FREE AMINO ACIDS PRESENT IN SOME SPECIES OF LYCOSID AND ARGIOPID SPIDERS (ARANEAE)

by

M.F. DUTTON and P.A. WALKER

Department of Biology
The Polytechnic
Wolverhampton

Little recent work seems to have been done on the comparative biochemistry of spiders, except for the numerous studies on venoms (Browning, 1963-7). For this reason we have been examining various free metabolites present in some species belonging to the Lycosidae and Argiopidae. It is hoped to extend these studies later to problems in taxonomy and phylogenetic relationships.

The spiders studied were Lycosa amentata (Clerck), L.rubrofasciata (Ohlert) and Pirata hygrophilus Thorell among the Lycosidae; and Meta segmentata (Clerck), Araneus diadematus Clerck and A.quadratus Clerck among the Argiopidae. With the exception of L.rubrofasciata, which was collected from the Breckland region of East Anglia, the specimens were obtained from the environs of Wolverhampton.

Before analysis the samples (containing 5 - 15 individuals, depending on availability and weight), were blotted dry on filter paper, and desiccated over phosphorous pentoxide in vacuo. The samples were then weighed and the lipid fraction was extracted by grinding the sample in a mortar with three lots of chloroform (5 ml). The chloroform extracts were removed by centrifuging and filtration, from the residue which was air dried. The residue was then extracted repeatedly with portions of aqueous ethanol (70 w/w) (5 ml), which removed compounds of low molecular weight such as amino acids and sugars.

Three such extractions completely removed free amino acids from the residue. The residue was separated from the ethanolic extracts by centrifuging, and stored at 0°C for further investigations. The ethanolic extracts were combined and evaporated to dryness in vacuo at 20°C. The resultant residue was made to 2 ml in distilled water and subjected to thin-layer chromatography for amino acids and other ninhydrin-positive compounds, after the method of Haworth and Heathcote (1969). Various staining techniques for certain amino acids were also applied and a further solvent system, ie. liquified phenol, was used to identify cysteic acid, (Smith, 1960).

The results of the chromatographic analysis are expressed in tables 1 and 2, and Figs. 1 and 2. They show that there is a distinct difference in the amino acid composition between the families examined. In the case of the Argiopids, up to 22 amino acids and five other unknown ninhydrin-positive compounds were present in relatively high concentrations, generally around 10 umols/g or above. In the Lycosids, only 19 amino acids were detected and one unknown, many of which were at a lower concentration than in the Argiopids, the only exceptions being glutamic acid, glycine, alanine, arginine and taurine. There seems to be little significant difference between the species in the families examined, apart

ible to demonstrate the presence of a compound having the same R_F values as glucose in all three species examined.

The amino acids retained on the 'Zeocarb 225' were eluted with 2N ammonia and the fraction was chromatographed on thin-layers as before. As was expected taurine and arginine were absent, arginine being retained on the resin. In addition the unknown compound III was also missing from the chromatogram.

On eluting the 'Deacidite FF' with 2N acetic acid, a fraction was obtained which was shown (by thin-layer chromatography) to contain taurine and compound III. From this result it was apparant that compound III is a stronger acid than 'Zeocarb 225' c.f. taurine and for this reason probably contains a sulphonic acid group. Phosphate is ruled out as it gives a negative result with the special phosphate staining agent. It has also been found that compound III is hydrolysed by treatment with 6N hydrochloric acid at 100°C, as it disappears from solutions treated in this manner as shown by thin-layer chromatography.

From these preliminary results it is evident that there is a distinct difference in free amino acid composition between the two families of spiders examined. The differences between species in the same genus however, seem to be only minor and for this reason comparison of amino acid patterns in intrageneric species for taxonomical purposes seems unfeasible, though differences at higher taxonomic levels may shed some light on the phylogeny of spiders. In addition, it is difficult to assign any significance to the amino acid patterns found as they must be at least partly determined by diet. For such studies to be fully meaningful the food intake should be controlled or monitored. It is assumed for the purposes of this study that by taking a number of random samples and because of the polyphagous feeding habits of spiders in nature, any major fluctuations in individuals will be cancelled out.

References:

BROWNING,E. 1963-67: Zoological Record, Section 12, 100-104.

HAWORTH,C. and HEATHCOTE,J. 1969: J.Chromatog., 41 : 380.

SMITH,I. 1960: Chromatographic and Electrophoretic Techniques. Vol. 1. Heinemann, London.

Nomenclature for TABLES 1 and 2

Special Staining Tests: Per = periodic test for serine: Pa = Pauly's reagent: Sa = Sakaguchi test: E = Erlich's reagent: I = Isatin: oP = o-phthalaldehyde test for taurine.

Where indicated on the table, these tests were used to confirm that particular amino acid.

V = visible in daylight: Ph = Liquified phenol solvent system used to demonstrate the presence of cysteic acid.

? = This indicates uncertainty of the assignment of the particular amino acid to a spot on the chromatogram.

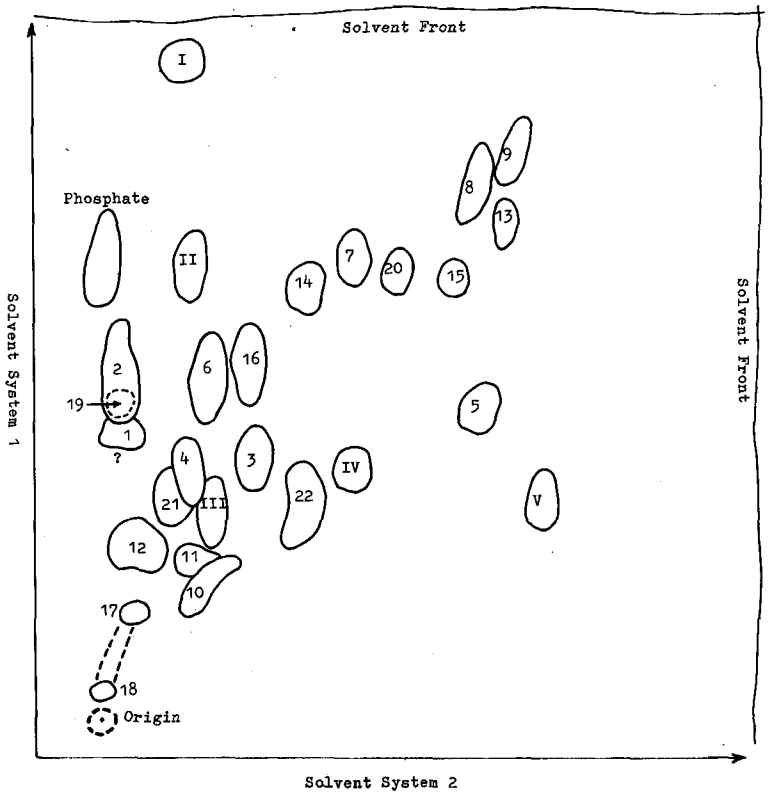

Fig. 1

Thin-Layer Chromatogram of the Free Ninhydrin-Positive Compounds Present in Araneus diadematus (Sample 1)

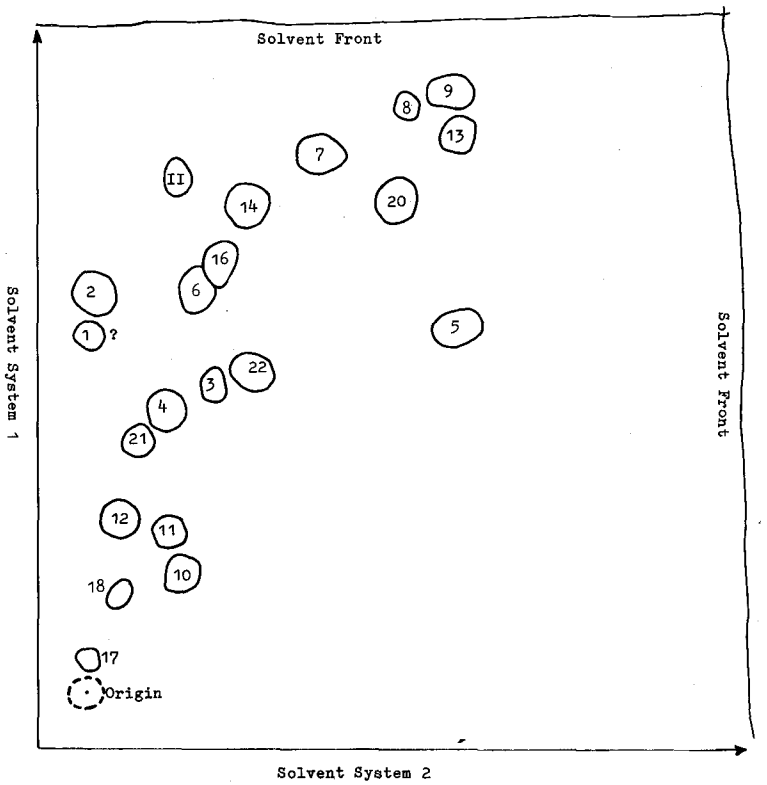

Fig. 2

Thin-Layer Chromatogram of the Free Ninhydrin-Positive Compounds Present in Lycosa amentata (Sample 2)

✓ = This indicates the presence of phosphate.
\+ = < 2.0 umols amino acid per g. of original material.
++ = 2.0-10.0 umols amino acid per g. of original material.
+++ = > 10.0 umols amino acid per g. of original material.

N.B. These concentrations were found by direct visual observation of the intensity and size of the amino acid spot, compared with standards run under the same conditions. They are intended to give an indication of relative concentrations and not an accurate assay.

\- = None detected.

Solvent system 1: 2-Propanol - Butanone - InHydrochloric acid
(60 : 15 : 25 v/v)

Solvent system 2: 2 Methyl propanol - 2 - Butanone - Propanone - Methanol - Water - (0.88) Ammonia.
(40 : 20 : 20 : 1 : 14 : 5 v/v)

****

A NOTE ON THE LONGEVITY AND MOLT CYCLE OF TWO TROPICAL THERAPHOSIDS

by W.J.BAERG and W.B.PECK.

It has been commonly accepted that a long life span is generally typical in spiders of the "primitive" taxa (Bristowe, 1958; Savory, 1966). Actual records confirming the upper limits of old age, however, are relatively rare. It is thence perhaps noteworthy to add the records of two tropical theraphosids which were kept in captivity as adults for longer than ten years.

Phormictopus cancerides (Latreille)

Of four specimens of Phormictopus cancerides that Prof. R. B. Cross brought to the senior author from Port au Prince, Haiti, in August 1959, the single male and one female died within a month. A second female lived for 15 months in captivity, and the third lived 11 years and four months, dying in December 1969. At the time that these spiders were received, it was recorded that this latter female, though small, appeared to be mature. Because mygalomorphs lack an epigynal plate and because other morphological manifestations of maturity in most of them are not known, it is not possible to determine with any degree of certainty whether or not a living female is sexually mature. Nothing is known of the life history of P.cancerides, but assuming that its period of development at least approximates that of Dugesiella hentzi (Girard) (Baerg, 1958 & 1963), and that it was mature, it was estimated that this female was 11 to 13 years old when captured. Granting the accuracy of this estimate, this spider was probably 22 to 24 years old at the time of its death.

Whereas captive tarantulas of several genera have frequently been known to become very docile and accept human handling with no evident distress, this specimen retained a defensive, almost aggressive, demeanor throughout its long captivity. Until shortly before dying, when it

from the absence of the 2 ninhydrin-positive compounds IV and V in Meta segmentata which were found in the two Araneus species examined.

Amino Acid		Araneus diadematus			Araneus quadratus		Meta segmentata	
		1	2	3	1	2	1	2
1 Aspartic acid	?	+	-	++	+	++	+	+
2 Glutamic acid		+++	+++	+++	+++	+++	+++	+++
3 Serine	Per	+++	+++	+++	+++	+++	+++	+++
4 Glycine		++	++	+++	+++	+++	+++	+++
5 Threonine		+	++	++	+	++	++	+
6 Alanine		+++	+++	+++	+++	+++	+++	+++
7 Valine		+	+++	+++	+	++	++	++
8 Isoleucine		++	+++	++	++	+++	++	++
9 Leucine		+	+++	++	+++	+++	++	++
10 Histidine	Pa	++	++	++	++	+++	++	+
11 Lysine		+	?	+++	++	++	+	+++
12 Arginine	Sa	+++	++	+++	+++	+++	+++	+++
13 Phenyl alanine		++	+	++	++	+++	+++	++
14 Tyrosine	Pa	+	++	+	++	-	++	+
15 Tryptophan	E	+	++	+	++	+	+	+
16 Proline	I	++	+++	++	++	+++	+++	++
17 Cysteine	I	+	+	+	+	+	++	+
18 Cystine		+	+	+	+	+	+	+
19 Cysteic acid	Ph	+	+	+	++	+	++	+
20 Methionine		+	++	++	++	+	++	++
21 Glutamine		++	+++	+++	+++	++	+++	+++
22 Taurine	oP	++	+++	+++	+++	+++	+++	+++
I		+	+	-	+	-	+	-
II		+	+	+	+	-	+	+
III	oP	++	?	++	+++	+++	+++	+++
IV	V	+	+	+	+	+	-	-
V		+	+	-	+	-	-	-
Phosphate		✓	✓	✓	✓	✓	✓	✓

TABLE 1

Free Amino Acids Present in Members of the Family Argiopidae

Amino Acid	Lycosa amentata		Lycosa rubrofasciata	Pirata hygrophilus	
	1	2		1	2
1 Aspartic acid?	-	+	++	-	-
2 Glutamic acid	+++	++	+++	+++	+++
3 Serine	-	+	++	+++	+++
4 Glycine	++	++	+++	+++	+++
5 Threonine	-	+	-	+	++
6 Alanine	+++	+++	+++	+++	+++
7 Valine	-	+	-	+	+++
8 Isoleucine	-	+	-	+	+
9 Leucine	-	+	-	++	++
10 Histidine	+++	+	-	+	+
11 Lysine	-	+	-	+	+
12 Arginine	+++	+++	+++	+++	+++
13 Phenyl alanine	-	+	-	+	++
14 Tyrosine	-	+	-	+	+
17 Cysteine	-	++	++	++	-
18 Cystine	-	++	++	++	-
20 Methionine	-	+	-	+	+
21 Glutamine	-	+++	-	+	+
22 Taurine ?	++	+++	++	+	++
II	-	+	++	+	++

TABLE 2

Free Amino Acids Present in Members of the Family Lycosidae

In order to investigate further the aqueous ethanol extract of the species of Argiopid examined, each fraction was chromatographed on ion exchange resins, as described in Smith (1960) for desalting physiological solutions. The fraction was passed down a column of 'Zeocarb 225' which retained all the amino acids apart from taurine. The taurine, neutral compounds and other anions, were eluted from the column with distilled water. The resultant eluate was then passed down a column of 'Deacidite FF' which retained taurine and other anions and allowed the neutral compounds to be eluted with distilled water. This fraction containing the neutral compounds was evaporated in vacuo at 20°C and subjected to paper chromatography for sugars (Smith, 1960). The solvent systems used were propan-2-ol, water (8:2) v/v and propan-1-ol ethyl acetate, water (7:1:2) v/v. The localising agent was alkaline silver nitrate. This system was not altogether satisfactory, but it was poss-