THE OCCURRENCE OF L-LANTHIONINE IN THE AMINO-ACID POOL OF INSECTS

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During the course of investigations into the amino acid metabolism of insects the presence of an unusual ninhydrin reacting compound was detected chromatographically in protein free extracts of haemolymph from Bombyx mori (silkworm) and Antherea pernyi (oak silkworm). This compound has been identified as L-lanthionine and this preliminary report describes the method of isolation and presents evidence for this conclusion.

Materials and Methods

Haemolymph was collected from 10 day old pupae of laboratory-reared Bombyx mori and from Antherea pernyi in the diapause state. The latter material was a gift from Dr. T.D.R. Grace, Division of Entomology, C.S.I.R.O., Canberra. Protein free extracts were prepared from the haemolymph samples by the addition of perchloric acid and the precipitated protein was removed by centrifugation. The solutions were chilled, neutralized with KOH and, after removal of the precipitated $KClO_4$ by centrifugation, were desalted by passage through Dowex - 50 - H^+ ion exchange resin. The retained amino acids were eluted with 2.5 N ammonium hydroxide. The eluates were evaporated to dryness in vacuo, the residues dissolved in water and the resultant

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solutions used as starting materials for subsequent investigations.

When chromatographed on paper in two dimensions using ethanol: formic acid: water (7:1:2 by vol.) and phenol: water (4:1 by vol.) and treated with ninhydrin, two ninhydrin reacting spots were revealed as having low mobilities in both solvents. The positions occupied by these ninhydrin reacting compounds were similar to but distinct from those occupied by serine- and threonine-ethanolamine phosphates and lombricine. (cf. Rosenberg and Ennor, 1959; Rosenberg et al., 1962). A small amount of each compound was subsequently obtained by elution of the appropriate areas from non-ninhydrin treated chromatograms and shown chromatographically to be substantially free from other ninhydrin reacting compounds. Both compounds gave a negative result for the presence of - SH and -S-S- groups and a positive result with Platinic-iodide (Smith, 196 After fusion with sodium the products were shown to contain sulphur by spot tests (Feigl, 1960). A thio-ether linkage was therefore suspected. Treatment of both samples with Raney-Nickel (Berridge et al., 1952) led to their destruction and to the appearance in the reaction mixtures, as demonstrated chromatographically, of two ninhydrin reacting products in the one case and one such product in the other. In the first case these products have been identified as alanine and α-amino-η-butyric acid and in the second case as alanine. This evidence strongly suggested that the original compounds were cystathionine and lanthionine respectively and procedures were developed to separate these from the original extracts on a larger scale to permit adequate identification.

These procedures have involved absorption on Amberlite CG-120 Type 1-H^+ (100 - 200 mesh) ion exchange resin, gradient elution with perchloric acid and subsequent purification of the fractions

using water elution of the material absorbed on columns of Dowex - 50 - NH_4^+ (100 - 200 mesh). The compounds were finally crystallized from water. From 50 ml. of Antherea pernyi haemolymph about 25 mg. of L-lanthionine and 8 mg. of L-cystathionine were obtained; in the case of Bombyx mori pupae the amounts of these two compounds were 7 mg. and 5.5 mg. respectively from 11 ml. of haemolymph. Cystathionine has been positively identified as the L-enantiomorph but since its presence in the haemolymph from Bombyx mori has been previously reported (Kondo, 1959) the evidence for its identification is not presented in this communication. It is worthy of note however that it was believed to be absent from the haemolymph of Antherea Pernyi (Kondo, 1962).

Certain physical and chemical characteristics of the isolated lanthionine have been determined. Anal. Calcd. for $C_6 H_{12} O_4 N_2 S$: C, 34.61; H, 5.80; N, 13.46; S, 15.40. Found C, 34.63; H, 5.97; N, 13.64; S, 15.20 $[\alpha]_{313}^{22} = -57.7°$ (C=2% in 0.1 N NaOH). An authentic synthetic sample of L-lanthionine prepared according to Schöberl and Wagner (1947) gave $[\alpha]_{313}^{22} = -57.97°$ (C=2% in 0.1 N NaOH). On treatment with Raney-Nickel the isolated sample yielded alanine as the only ninhydrin positive degradation product. After treatment with acid ninhydrin both isolated and synthetic material gave identical absorption maxima.(Work, 1957). The infra-red spectrum of the isolated sample was similar to that of the synthetic material (Fig. 1) and in marked contrast to that of meso-lanthionine. (Blackburn and Lee, 1955).

Futher evidence supporting the contention that the isolated lanthionine was of the L-configuration has been obtained by tests with L-amino acid oxidase from snake venom and with D-amino acid oxidase isolated from hog kidney. When a sample was incubated

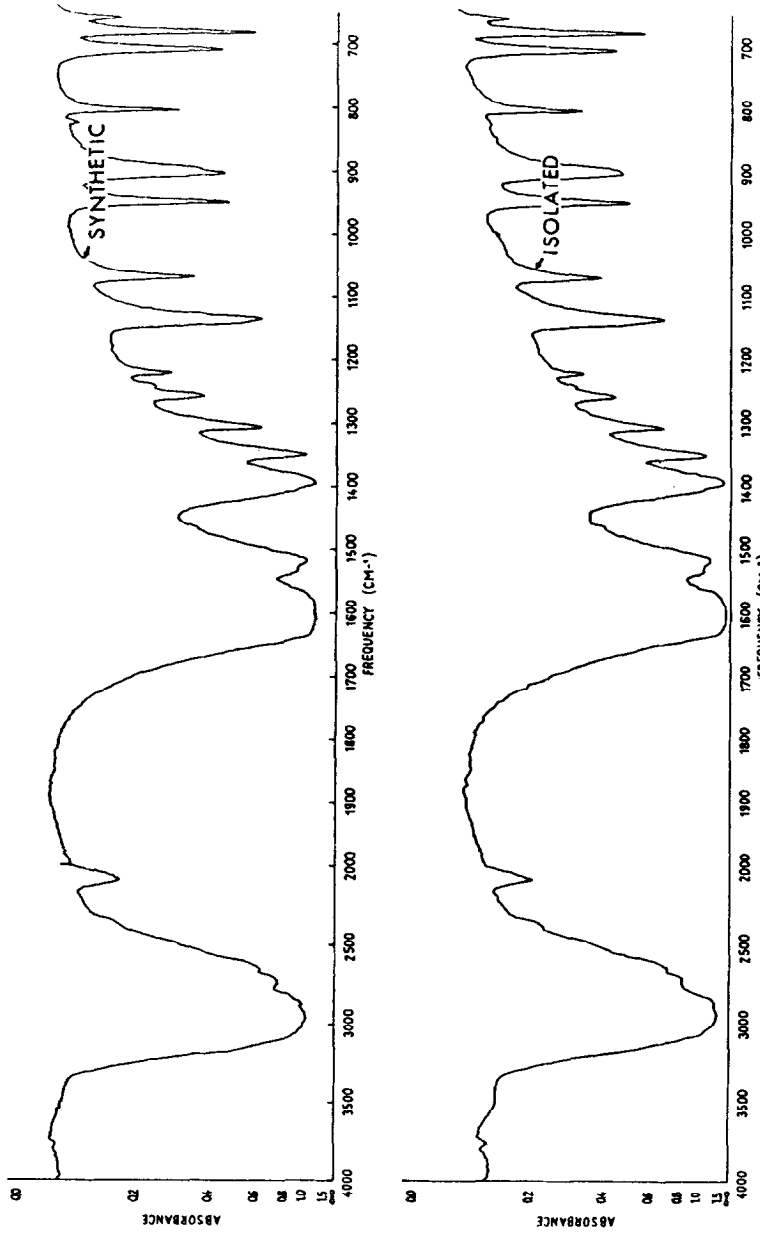
SYNTHETIC
ISOLATED
ABSORBANCE
FREQUENCY (CM-1)
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700

Fig. 1

with the latter enzyme for 6 hours there was no detectable uptake of oxygen and there was a complete recovery of the sample as determined on an automatic amino acid analyzer (Beckman - Model 120B). By contrast, treatment of a sample with the L-amino acid oxidase preparation led to oxygen uptake and complete disappearance of the compound as similarly analysed.

Lanthionine is well known as an acid hydrolysis product of alkali-treated proteins and is generally regarded as being an artifact arising from the decomposition of cystine residues. (cf. Dowling and Maclaren, 1964). As far as we are aware the presence of lanthionine as a free amino acid in biological material has not been previously described although it has been reported to be present in acid hydrolysates of locust wing muscle by Stein (1955). However, in this case the compound was not isolated nor was adequate proof of its presence given. Some preliminary experiments have been carried out on the distribution of lanthionine and it is believed to be fairly widely distributed throughout the Insecta. Experiments with isotopically labelled materials have shown that it is rapidly synthesized in vivo from methionine, cysteine and serine and it is hoped that it will be possible to report its mode of biosynthesis in the near future. Full details of the work reported in this communication will be submitted for publication elsewhere.

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