Notes

[Chem. Pharm. Bull. 14(9)1049~1051(1966)]

UDC 595.787.04 : 547.92

Mieko Suzuki (née Saito)*1 and Nobuo Ikekawa*2 : Studies on the Sterol of *Bombyx mori*. V.*3 Lupeol in Silkworm Blood.

(*The Sericultural Experiment Station, Ministry of Agriculture and Forestry*1 *and The Institute of Physical and Chemical Research*2)

In the previous communication of this series,[1] the demonstration of several unknown substances in the unsaponifiable fraction of silkworm larva by the gas chromatographic analysis was reported. Among these unknown substances, compound B and C in Fig. 1 were determined as hydrocarbons and the composition of silkworm hydrocarbons have already been reported.[2] This paper deals with the isolation and identification of lupeol eluted after β-sitosterol by gas chromatography (substance A in Fig. 1) from a unsaponifiable fraction of larval blood of silkworm. On triterpene in the silkworm and mulberry tree α-amyrin was isolated from mulberry bark,[3] a triterpene ($C_{30}H_{50}O$) from mulberry leaf[4] and lupeol from mulberry leaf,[5] from silkworm soil[5] and from silkworm

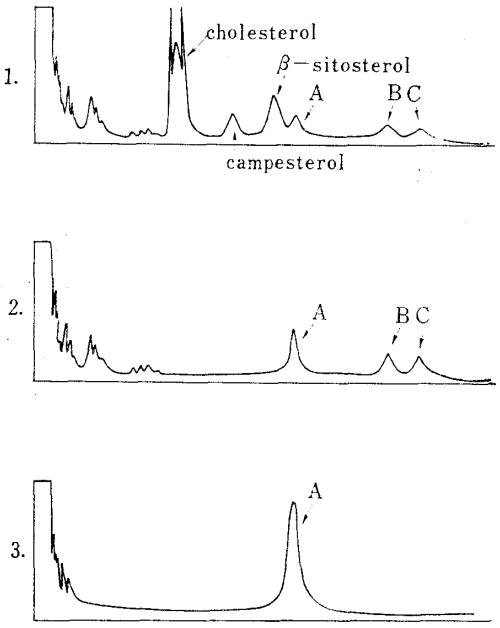

Fig. 1. The Gas Chromatograms of Unsaponifiable Fraction (1), Unsaponifiable Fraction without Sterol (2), and Triterpene Fraction obtained by the Column Chromatography (3), from the Blood of Silkworm Larvae

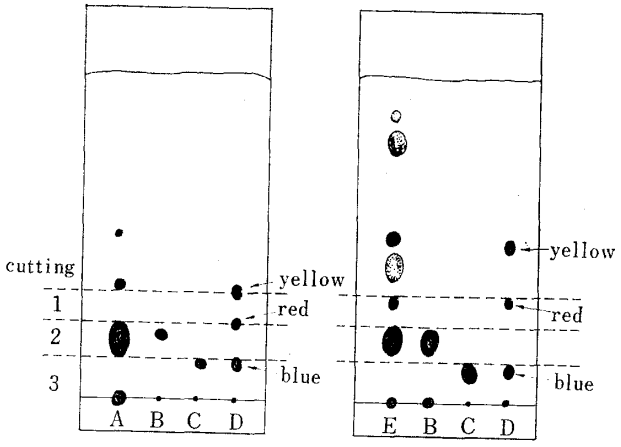

Fig. 2. The Thin Layer Chromatograms of Triterpene Fractions from the Silkworm and Mulberry Leaf

A. Sample from silkworm.
B. Lupeol, α-amyrin, β-amyrin.
C. β-Sitosterol, cholesterol.
D. Marker dye.
E. Sample from mulberry leaf.

*1 Wada, Suginami-ku, Tokyo (鈴木美枝子).
*2 Komagome, Bunkyo-ku, Tokyo (池川信夫).
*3 Part Ⅳ. N. Ikekawa, M. Suzuki, M. Kobayashi, K. Tsuda : This Bulletin, **14**, 834 (1966).

1) M. Suzuki, E. Akaike, K. Tsuda, N. Ikekawa : *Ibid.*, **14**, 830 (1966).
2) M. Suzuki, N. Ikekawa, E. Akaike, K. Tsuda : *Ibid.*, **14**, 837 (1966).
3) M. Oku : J. Agri. Chem. Soc. Japan, **12**, 555 (1936).
4) T. Ito, K. Kawashima, M. Nakahara, K. Nakanishi, A. Terahara : J. Ins. Physiol., **10**, 225 (1964).
5) M. Goto, M. Kamada, S. Imai, T. Murata, S. Fujioka, E. Fujita, Y. Hamamura : Annual Report of the Takeda Research Lab., **24**, 55 (1965).

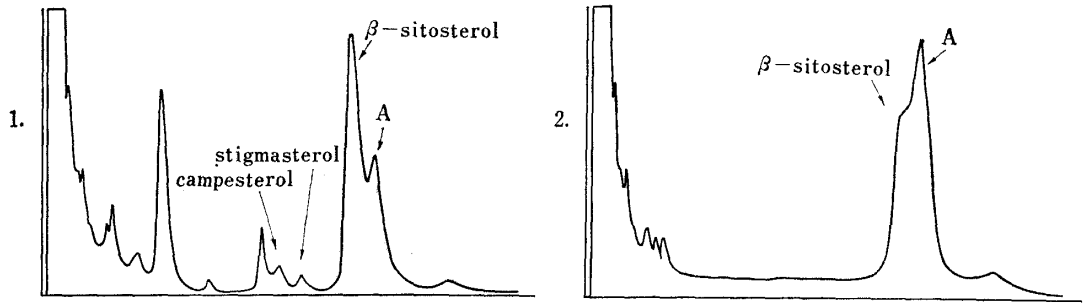

Fig. 3. The Gas Chromatograms of Unsaponifiable Fraction (1) and Triterpene Fraction obtained by the Florisil Column Chromatography (2), from the Mulberry Leaf

cocoon.[5,6] However, nothing is yet reported about triterpene in silkworm blood. Generally, triterpene is distributed in plant kingdom but not in animal kingdom, except for the recent report[7] on the isolation of a triterpene alcohol, tetrahymanol, from the ciliated protozoa.

After removal of sterol as digitonide from the unsaponifiable fraction of larval blood, the residue was fractionated by silica gel column. A triterpene alcohol was eluted after hydrocarbons and ketonic substances. In the process of the fractionation each of aforegoing material was checked by gas chromatography (Fig. 1). The substance which appeared to be triterpene was separated with thin layer chromatography, giving the same Rf value on the chromatogram with those of so-called triterpene alcohol : lupeol, α-amyrin and β-amyrin (Fig. 2). The crude crystals eluted from the main spot of the chromatogram were repurified by thin layer chromatography to give the needles, m.p. 212~213°. The retention time of this material in gas chromatography was identical with lupeol as presented in Table I. Lupeol and α-amyrin were separated on the column packing of XE-60, but not on that of SE-30 or QF-1. The triterpene alcohol gave m/e 426 (M^+ peak), m/e 408 (M^+-H_2O peak) and other peaks corresponding to lupeol in mass spectrometry. The infrared spectrum of this substance was also identical with that of lupeol.

The unsaponifiable fraction of mulberry leaf was separated by florisil column. The triterpene alcohol fraction which was eluted with hexane (Fig. 3-2), was purified with

Table I. Relative Retention Times of Triterpene from Silkworm and Mulberry Leaf

Compounds	Column				
	QF-1[a]		SE-30[b]		XE-60[c]
	free	acetate	free	acetate	free
Cholesterol	1.00 (6.0 min.)	—	1.00 (8.0 min.)	—	1.00 (12.8 min.)
Campesterol	1.33	2.11	1.31	1.91	—
β-Sitosterol	1.58	2.58	1.62	2.44	—
Lupeol	2.00	3.34	—	2.41	1.95
α-Amyrin	1.98	3.34	1.74	2.40	1.88
Mulberry leaf triterpene	2.00	3.34	—	2.41	1.95
Silkworm triterpene	2.00	3.34	—	2.42	1.95

a) 2% QF-1, column temp., 224°; N_2, 90 ml./min.
b) 0.75% SE-30, column temp., 232°; N_2, 90 ml./min.
c) 1.5% XE-60, column temp., 222°; N_2, 80 ml./min.

6) M. Oku : J. Agri. Chem. Soc. Japan, **10**, 46 (1934).
7) F.B. Mallory, J.T. Gordon, R.L. Conner : J. Am. Chem. Soc., **85**, 1363 (1965).

silica gel column followed by thin layer chromatography by the same procedure as used in the isolation from larval blood. The crystalline material thus obtained was identical with lupeol as shown by means of gas chromatography (Table I), mass and infrared spectrometry. From the measurement of peak areas on gas chromatogram of unsaponifiable fraction from mulberry leaf (Fig. 3-1) the ratio of four compounds, campesterol,[8] stigmasterol (unidentified), β-sitosterol[8] and lupeol was calculated as 4:3:65:28. These facts suggest that lupeol in the blood may be originated from the mulberry leaf. It is interesting that lupeol was found in blood without undergoing decomposition in the insect body.

Experimental

Material——The 5th instar larva of 6 days old of F_1 hybrid between two races, J. 124 and C. 124, and mulberry leaves from Ichinose race were used as materials. The blood of 1.361 g. was collected in methanol from 4.382 larvae which were injured at caudal legs.

Isolation of Lupeol from the Silkworm——The larval blood was homogenized and extracted with methanol. After removal of the methanol, the residue was refluxed with ether and the ether extract was saponified with 10% KOH in ethanol. Sterol was separated as digitonide from unsaponifiable fraction and non-sterol substance, 423.8 mg., was dissolved in a small amount of hexane, then the insoluble substances were removed by filtration. Gas chromatogram of this fraction was shown in Fig. 1-2. The hexane solution was fractionated by silica gel (Mallinckrodt) column with the solvents of hexane (400 ml.), 5% benzene in hexane (300 ml.), 10% benzene in hexane (500 ml.), and 20% benzene in hexane (500 ml.). Gas chromatographic analysis showed that the fraction eluted with 10% benzene in hexane contained triterpene. This fraction was developed by thin layer chromatography on silica gel G using chloroform system. The main spot, showing the same Rf value as those of lupeol, α- and β-amyrin, was eluted with tetrahydrofuran. The crude crystalline material obtained by this procedure was purified repeatedly by the same procedure.

Recrystallization from hexane-acetone gave needle crystals, m.p. 212~213°. The infrared spectrum of this substance was identical with that of lupeol: 1740 cm^{-1}(m), 1640 cm^{-1}(m), 883 cm^{-1}(s) (end methylene group), 1470 cm^{-1}(s), 1042 cm^{-1}(m), 1015 cm^{-1}(m) (3β-OH). The mass spectrum which was taken by Hitachi Mass Spectrometer Model RMU-6D was also identical with that of lupeol.

Isolation of Lupeol from Mulberry Leaf——The mulberry leaf powder was extracted with ether for a week. The extract was dissolved in acetone and the acetone soluble fraction was concentrated to 1/5 volume of the starting solution. After removal of precipitate, the supernatant was saponified with 10% KOH in ethanol. The unsaponifiable fraction was fractionated by a florisil column with hexane. The first elute contained hydrocarbons and triterpene alcohol was separated by silica gel column with the same procedure as described above. Needle crystals were obtained by the purification of thin layer chromatography. The melting point, infrared spectrum and gas chromatographic retention time of this material were identical with those of lupeol.

Gas Chromatography——A Shimadzu gas chromatograph Model GC-1B with hydrogen flame ionization detector was used in this study. The column was U-shape stainless steel, 1.5 m. × 4 mm. i.d. The column packings used for this work were made as following: Gas Chrom P (80~100 mesh) was washed with 36% HCl, siliconized with dimethyldichlorosilane in toluene, and coated with 2% QF-1 (D.C. fluorinated alkyl silicone polymer) in toluene, 0.75 % SE-30 (G.E. methyl silicone gum) in toluene and 1.5% XE-60 (G.E. cyanoethyl methyl and dimethyl silicone gum) in acetone, respectively.

(Received January 14, 1966)

8) K. Miyauchi, M. Saito, N. Ikekawa: Bull. Sericul. Exp. Station, **18**, 431 (1964).