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BIOCHEMICAL BUILDING BLOCKS OF A LEPIDOPPERAN INSECT, *SCHOENOBIUS INOTATA*

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ABSTRACT

Schoenobius innate, a pheromone producing insect, commonly known as rice stem borer, was studied for its biochemical composition namely lipid, carbohydrate and amino acids. The studies involved the application of column, thin layer and gas liquid chromatography besides mass and proton magnetic resonance spectrometry. Amino acid analysis was performed on automatic amino acid analyser. No such report on this species has so far appeared.

Introduction

Schoenobius inotata (Pyralidae-Lepidoptera) commonly known as rice stem borer is a small orange yellow to off white moth with black dot on the fore wings and is widely distributed throughout Pakistan. It's larvae arises from the eggs laid down on the under sides of the leaves of rice plant, feed for a few days on these leaves and then, bore into the stem and feed on the soft tissues inside to cause "dead heart", "dead shoot" and/or "white head" and even kill the plant (Haque, 1970).

Much biochemical work has been carried out on lepidopteran insects (Yoo and Jeong, 1988; Bean and Shirk, 1988; and Barrett, 1974) but unfortunately it is restricted to only a few insect types like silk worms (Kato *et al.*, 1985; Shimada *et al*, 1987) and tobacco plant worms (Webb and Riddiford, 1986; Cole and Wells, 1990) though discretely, few studies on free amino acids (Ramakrishnan and Raghavan, 1978; Mathur and Tiwari, 1976; Lazar and Mohammad, 1988), sugars (Fell, 1990, Minder *et al*, 1984, Franke *et al.*, 1987) and lipids (Basalingappa *et al.*, 1982; Gherghel and Petruta, 1986; Kaplan *et al.*, 1986) of other lepidopteran insects can be found in the literature, no such report is available on *S. inotata.* The biochemical composition of this specie is being presented in this paper for the first time.

Experimental

Sample collection:

Larvae were collected from lower Sindh area in the months of September, October and November. After careful field surveys, Thatta, Ghulamullah, Sujawal and Jungshahi areas were chosen for collection because of heavy infestation. Affected stems, which are indicated by decolourization at the fruit bearing end of the plant were ripped open and

hidden larvae were forger tapped into separate sample bottles whose rubber lids were finely perforated for air passage. Only fully grown larvae (last instar) were used in this study.

In the areas where rice fields had already been cut, the stubbles were dug out and searched carefully for larvae. In heavily infested areas 70-80% of stubbles were inhabited by larvae.

Sample bottles with alive larvae were transported from fields to laboratory directly and processed for study.

Extraction of organic substances:

Fully grown gut emptied larvae of approximately equal weight and size were asphyxiated as cited by Cook *et al* (1984). They were then delicately washed with double distilled water, blotted dry on soft tissue papers, pooled and homogenized to make 10% homogenate in double distilled water using an electrical homogenizer, Janke and Kunkel, IKA Labortechnik Ultra-Turrax T-25. The temperature was between 15°-18°C.

The total homogenate thus obtained was extracted thrice with a mixture of chloroform: methanol (2:1 v/v) (Folch *et al*., 1957) and all three extracts were pooled and evaporated. Lipid fraction that resulted was weighed and analyzed for fatty acids

Aqueous phase remaining after extraction was briefly centrifuged at 3000 rpm to remove insoluble particles and subjected to column chromatography on cation exchange resin IR 120 x 8, as described by Kaludi *et al*. (1985). The eluates obtained from acetic acid and water were pooled, evaporated and analyzed for carbohydrates. Ammonia fraction was analyzed for amino acids.

Fatty acid analysis:

Lipid content was analyzed for fatty acids after converting total lipid fraction to fatty acid methyl esters through transmethylation using the method of Hammond and Lundberg (1953) with some modification. Methanolic sulfuric acid (85:15, v/v) was used and the process was continued for 2 hr at 80°C. Petroleum ether Analar (b.p. 40-60°C) and water were used to extract the transmethylated products. The etherial layer was drawn from above by a dropper into a small tube. Three such extractions were made, combined and the solvent was finally evaporated. The transmethylated samples were transferred into small tubes and stored in a refrigerator (Salam *et al*, 1970).

Methyl esters were analysed on Shimadzu Model GC-14A Gas chromatograph equipped with a flame ionization detector annexed with Shimadzu Integerator Model C-R6A chromatopac. Methyl esters were injected into a glass column (5ft 10 in x 0.2 in;

20% DEGS coated on 80-100 mesh with chromosorb WHP) having column temperature programmed at 150°C -5°C/5 min - 250°C. Nitrogen flow rate was 22 ml / min, hydrogen 20 ml / min and chart speed 1.6 mm / min. The identification of peaks of fatty acids was carried out by cochromatography with standards (SUPELCO) and relative retention times.

Carbohydrate analysis:

A portion of acetic acid / water fraction was treated with acetone in order to check the presence of polysaccharides (Kaludi *et al,* 1985). Remaining fraction was concentrated by evaporation under reduced pressure and subjected to thin layer chromatography on silica coated plates size 24x18 ans. Three different solvent systems (Kaludi *et al.,* 1985) (a) butanol: acetic acid: water (4:1:5 v/v) (b) ethyl acetate:pyridine:water (10:4:3 v/v) and (c) propanol: pyridine: water: acetic acid (8:8:4:1 v/v) were used for resolution: Identification was done by cochromatography with standard sugars from E. Merck and Fluka and orcinol was used as colour developing agent (Kimmich, 1980). Each identified sugar was subjected to mass spectrometry and proton magnetic resonance (Finnigan MAT-112 spectrometer coupled with PDP11/34 computer system and Bruker AM-300 spectrophotometer.

Amino acid *analysis:*

The eluate obtained with 0.5N ammonia solution was evaporated under reduced pressure at 4°C to afford free amino acids which were analyzed by paper chromatography and then authenticated on Biotronik Amino acid Analyzer LC-6001.

Results and Discussion

The field collection data of larvae is shown in Table-1. The results of fatty acid, carbohydrate and free amino acids profile have been presented in Tables-2, 3 and 4 respectively.

Table-1
Weekly collection data of larvae

S.No.	Month	Place	No of fields visited	Wt. of larvae collected
1	Sept.	Ghulamullah	4	8 gms
2	Sept.	Ghulammullah	3	6 gms
3	Oct.	Jangshahi	6	4 gms
4	Oct.	Thatta Sujawal	3	6 gms
5	Oct.	Thatta Sujawal	4	4 gms
6	Oct.	Ghulamullah	6	15 gms
7	Nov.	Ghulamullah	3	12 gms
8	Nov.	Ghulamullah	4	12 gms
9	Nov.	Ghulamullah	4	15 gms
10	Nov.	Ghulamullah	7	2 gms.

Fatty adds analysis (Table-2) shows the presence of fatty acids ranging from caprylic acid ($C_{8:0}$) to arachidic acid ($C_{20:0}$) in saturated fatty acids. In unsaturated types only three acids are found namely palmitoleic acid ($C_{16:1}$), oleic acid ($C_{16:1}$) and oleic acid ($C_{18:2}$). In saturated adds, Stearic acid (C18;o) is most abundant followed by palmitic acid ($C_{16:0}$). Arachidic acid ($C_{20:0}$) is found in relatively small quantity. In unsaturated acids, oleic acid ($C_{18:2}$) is the forerunner with palmitic acid ($C_{16:1}$) and linoleic acid ($C_{18:2}$) to follow respectively. Unsaturated fatty acids, in over all, make 34% of the total fatty adds

In carbohydrates (Table-3) β-D-xylose, α-D-glucose and maltose were identified and characterized by their co-tic with standard samples, field desorption mass spectra revealing molecular ion peaks at m/z 150, 180 and 342, respectively, and the proton magnetic resonance spectra exhibiting the anomeric protons at 431, 5.10 5.14 and 5.67 respectively (Kazmi *et al.,* 1990). Existence of xylose in β-form, is confirmed by its large coupling constant (8.16 Hz) in proton magnetic resonance spectra. The absence of insoluble fraction as observed upon addition of acetone to water extracts indicated that no soluble polysaccharides are present in this species.

Free amino acid pattern showed the presence of 17 amino acids which are presented in Table-4 along with their concentration in μM /ml and μ M/larva.

Table-2

Fatty acid composition (weight %) of total lipids of adult larvae of *S. inotata.*

No.	Systematic name	Common name	Molecular Formulae	R.R.T.	Weight%
	Saturated Acids				
1	Octanoic acid ($C_{8:0}$)	Caprylic acid	$C_8H_{16}O_2$	0.33	6.10
2	Decanoic acid ($C_{10:0}$)	Capric acid	$C_{10}H_{20}O_2$	0.66	4.81
3	Dodecanoic acid ($C_{12:0}$)	Lauric acid	$C_{12}H_{24}O_2$	1.00	5.80
4	Tetradecanoic acid ($C_{14:0}$)	Myristic acid	$C_{14}H_{28}O_2$	1.33	3.95
5	Hexadecanoic acid ($C_{16:0}$)	Palmitic acid	$C_{16}H_{32}O_2$	1.65	19.25
6	Octadecanoic acid ($C_{18:0}$)	Stearic acid	$C_{18}H_{36}O_2$	1.80	25.53
7	Eicosanoic acid ($C_{20:0}$)	Arachidic acid	$C_{20}H_{40}O_2$	2.08	0.97
	Unsaturated acids				
8	Hexadecenoic acid ($C_{16:1}$)	Palmitoleic acid	$C_{16}H_{30}O_2$	1.71	8.98
9	Octadecenoic acid ($C_{18:1}$)	Oleic acid	$C_{18}H_{34}O_2$	1.90	21.21
10	Octadecadienoic acid ($C_{18:2}$)	Linoleic acid	$C_{18}H_{32}O_2$	1.93	3.40

*R.R.T = Relative retention time with respect to palmitic acid.

Studies on carbohydrates show the presence of monosaccharides fl-D-Xylose, a-D-glucose and disaccharide, maltose. No soluble polysaccharides are indicated. Among disaccharides, interesting feature is the absence of trehalose in this species in grown up larvae. Trehalose is a normal constituent in many insects (Alumot *et al*, 1969). May be that trehalose is present in this species in adult moth but not in growing or grown up larvae. In adult moth, trehalose is reported to have formed from glucose in phornia regina (Diptera) (Evans and Dethier, 1957). It may be possible that, either, in this species only adult moth have trehalose or, contrary to other insects, *S. inotata* does not include trehalose to sugar itself. Studies on adult moth can throw light on this maze. Existence of xylose in β-form is also a peculiar feature of this specie.

Table-3

Separation and identification of carbohydrate content of adult *S. inotata* using different solvent systems.

	SOLVENT SYSTEM						
	BAW Rf		EPW Rf		PPWA Rf		Molecular ion (M^+)
Sugar	std	sam	std	sam	std	sam	
β-D-xylose	0.342	0.340	0.354	0.355	0.325	0.326	150
α-D-Glucose	0.228	0.227	0.331	0.331	0.331	0.332	180
Maltose	0.141	0.143	0.294	0.292	0.208	0.210	342

	PMR(s) anomeric proton
β-D-xylose	4.51 (IH, d, J = 8.16 Hz)
α-D-Glucose	5.13 (1H, d, J = 3.62 Hz)
Maltose	5.15 and 5.67 (each 1H, d, J = 3.68 and 3.71 Hz)

R_{fstd} : Rf values of standard sugars
Rf_{sam} : Rf values of identified sugars
BAW: Butanol: acetic acid: water, upper layer (4:1:5 v/v)
EPW: Ethyl acetate: pyridine: water (10:4:3 v/v)
PPWA: Propanol: pyridine: water:acetic cid (8:8:4:1 v/v).

Table-4

Free amino acids in adult larvae of *S. inotata*

Amino acid	Concentration in μ Mole/ml	Concentration in μMole/larvae
Cys/Cysteic acid	21.28	0.060
Asp	6.4	0.017
Thr	2.32	0.006
Ser	0.88	0.002
Glu	11.23	0.030
Pro	0.0004	0.000006
Gly	4.28	0.011
Ala	6.61	0.017
Val	1.23	0.003
Met	0.98	0.002
Ile	2.44	0.006
Leu	1.23	0.003
Tyr	0.29	0.001
Phe	0.33	0.001
Hist	0.98	0.0026
Iys	9.88	0.026
Arg	0.02	0.00005
Total	70.52	0.18765

Fatty acid profile shows high percentage of stearic acid ($C_{18:0}$) and palmitic acid ($C_{16:0}$) in saturated series, whereas of three unsaturated fatty acids present, oleic acid ($C_{18:1}$) is fore runner followed by palmitoleic acid ($C_{16:1}$) and linoleic add (C1E:2). Contrary to sugars, fatty acids represent a wide spectrum ranging front Cs to C2o. Such heterogenity in fatty acids is interesting.

Among lepidoptera, essential fatty acids deficiency is most apparent as a characteristic failure of ecdysis at the pupal to adult moth (Stanley *et al*, 1984). Linolenic acid ($C_{18:3}$) and certain C_{20}/C_{22} analogues with the triennia n-3 structure have special significance for ensuring the emergence of normal adults. Among dienoic acids, linoleic ($C_{18:2}$) and $C_{20:2}$ are also thought important for normal adult emergence (Dadd, 1983). In our studies, we have found oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and palmitoleic acid

($C_{16:1}$). Presence of palmitoleic acid is a peculiarity since, to our knowledge, this fatty acid is obscure in other lepidoptera speices. We could find no $C_{20:3}$ or C_{22} polyunsaturates in *S. inotata* though all these are required for normal moth development and emergence. However, as chain elongation and shortening to interconvert fatty acids both appear normal phenomena in lepidopteran insects (Stanley *et al*., 1984), it is not a wild thought to assume that these three fatty acids play as architectural blocks for synthesis of higher unsaturated fatty acids.

The high titre of amino acids (7052 μMole/ml) in these insects is a special characteristic of this class. This high titre has been related with protection of insect during dehydration and during cold season (Morgan and Chippendale, 1983). Lysine which makes 14% of all 17 amino adds identified in *S. inotata* larvae in this study is reported to have function in protection against toxic salutes produced during cold season. In addition proline, serine, alanine, histidine, threonine and glutamine have been related with survival in winter season (Morgan and Chippendale, 1983). Our results are consistent with already reported data in the literature (Mansingh, 1967; Mitsuhashi, 1978) with the exception of proline which was found low in our studies. This may be due to the fact that *S. inotata* larvae grow inside the rice stems where there is always a constant humid atmosphere. A high proline is predominant in dry season (Levitt, 1980) and as such the low concentration of proline in this specie is quite justified.

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