AMINO ACID CONTENT OF MARINE BORERS

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Although a great amount of study on the prevention of marine borer attack on wooden structures has been made in the last century, relatively little is known of the biochemical make-up of these organisms. The only published investigation of the amino acid content seems to be that of Lasker and Lane (1953) who reported eight amino acids present in *Teredo bartschi* Clapp. In view of the current idea (Fox, 1956) that primitive organisms have proteinaceous matter similar to that found in higher forms of life, it does not seem likely that several commonly occurring amino acids would be absent from the protein structure of *Teredo.*

In the present investigation *Teredo diegensis, Bankia setacea* (another member of the Teredinidae family) and two species of the crustacean borer *Limnoria* were investigated to determine their amino acid content. In all cases considerably more than the eight amino acids reported by Lasker and Lane were found to be present. In addition, southern yellow pine, redwood, and greenheart (*Ocotea rodiaei*) were hydrolyzed for identification of the amino acids present. The latter species of wood has been reported to possess a high protein content (Marchán, 1946) and to be highly resistant to marine borer attack (Baldwin, 1938).

Materials and Methods

The marine borers investigated were *Teredo diegensis, Bankia setacea,* and *Limnoria tripunctata* and *quadripunctata.* The first two were removed from both pine and redwood blocks, the *Limnoria tripunctata* from a creosoted log, and the *quadripunctata* from a redwood block in the same waters. Whole adult animals taken from the harbor and *Teredo* larvae produced in the laboratory were hydrolyzed. The larvae were collected by screening the overflow sea water from a tank containing pine blocks infested with *Teredo.* In addition to analyzing whole animals, *Bankia* was dissected into viscera, mantle, and gill, and *Teredo* into viscera, mantle, and reproductive organ containing larvae, and each section was analyzed individually. These dissected parts were extracted with 80% alcohol prior to hydrolysis in order to separate the free amino acids from those bound in the protein structure of the animals. The extracts were concentrated, the fatty substances extracted with chloroform, and the residues spotted for chromatography without further treatment.

Acid hydrolysis was in all cases effected by heating with 6 N HCl in a boiling water bath for twenty hours. Excess of HCl was removed by repeated evaporation to dryness under reduced pressure. Two-dimensional chromatograms using Whatman No. 1 filter paper with phenol solvent (Block, 1950) in the first direction and lutidine-collidine (Dent *et al.,* 1947) in the second were routinely run by the capil-

lary ascent method of Williams and Kirby (1948). The leucines were separated on a one-dimensional descending chromatogram using water saturated *tert*-amyl alcohol in an atmosphere of diethylamine. Confirmation of the identity of each spot was made by running suitable quantities of authentic amino acids simultaneously. The amino acids were revealed with the ninhydrin reagent of Levy and Chung (1953). Histidine and tyrosine were further identified by Block's (Block *et al.,* 1952) modification of the Pauly reaction; arginine, by one-dimensional chromatography using the solvent system of 77% alcohol and diethylamine (Block and Bolling, 1951); and the sulfur-containing amino acids, with the platinic iodide reagent (Toennies and Kolb, 1951). Dent's (1948) hydrogen peroxide treatment likewise proved to be useful in identifying the sulfur-containing amino acids.

A spot corresponding to lanthionine was eluted by the method of Dent (1947) from an unsprayed two-dimensional chromatogram. It was then spotted on a one-dimensional descending paper and run for five days using *n*-butyl alcohol-acetic acid (Partridge, 1948) as the solvent.

Glutamine, which was found to occur as a free amino acid in the Teredinidae, was further identified by elution (Dent, 1947) from an unsprayed two-dimensional chromatogram, by hydrolysis with acid, and by rechromatography. Glutamic acid was the only amino acid found on this latter chromatogram.

The barium hydroxide hydrolysis method of Levy and Chung (1953) was used to detect tryptophan which is unstable to acid hydrolysis.

The three woods investigated were hydrolyzed with 6 N HCl in a boiling water bath for twenty hours. Before spotting, each hydrolyzate was partially purified by adsorption on a column of the hydrogen form of Dowex 50-X8 (200 to 400 mesh), washing with water, and elution with 4 N ammonia. A synthetic mixture of amino acids was similarly treated.

Results

The amino acids found in the acid hydrolyzates of whole living organisms are summarized in Table I for each of the species studied. Taurine is also listed in this Table, as its spot was readily detected in these hydrolyzates. When the freshly-ground tissues of the Teredinidae were treated with 80% alcohol, taurine and β-alanine were extracted into the alcohol. All other amino acids listed in Table I were found in the hydrolyzates of the 80% alcohol-insoluble residues.

The alcoholic extracts of the dissected Teredinidae fragments all gave readily identifiable spots for α-alanine, β-alanine, glutamic acid, glycine, and taurine. In addition, some of the fragments, notably the mantle, gave very faint tests for some of the other amino acids in Table I, but these were not further investigated. The *Bankia* gill, the *Teredo* reproductive organ, and the mantles from both organisms were found to contain glutamine.

The acid hydrolyzates of both species of Teredinidae removed from redwood gave a spot corresponding to lanthionine, while those from pine did not. The spot gave a positive sulfur test with the platinic iodide reagent and proved to be chromatographically indistinguishable from lanthionine in the solvent systems used.

An unidentified spot was found in the acid hydrolyzates of both species of *Limnoria*. It is designated "A" in Table I. The spot persisted even when the tissues were hydrolyzed for an additional twenty-four hours. It was located on a

TABLE I

Amino acids in acid hydrolyzates of whole organisms

Amino acid	TP	TR	TL	BP	BR	LT	LQ
α-Alanine*	X	X	X	X	X	X	X
β-Alanine	X	X	X	X	X	O	O
Arginine*	X	X	X	X	X	X	X
Aspartic acid	X	X	X	X	X	X	X
Cystine-cysteine	X	X	X	X	X	X	X
Glutamic acid	X	X	X	X	X	X	X
Glycine	X	X	X	X	X	X	X
Histidine	X	X	X	X	X	X	X
Isoleucine	X	X	X	X	X	X	X
"Lanthionine"	O	X	O	O	X	O	O
Leucine*	X	X	X	X	X	X	X
Lysine	X	X	X	X	X	X	X
Methionine*	X	X	X	X	X	X	X
Phenylalanine*	X	X	X	X	X	X	X
Proline*	X	X	X	X	X	X	X
Serine	X	X	X	X	X	X	X
Taurine	X	X	X	X	X	X	X
Threonine	X	X	X	X	X	X	X
Tyrosine*	X	X	X	X	X	X	X
Valine*	X	X	X	X	X	X	X
Spot "A"	O	O	O	O	O	X	X

* Reported by Lasker and Lane to be present in *Teredo bartschi* Clapp.

X Present in readily detectable amounts.

O Not present in readily detectable amounts.

TP—*Teredo* from pine; TR—*Teredo* from redwood; TL—*Teredo* larvae; BP—*Bankia* from pine; BR—*Bankia* from redwood; LT—*Limnoria tripunctata*; LQ—*Limnoria quadripunctata*.

two-dimensional phenol and collidine paper between the spots for threonine and tyrosine. The R_f value in phenol was considerably lower when run in an atmosphere of acetic acid (Dent, 1948) than in an atmosphere of ammonia.

Tryptophan was not found in any of the alkaline hydrolyzates. This may have been because of its relative insensitivity to the ninhydrin reagent as compared to other commonly occurring amino acids.

The amino acids found in the three species of wood are listed in Table II. The greenheart hydrolyzate gave a much stronger phenylalanine spot than did the hydrolyzates of either pine or redwood. When a synthetic mixture of amino acids was treated with Dowex-50, all but lanthionine were eluted with ammonia.

DISCUSSION

These data indicate that there are at least eleven amino acids present in *Teredo diegensis* in addition to the eight reported present in *Teredo bartschi* Clapp by Lasker and Lane. It is considered unlikely that so many additional amino acids would occur in the one species and not the other.

The fact that taurine and β-alanine are not present in any of the hydrolyzates of tissues that had been extracted with 80% alcohol indicates that these compounds are

not present in the protein of the borers investigated. Seventeen amino acids are common to each species. All the amino acids seem to be distributed throughout all the Teredinidae sections. In addition, spot "A" was detected in the hydrolyzates of both species of *Limnoria,* and a spot corresponding to lanthionine was found in the hydrolyzates of both species of Teredinidae which had been removed from redwood. A corresponding spot was not found in the hydrolyzates of animals removed from pine. Cystathionine is reported (Dent, 1948) to have chromatographic properties similar to those of lanthionine. Neither of these two amino acids has been found in nature (Dent, 1948).

TABLE II

Amino acids readily detected in acid hydrolyzates of woods studied

α-Alanine	Lysine
Aspartic acid	Phenylalanine
Glutamic acid	Proline
Glycine	Serine
Hydroxyproline	Threonine
Isoleucine	Valine
Leucine	

The three species of wood studied contain thirteen amino acids in common. Hydroxyproline was found in all three woods but in none of the marine borers. A spot corresponding to lanthionine was not found in the chromatograms of the acid hydrolyzed redwood, but it was shown that lanthionine is not eluted with the other amino acids when a synthetic mixture is similarly treated.

SUMMARY

1. Eighteen naturally-occurring amino acids and taurine have been identified chromatographically in the acid hydrolyzate of *Teredo diegensis* and *Bankia setacea,* and seventeen naturally-occurring amino acids and taurine have been identified chromatographically in *Limnoria tripunctata* and *quadripunctata.* A spot corresponding to lanthionine has been found in the above Teredinidae living in redwood blocks but not in those living in pine blocks. Glutamine has been found in the alcoholic extract of freshly-ground Teredinidae sections.

2. Three species of wood have been hydrolyzed with acid, and all were found to contain the same thirteen amino acids.

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