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ROLE OF PTERIDINES IN CHAEMOTAXONOMY OF IMMATURES OF TREEHOPPERS (HOMOPTERA: MEMBRACIDAE)+

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Four different pteridines, viz. xanthopterine, *iso*xanthopterine, biopterin and pterine, were separated through paper chromatography from whole body squash, from squashed body parts, and haemolymph of the immatures of four different species of treehoppers using seven different solvent systems. Whole body squash and squashed body parts of the third instar immatures of all the species gave the most characteristic patterns in *n*-butanol. Rf values were found to be similar to those of pteridines provided by Blakley [1]. Pterine and biopterin were found only in *Oxyrhachis taranda* Fabr. and only the latter in *Oxyrhachis* sp. in addition to xanthopterine and isoxanthopterine in both species and also in *Gargara contraria* Distant but only the former in *G. nigroapica* Funk.

INTRODUCTION

Since Hadorn and Mitchell [2] first discovered the fluorescent compounds in the eye colour mutant of *Drosophila* and these were isolated and identified by Forrest and Mitchell [3, 4], their presence has been demonstrated in a number of insect groups in different parts of their bodies [5, 6, 7, 8, 9, 10 and 11]. It was found that several pteridines could be correlated with the taxonomic positions in Diptera and later Micks *et al.* [12] were able to separate morphologically indistinguishable strains of *Aedes aegypti* on the basis of chromatographic patterns of pteridines. Although Bhalla [7] found striking qualitative and quantitative differences in mosquitoes on the basis of pteridines, yet in some identical phenotypes he found different patterns but in some distinguishable phenotypes similar patterns.

Within Hemiptera Good and Johnson [13] and Forrest *et al.* [14] reported them in *Oncopeltus* of Heteroptera and in cicadids of Homoptera. Bhalla [7] found an increase or decrease and in some cases entire disappearance of pteridines with age. Hudson *et al.* [15] showed that xanthopterine increases tenfold during embryonic development in *Oncopeltus* and that in later stages *iso*xanthopterine and xanthopterine occur chiefly in the epidermis but also in the fat bodies, in the dried excreta and in the uric acid. Membracid treehoppers in general and their immatures in particular in many cases have been regarded very difficult to differentiate [16, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26], but with reference to these fluorescent compounds the group is entirely unexplored and this necessitated the present investigation.

MATERIALS AND METHODS

Of the four species presently studied, *Gargara contraria* Distant and *G. nigroapica* Funkhouser, were collected from *Withania somnifera* L., while *Oxyrhachis taranda* Fabr. and *Oxyrhachis* sp. were collected from *Acacia modesta* Wall. Species were maintained in laboratory on their respective host plants as well as on a common host plant, *Prosopis specigera* L., in separate cages. Immature material for chromatography was prepared by pressing the nymphs with a clean glass slide on a chromatographic paper and the debris was removed with fine forceps.

In the immatures from which anal tube and legs had been removed with the help of fine forceps, the head, thorax and abdomen were squashed separately as described for the whole body squash and used for chromatography. Also the abdomen of an immature was pricked with a fine sterile needle and the exudating haemolymph was brought into contact with the chromatographic paper. All chromatograms were prepared using Whatman No. 1 chromatograph paper. Samples were applied along a line 1 cm from the edge of the paper and subjected to ascending chromatography in glass jar (60 x 60 x 60 cm) using different solvent systems (1) *n*-butanol, acetic acid, water (4: 1:

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1); (2) *n*-butanol, acetic acid, water (15: 3: 7); (3) 3% ammonium chloride; (4) 5% citric acid; (5) *n*-propanol, 1% ammonia solution (2:1); (6) -*sec*-butanol, formic acid, water (8:2:5); (7) *n*-butanol, acetone, water (4:5:1).

Chromatography was continued for 4 hr. with the solvent systems 1,2,5,6, and 7 but only for 1 hr in the solvent systems 3 and 4. The chromatograms were air-dried and examined under ultraviolet light. The characteristic fluorescent patterns observed were marked with a carbon pencil and R_f values of characteristic spots were calculated. R_f values of different peteridines were compared with the data provided by Blakley [1].

RESULTS

All the four species tested produced characteristic fluorescent patterns of varying degrees of individuality from the first to the fourth stages of immatures in chromatograms prepared from the immature materials as described under Materials and Methods. Solvent systems 5 and 6 gave unsatisfactory results. Best results were obtained with all other solvents except 4, and in each case useful information was obtained.

In all the species examined, the 5th stage showed deterioration in the characteristic fluorescent patterns of a species and therefore the identification of species with the help of 5th instar chromatograms was not possible. Fourtunately the latter stages of membracid species can be identified easily visually [26 and 27].

In all the spcies the 3rd stage immatures gave the most characteristic patterns in a particular solvent system, especially in the chromatograms developed in the solvent system containing *n*-butanol. These chromatograms exhibited 1 to 3 prominent spots characterising a species. R_f values of the most characteristic spots were found to be similar to those of pteridines provided by Blakley [1]. In Fig. 1. are illustrated the fluorescent chromatograms of the whole 3rd stage immature from the four membracid species developed in solvent system. 1. In Table 1 R_f values of the one or more spots near start of chromatograms developed in different solvent systems are compared with some pteridines. Squashes of different body parts, especially those mentioned in the section on Materials and Methods, and haemolymph also gave the same patterns as whole body squashes. However, haemolymph gave very faint spots.

DISCUSSION

Presently four different pteridines, viz. xanthopterine, *iso*xanthopterine, biopterin and pterine are identified in

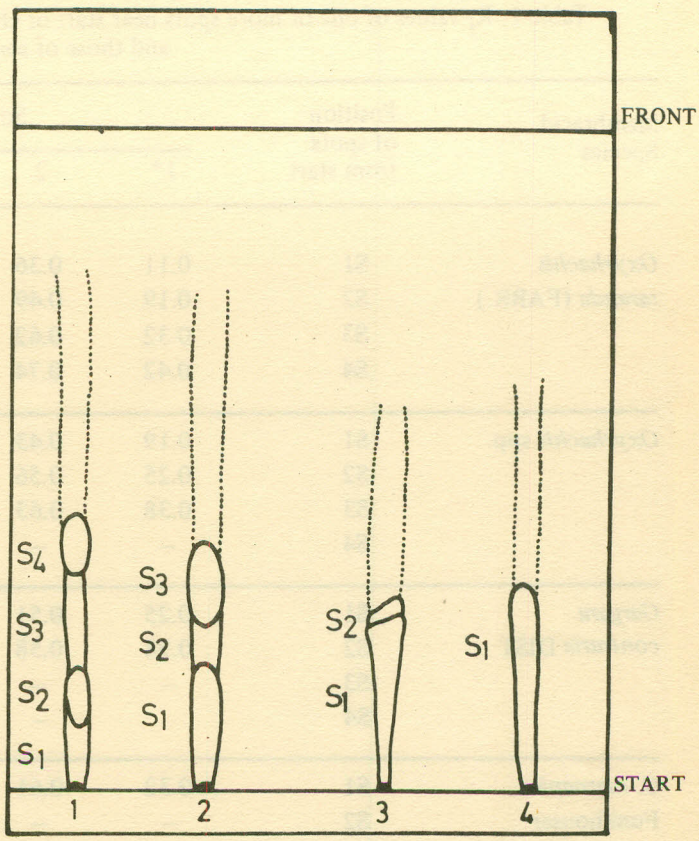

Fig. 1. Fluorescent chromatograms of the 3rd stage immature from four species, viz. *Oxyrhachis taranda*, *Oxyrhachis* sp. *Gargara contraria* and *G. nigroapica* developed in solvent system 1, viz. *n*-butanol, acetic acid and water (4: 1: 1).

the immatures of different species of membracids. Xanthopterine and *iso*xanthopterine were also found in *Oncopeltus* of Hemiptera [15] and the former in different species of Lepidoptera [10]. Biopterin has also been found in mosquitoes.

Hudson *et al.* [15] reported that xanthopterine increases tenfold in the course of embryonic development in *Oncopeltus* (Heteroptera) and in nymphal stages of the same species *iso*xanthopterine and xanthopterine occur chiefly in the epidermis but also in the fat bodies, dried excreta and in the uric acid. In view of these findings, pteridines which are important excretory products may well be widely distributed in Hemiptera.

Ahmad *et al.* (unpublished work) have observed that in membracids undigested food material accumulates in the hinder part of the midgut throughout immature life. Further, a complex mass of malpighiamtubules and hind intestine are responsible for the removal of excess water from midgut and haemolymph [28]. Excretion of immatures of membracids also contains small amounts of honey

Table 1. R_f values of one or more spots near start of chromatograms developed in different solvent systems and those of some pteridenes

Membracid Species	Position of spots from start	Solvent systems						
		1*	2	3	4	5	6	7
Oxyrhachis taranda (FABR.)	S1	0.11	0.36	0.56	0.12	–	–	0.1
	S2	0.19	0.49	0.61	0.54	–	–	–
	S3	0.32	0.62	0.66		–	–	–
	S4	0.42	0.74	–	–	–	–	–
Oxyrhachis spp.	S1	0.19	0.43	0.54	0.12	–	–	0.11
	S2	0.25	0.56	0.62	0.54	–	–	–
	S3	0.38	0.63	0.66	–	–	–	–
	S4	–	–	–	–	–	–	–
Gargara contraria DIST	S1	0.25	0.51	0.54	0.18	–	–	–
	S2	0.28	0.58	0.68	0.54	–	–	–
	S3	–	–	0.71		–	–	–
	S4		–	–	–	–	–	–
G. nigroapica Funkhouser	S1	0.32	0.61		0.16	–	–	0.6
	S2	–	–	–	0.54	–	–	–
	S3	–	–	–	–	–	–	
	S4	–	–	–	–	–	–	–
Pteridines								
Xanthopterine		0.36	–	0.47	–	0.15	0.62	–
Isoxanthopterine		0.15	–	0.33		0.17	0.42	
Biopterin		0.30	–	0.66	–	0.31	0.60	–
Pterine		0.29	–	0.49	–	0.31	0.55	–

+ Described under Materials and Methods.
* Position of spots illustrated in Fig. 1.

dew which attracts attending ants [29]. Wieland *et al.* [30]. have suggested their connection with purine metabolism. There is a possibility that most of the excretion in the immatures of membracids is through storage of pteridines in different parts of body. Such a possibility in some Lepidoptera has also been pointed out by Harmsen [31]. Probably the interspecific differences in the composition of pteridines are responsible for characteristic fluorescent patterns observed presently in chromatograms prepared from the immature squashes of different membracid species. It is hoped that in future studies this technique would be used as a basis for the identification of immatures of several sibling and sympatric membracid species [32 and 33].

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