AMINO ACID COMPOSITION OF THE FIBRES OF SOME SILK WORM SPECIES (*PHILOSAMIA RICINI, ANTHERAEA MYLITTA* AND *ATTACUS ATALUS*)

Introduction

ALTHOUGH the finest natural silk is produced by the mulberry leaf-consuming *Bombyx mori* which has been the universal topic of biochemical research, there are several other Lepidopterans that spin silk. All the eight species of *Antheraea* (*Antheraea mylitta, papia, roylei, andamana, prithii, knyvetti, sivalika* and *halferi*) produce the world famous tasar silk and the eri worm *Philosamia ricini* another species, spins silk that is claimed second in quality only to mulberry silk.

Silk is synthesized in the nature larva by a pair of silk glands situated on either side of the mouth of the insect. They are long tubular structures 25–40 cm long and about 2–3 mm wide and fill the entire coelonic cavity of the insect.

Silk, the thread extruded by the silk worm consists of two types of protein—the filamentous fibroin and sericin the cementing material coated on the filaments which binds them together. The amino acids required for silk synthesis are derived from the heaemolymph [1].

Significant qualitative and quantitative changes observed in both the silk glands and haemolymph of *P. ricini*[2] and in silk gland of *A. mylitta* (unpublished)[3] during fifth instar larval development and spinning period induced us to study the amino acid composition of the silk fibrs of the above said insects and of another Lepidopteran species *Attacus atalus.*

Materials and Methods

Fifty mg of each of the thoroughly cleaned and dried silk fibres were subjected to both acid (5 ml, 6N HCl) and alkaline (5 ml, 6N NaOH) hydrolysis in a sealed tube under nitrogen at 100°–110° C for about 24 h till the hydrolysates gave negative biuret reaction.

The acid-free hydrolysate (pH 4) after repeated distillation *in vacuo* was subjected to two-dimensional chromatography[4] employing Whatman No. 1 filter sheets with phenol-ammonia (80%, v/v) and butanol-acetic acid-water (12 : 3 : 5, v/v/v) as irrigating solvents. Individual amino acids in both the hydrolysates were estimated by Lee and Takayashi's method[5], while the total free amino acids were determined by Rosen's method[6]. The alkaline hydrolysate was used for detection of tryptophan. Specific spray reagents[7–11] were employed for indentification of individual amino acids.

Results and Discussion

Table I represents the amino acid composition of the three silk fibres analysed.

Presence of alanine, arginine, aspartic and glutamic acids. glycine, histidine, isoleucine, leucine, lysine,

TABLE I

Amino acid composition (mg/100 mg) of silk fibre*

Amino acid	*P. ricini*	*A. mylitta*	*A. atalus*
Alanine	11·8 ± 0·3	8·8 ± 0·3	8·5 ± 0·5
Arginine	4·9 ± 0·1	2·7 ± 0·2	4·0 ± 0·2
Aspartic acid	6·3 ± 0·2	4·7 ± 0·9	4·4 ± 0·2
Gultamic acid	7·8 ± 0·7	4·6 ± 0·1	7·9 ± 0·1
Glycine	9·3 ± 0·3	12·1 ± 0·1	11·3 ± 0·1
Histidine	9·0 ± 0·3	7·0 ± 0·01	6·6 ± 0·5
Lysine	5·1 ± 0·1	4·6 ± 0·3	4·2 ± 0·2
Leucine-Isoleucine	5·1 ± 0·1	4·6 ± 0·2	4·2 ± 0·2
Proline	2·5 ± 0·3	2·8 ± 0·2	2·6 ± 0·25
Phenylalanine	2·8 ± 0·3	2·2 ± 0·2	4·0 ± 0·25
Serine	11·8 ± 0·7	10·6 ± 0·1	13·3 ± 0·6
Tyrosine	5·2 ± 0·1	10·5 ± 0·3	9·2 ± 0·3
Threonine	3·5 ± 0·3	8·9 ± 0·5	10·2 ± 0·7
Tryptophan	Trace	Trace	Trace
Valine	Trace	3·8 ± 0·1	Trace
Total free amino acids **	95	99	97

*Expressed as mean values ± standard error of four samples of fibres analyzed in each case.

** Expressed in terms of glycine.

TABLE I A

Bombyx mori* (*Reference data*)

(Amino acid composition of polypetides in fibroin and sericin fractionated by gel electrophoresis at acid pH in 4m urea) (mole %).

Amino acid	Fibroin	Sericin
Alanine	29·3	4·6
Arginine	0·5	2·3
Aspartic acid	1·3	14·5
Cystine (half **	0·2	0·3
Glutamic acid	1·0	4·8
Glycine	44·5	13·9
Histidine	0·2	1·6
Isoleucine	0·7	0·7
Leucine	0·5	1·2
Lysine	0·3	8·4
Methionine	0·1	0·1
Proline	0·3	0·4
Phenyl aline	0·6	0·4
Serine	12·1	32·3
Tyrosine	5·2	2·6
Threonine	0·9	8·4
Valine	2·2	3·2

* Reproduced from Lucas, F. and Rudall, K. M., In *Comprehensive Biochemistry*, 1968, 26B, 475-558, (cited under reference No. 13).

** S-Carboxyamidomethylated cysteine.

phenylalanine, proline, serine, threonine and tyrosine was detected in measurable quantities. Valine although present in appreciable quantity in the tasar silk fibre of *A. mylitta*, was detected only in traces in the fibres of *Attacus atalus* and *Philosamia ricini*. The sulphur containing amino acids, methionine and cystine were conspicuous by their absence in all the three fibres. This is in line with the previous findings in the spider silk[12]. On the contrary, cystine was detected in *B. mori* in measurable amounts in both the protein fractions of the silk *i.e.*, silk, sericin and fibroin[13]. Also Gamo *et al.*[14], demonstrated the presence of SH groups in the *B. mori* silk fractions.

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Biochemistry Department, The University, Allahabad 211 002, *February* 25, 1978.

RADHA PANT.
BALAGOPALAN UNNI.

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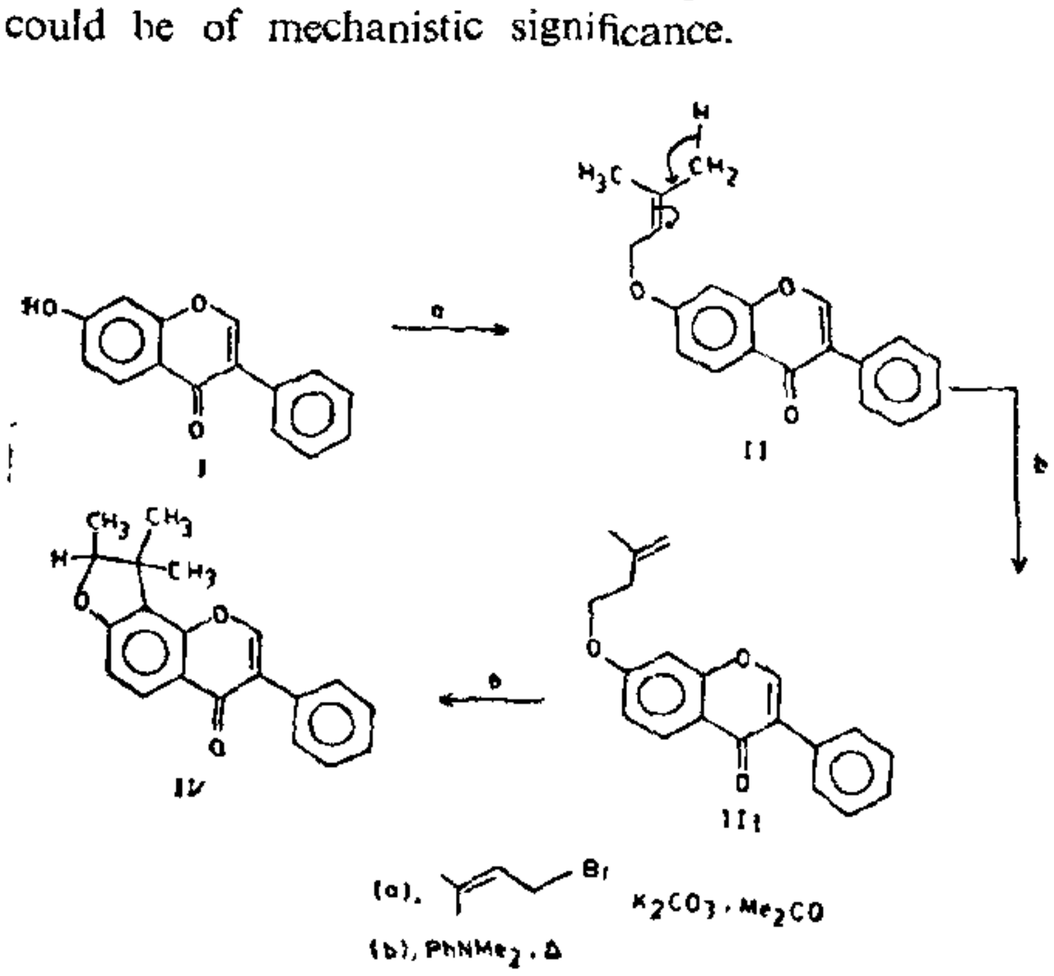
A NOVEL OBSERVATION IN THE CLAISEN REARRANGEMENT OF 7-PRENYLOXYISOFLAVONE

IN continuation of our work on the Claisen reararngement of cinnamyl and prenyl ethers of complex polyphenols[1–4], the rearrangement of 7-prenyloxyisoflavone in refluxing *N*, *N*-dimethyl aniline was studied. As the results obtained were different from the earlier reports, our observations are reported here.

7-Prenyloxyisoflavone (II) prepared from 7-hydroxyisoflavone[5] (I) by refluxing, with prenyl bromide in acetone and potassium carbonate was crystallised from benzene-light petroleum mixture as colourless crystals, m..p 155–56° C; λ_{max} (MeOH) 248 and 300 nm (log ϵ 4·53 and 4·21 respectively) (Found: C, 78·0; H, 6·1. $C_{20}H_{18}O_3$ requires C, 78·4; H, 5·9%); 60MHz NMR ($CDCl_3$): δ 1·75 [2s, 6H, $=C(CH_3)_2$], 4·40 (d, J = 7Hz, 2H, $-OCH_2-$), 5·40–5·48 (m, 1H, $=CH-$) 6·78, 6·81 (2d, J_o = 9Hz, J_m = 2·5Hz, 1H, H–6), 6·87 (d, J_m = 2·5 Hz, 1H, H–8), 7·35–7·41 (m, 5H, $-C_6H_5$), 7·81 (s, 1H, H–2) and 8·10 ppm (d, J_o = 9Hz, 1H, H–5).

The above prenyloxyisoflavone (II) when refluxed with *N*, *N*-dimethylaniline for 3 h gave a product in almost quantitative yield, insoluble in aq. Na_2CO_3; m.p. 126–28° C. The NMR spectrum showed the resonance signals of all the three aromatic hydrogens of ring A as in the parent ether (II). However, the prenyloxy unit of II was found in the form of 3-methyl-3-butenyloxy form, showing two triplets at δ 2·48 and 4·12, a singlet of an unsaturated methyl group at δ 1·75 and a multiplet of two olefinic hydrogens at δ 5·27. Hence, the structure of this product must be 7- (3-methyl-3-butenyloxy) isoflavone (III) and it represents an allylic arearrangement of the hydrogen atom of the prenyloxy group as shown in II. Such a rearrangement has not been reported earlier and could be of mechanistic significance.



When III was heated for a longer period, it gave two products. The minor compound was found to be simple 7-hydroxyisoflavone (I) and the major compound crystallised from ethyl acetate-light petroleum mixture to give 4″, 4″, 5″-trimethyl-4″, 5″-dihydrofuro (2″, 3″: 7, 8) isoflavone (IV), m.p. 136–137° C; n.m.r. spectrum: δ 1·27, 1·51 [6H, s, $>C(CH_3)_2$], 1·37 (3H, d, J = 6·5Hz, CH_3-CH-O), 4·51 (1H, q, J = 6·50Hz, $-O-CH-CH_3$), 6·77 (H, d, J = 9Hz, *H*–6), 7·23–7·53 (5H, m, $-C_6H_5$), 7·87 (1H, s, *H*–2) and 8·10 ppm (1H, d, J = 9Hz, *H*–5).