas chromatograph used was Shimadzu gas chromatograph Model GC-1B with hydrogen flame ionization detector, using the column packing of 1.5% SE-30 (G.E. methyl silicone gum) on Gas Chrom P (80～100 mesh, acid-washed and siliconized) and U-shape stainless steel column (1.5 m. × 4 mm., i.d.). Column temperature was 235° and nitrogen flow rate was 75 ml./min.

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Summary

The quantitative analysis of sterols in the silkworm was carried out by means of gas chromatographic technique. The amount of sterol in whole body was 0.59 mg. and sterol percentage of live weight was 0.066%, as an average, showing that sterol percentage in unfeeding silkworm was larger than that in feeding larva. Furthermore sterol percentage (0.024%) of blood in the silkworm was lower than that in mammals. The amount of cholesterol calculated from total sterol in the whole body was larger than threshold amount of brain hormone cholesterol in the silkworm for the induction of imaginal development. On the other hand, the critical period of the brain hormone for the imaginal development was coincided with time of sudden increase of blood sterol.

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