THE CUTICLE OF *ACARUS SIRO* L. (= *TYROGLYPHUS FARINAE*)

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INTRODUCTION

The cuticle of *Acarus siro* is of two distinct types. That of the appendages is similar in appearance to the sclerotized cuticle of many adult insects; and there is no reason to suppose that it differs from this at all. The cuticle covering the opisthosoma, however, is exceedingly thin and colourless, the dorsal plate having undergone extreme reduction. There is no visible distinction into endo- and exo-cuticle. The cuticle as a whole forms a thin stiff layer lying directly on the epidermis. It is not elastic and if mites are kept at low humidities grooves develop in the cuticle as the internal volume decreases by loss of water.

Since *Acarus siro* can be bred in large numbers it is a suitable animal for the investigation of the thin colourless cuticle of the opisthosoma.

METHODS

In order to obtain samples of cuticle, heavy cultures of *Acarus siro* were placed in funnels over water, in which the active instars of the mites were collected. This prevented the inclusion of any resting stages, which might contain untanned skeletal proteins. The mites so obtained were broken by slight grinding in a tissue homogenizer with a loose plunger. They were then digested at pH 7·2 with trypsin for 24 hr. The products of tryptic digestion were removed by repeated washing with distilled water. When samples of washings concentrated to 10% of their original volumes gave no ninhydrin reaction, it was considered that the residual material included only hardened skeletal fragments. Microscopic examination showed only empty pieces of exoskeleton and a few guanine (Hughes, 1950) excretory bodies.

The cuticular material thus obtained was analysed for various elements including nitrogen and sulphur. Some samples were hydrolysed by refluxing for 12 to 20 hr. with 5·0N hydrochloric acid or with 5·0N caustic soda solution under an atmosphere of nitrogen. Acid hydrolysates, after filtering, were evaporated to dryness under reduced pressure and then taken up in 10% isopropanol. Alkaline hydrolysates brought to neutrality were de-salted electrolytically. A flowing anode of 5% sulphuric acid and a cathode of mercury circulated by water were used. These hydrolysates were then analysed by paper chromatography. Other hydrolysates were analysed for nitrogen and sulphur. The chromatography was done on strips, or by two-way partition; some circular chromatographs were also made. The solvents used were water-saturated phenol and butanol-acetic-water 5:1:4.

Whole mites and cuticular material obtained as described were subjected to the chitosan test in order to determine the presence or absence of chitin in the skeleton. The quantities of material available were small, 0·2 g. of cuticle being the average value. In the present instance it has so far not been possible to attempt anything other than a qualitative analysis of this material. The smallness of the mites makes it impossible to obtain enough material of ecdysing stages to attempt an analysis of the untanned cuticular proteins.

RESULTS

1. The chitosan reaction is weakly positive except on the appendages where it is strongly positive (the skeleton here is thicker and includes hard brown sclerotin).

2. Quantitative analysis for elements:

Material	% Nitrogen	% Sulphur
Whole cuticle unhydrolysed	9·75–10	1·71–2·0
Acid hydrolysate, soluble fraction	6·0 – 6·5	0
Acid hydrolysate, insoluble humin	3·0 – 3·5	2·0
Alkaline hydrolysate	6·0 – 6·25	2·0

3. Table of amino acids identified:

Amino acid	Alkaline hydrolysate	Acid hydrolysate
Aspartic	+ +	+ +
Glutamic	+ +	+ +
Glycine	+ + +	+ + +
Serine	+	+ +
Threonine	–	Trace
Lysine	+	+
Arginine	–	+
Proline	+	+
Leucine	+	+
Valine	+	+
Tyrosine	+	+
Alanine	+ + +	+ + +

The depth of colour given in the ninhydrin reaction on development of the chromatographs has been indicated by + signs.

The acid hydrolysate also included glucosamine, and colorimetric estimation of phenols using Folin and Ciocalteu's reagent gave a ratio of

$$\frac{0{\cdot}6}{1{\cdot}0} = \frac{\text{Phenols of acid hydrolysate}}{\text{Phenols of alkaline hydrolysate}}.$$

Spectographic analysis of the hydrolysates showed absorption in the range 220–230 mμ with a plateau between 250 mμ and 270 mμ.

The acid hydrolysate produced a black humin. This was dissolved in cold 5·0N caustic soda solution, evaporated to dryness under reduced pressure and extracted with three successive lots of ethyl ether. The ether extract which was straw-coloured contained a substance which gave a positive reaction for sulphydryl groups with

sodium nitroprusside and a blue colour with Folin and Ciocalteu's reagent before the application of ammonia, indicative of a paraphenolic group. A similar phenol occurs in the alkaline hydrolysate.

DISCUSSION

The weak chitosan reaction of the skeleton, apart from the appendages, is in agreement with the fact that on hydrolysis the glucosamine reaction is no stronger than that given by the majority of individual amino acids. This indicates that the amount of chitin in the skeleton is relatively small.

The results of the analysis for elements are of special interest in that they showed the presence of sulphur in far larger quantities than the results of similar analyses of insect cuticle (Hackman, 1953). From the examination of sections of mites over a period of years it had been thought from staining reactions that this thin cuticle might be a protein material similar to keratin. This view was strengthened by the report (Beament, 1951) that the egg shells of the red spider mite, *Metatetranychus ulmi*, contain sulphur, and are plasticized to some extent by agents which destroy di-sulphide linkages.

However, when the results of chromatography of hydrolysates were compared with those obtained by treating white wool in an identical manner, this idea was seen to be erroneous. The wool hydrolysates gave considerable quantities of cystine which was entirely lacking from the hydrolysates of mite cuticle.

The sulphur in the acid hydrolysate is confined to the humin, a black intractable residue. It is there in combined form as it cannot be extracted by sulphur solvents. Since a phenolic substance, probably a paraquinone, is extractable from the humin and since this same extract gives a sulphydryl reaction it is possible that the sulphur is present as SH groups attached to a quinone, probably a paraquinone. The phenolic substance obtained, when chromatographed on strips with butanol-acetic-water solvent, gives a spot of R.F. value 0·85. Dennell (personal communication and in press) obtained from insect cuticle (treated with alkaline stannite under anaerobic conditions) a substance which under similar chromatographic treatment gives a spot R.F. 0·83. This substance gives every indication of being a paraquinone with amino groups attached.

The presence of lysine, in both alkaline and acid hydrolysates, precludes the presence of the particular type of quinone-amino acid bonding postulated for insect cuticles by Pryor (1940*a*, *b*) and Pryor, Russell & Todd (1947). In the insect cuticle the ϵ-amino group of lysine plays an important part in the formation of cross links between the protein molecules by the aromatic nuclei, the other amino groups concerned being mainly terminal ones. It is, moreover, known that when there is competition between a sulphydryl and an amino group in reactions with quinones, there is a preference for the sulphydryl to react (James & Weissberger, 1939; Wittle *et al.* 1953). Thus, if the protein of this particular cuticle contained sulphydryl groups, it could be tanned by quinones forming cross links with them similar to those occurring in insect sclerotin between quinones and the amino groups of neighbouring molecules.

Acid hydrolysis of a protein tanned in this way through quinone linkage with sulphydryl groups could not produce any cystine as it does from wool. It might conceivably produce cysteine if the linkages between quinone and sulphydryl group were easily broken. An essential feature of tanned protein is, however, that the cross bonding is not easily broken. The occurrence of sulphur still combined in the humin is evidence of the firmness of the linkage in this case.

If the linkage of quinone to sulphydryl groups of the protein were broken between the sulphur and the protein molecule by hydrolysis, there should appear in the hydrolysate the hydroxy-acid corresponding to cysteine, namely serine. This amino acid was not found in hydrolysates of insect sclerotin by Hackman (1953, 2). However, it appears in both acid and alkaline hydrolysates of the mite cuticle.

In alkaline hydrolysis under nitrogen serine is found in quantities comparable to other amino acids, although serine is known to be destroyed by boiling with caustic alkalis (Block & Weiss, 1956). This can only mean that serine is being slowly liberated by alkaline hydrolysis from some other compound, and in sufficient quantities to make good the losses involved in the process during the time of hydrolysis, if this does not continue too long. Alkaline hydrolysis prolonged beyond 24 hours leads to the disappearance of serine. It is known that cysteine will react by its sulphydryl groups with paraquinone to give substitution products (Mason, 1955; Fieser, 1941; Burton & David, 1952). A substance which is probably a paraquinone is extractable from the humin of acid hydrolysis. It is, therefore, here suggested, as a possible explanation of the experimental data, that in these mites the tanning process is basically the same as in insects, namely that enzymatically a quinone is produced from tyrosine or some other amino acid which contains a phenolic residue, and that this quinone then tans the protein produced by the epidermal cells by reaction with sulphydryl groups.

The essential difference between the typical hard sclerotin of insects and the thin white cuticle of these mites appears to be that, whereas the protein produced by the insect epidermis contains no sulphydryl groups, that of the mite does. Such a mechanism as is here postulated might well account for the sharp transitions from one type of cuticle to another, which are of frequent occurrence in arthropods. A basic tanning mechanism reacting with the epidermally produced proteins of cuticle might well be expected to produce different types of tanned cuticle, dependent on the type of protein with which it reacted.

It is not impossible that the aromatic nucleus of phenylalanine should provide the paraquinone nucleus (Dennell, 1958), and thus effect direct linkage between neighbouring protein molecules. Although tyrosine is present in both types of hydrolysate, no phenylalanine is found. Alanine is the amino acid giving the most marked ninhydrin reaction, equalled only by glycine.

Apart from the data supporting the suggestion that the cuticle is tanned through sulphydryl groups reacting with quinones, other differences from insect cuticle occur. No arginine or proline was obtained by Hackman (1953) from insect cuticles. These hydrolysates of *Acarus siro* cuticle include, of course, material from the legs and mouthparts which are pigmented. There is no reason to suppose that

these yellow-brown parts are not hardened with sclerotin in the same way as in insects. Thus it would appear that amino acids not present in insect sclerotin must come from the thin cuticle of the opisthosoma. The fact that hydrolysis of many samples, prepared in the way described, always yielded the same amino acids, rules out the occurrence of serine and lysine as contaminants. In the case of contamination one would expect to find other amino acids too, and to get variable analyses.

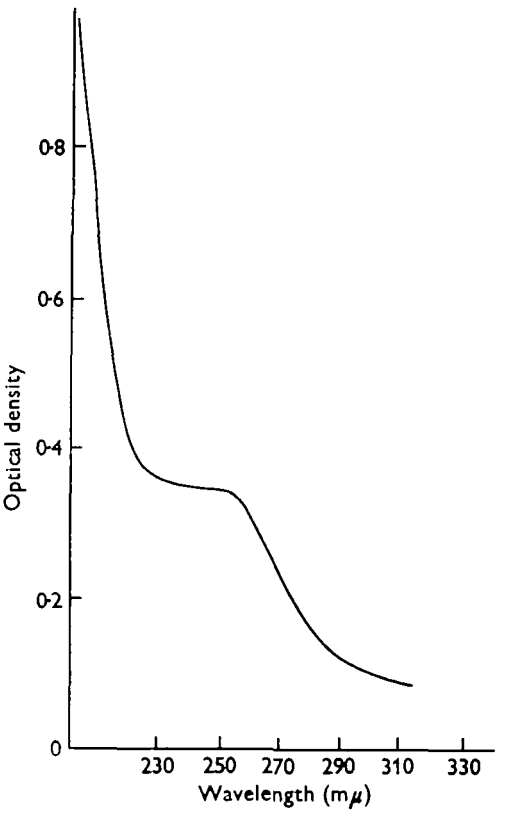

Fig. 1. Absorption spectrum of cuticular hydrolysates.

The estimation of phenolic substances gave results consistent with the hypothesis put forward here. The acid hydrolysate contains much less phenolic material than the alkaline one. This is to be expected since the humin includes phenolic material possibly combined with sulphur, and alkaline hydrolysis produces no such humin.

The light-absorption of the hydrolysates (Fig. 1) arises from the presence of phenols and it is interesting to note the close agreement of this curve with those obtained by Hackman (1953, 3, fig. 2) for cuticular protein tanned with or without the addition of catechol.

SUMMARY

1. The thin cuticle of *Acarus siro* contains a relatively large quantity of sulphur.

2. The sulphur does not form disulphide linkages between cysteine molecules as it does in keratin.

3. The presence of lysine amongst the products of hydrolysis shows that this cuticle is not tanned by linkage of quinone and amino groups.

4. The experimental data support the view that the cuticle is tanned by reaction of quinones and sulphydryl groups.

5. It is suggested that the quinone involved is a paraquinone, probably derived from phenylalanine.

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