Separation of Free Amino Acids and Derivatives in the Tsetse Fly *Glossina palpalis* (Diptera) by Ion-Exchange Chromatography

The insects are known to have an unusually high content of free amino acids. Numerous investigations demonstrated that the patterns of these components are both species- and stage-specific (for references, see CHEN[1]). In the tsetse fly *Glossina morsitans* BURSELL[2,3] showed that starvation and flight activity exert a remarkable influence on the concentration of certain amino acids. Of particular interest is his finding that proline can serve as energy reserve during flight, and its utilization is accompanied by a transamination reaction involving alanine and glutamic acid.

For studies of amino acids in insects the technique of paper chromatography has been most commonly used, mainly due to its high sensitivity and simple application. However, the filter paper has only a limited absorption capacity, and at concentrations without overloading the paper only components at relatively high levels can be detected. In this connection the method of column chromatography is superior; by using proper ion exchangers and elution systems an adequate separation of the substances can be achieved, even when larger quantities of samples are applied. Previous studies by employing this technique revealed the presence of a large number of peptides and phosphate esters in various insects in addition to amino acids[4–7]. For this reason and in view of the interesting results reported by BURSELL[3] we decided to carry out a more detailed column-chromatographic analysis of the free ninhydrin-positive components in the tsetse fly.

The pupae of tsetse flies *Glossina palpalis* employed in the present study were supplied by the Nigerian Institute for Trypanosomiasis Research, Kaduna. At the time of hatching fully differentiated flies were dissected out individually from the puparium. Males and females were not separated. These were immediately homogenized in cold absolute methanol, filtered at -27 °C, and followed by washing with 50% cold methanol. The pooled filtrate was taken to dryness in a flash evaporator at 26 °C. The dry residue was dissolved in 10 ml water, and the resulting solution was shaken with chloroform. After separation, the upper layer was collected and centrifuged. The final concentration of the clear extract was 0.33 g wet weight/2 ml. This was mixed with 1 part of a citrate buffer of pH 2.2. Two ml of the mixture were applied to the column of the automatic amino acid analyzer (Beckman, Model 120 B) for fractionation according to procedures given by SPACKMAN et al.[8].

A typical chromatographic pattern of all free ninhydrin-positive substances is shown in Figure 1. Under the present experimental condition a minimum of 37 fractions could be distinctly separated, and many of these occur in rather high concentrations. It is noteworthy that γ-aminobutyric acid, ornithine, ethanolamine and tryptophan are also present at distinct levels. In addition, a total of about 14 fractions in positions not occupied by common amino acids has been detected, and the corresponding peaks are numbered according to their order of elution from the column. Based on their chromatographic behaviour and our earlier experience from similar studies on *Drosophila*[6], fractions Nos. 1 and 2 are probably phosphoserine and glycerophosphoethanolamine respectively. The component (No. 3) eluted immediately ahead of taurine is phosphoethanolamine. Following taurine there are 3 further prominent peaks, the last one of which is poorly separated from aspartic acid. In later regions a number of minor fractions are visible, but owing to the extremely low concentrations their identification is difficult. It should be mentioned that paper chromatographic and high-voltage paper electrophoretic studies of these substances in the same material gave similar results.

As shown by the chromatogram in Figure 2, nearly all fractions other than amino acids disappeared after acid hydrolysis. This suggests that, in addition to phosphate esters, many of these are probably peptides. More detailed analyses of individual fractions are needed to establish

Distribution of free amino acids in adult *Glossina palpalis* and 3 other dipterous insects (μM/g wet weight and % total)

Amino acids	*Glossina palpalis*		*Drosophila melanogaster*		*Culex pipiens*		*Phormia regina*	
	μM/g	%	μM/g	%	μM/g	%	μM/g	%
Taurine	4.42	3.33	2.16	4.12	0.39	1.42	9.13	12.01
Methionine sulphoxide	–		–		1.99	7.24	3.43	4.51
Aspartic acid	3.51	2.65	0.05	0.10	0.17	0.62	0.46	0.61
Threonine	5.77	4.35	1.47	2.81	0.90	3.27	1.58	2.08
Serine + glutamine	11.79	8.89	4.28	8.17	2.51	9.13	6.30	8.29
Glutamic acid	12.90	9.72	2.08	3.97	1.23	4.47	4.37	5.75
Proline	3.98	3.00	6.65	12.70	2.45	8.91	21.30	28.03
Glycine	9.95	7.50	3.36	6.42	0.92	3.35	1.44	1.89
α-Alanine	28.79	21.70	15.47	29.54	2.95	10.73	8.42	11.08
Valine	8.87	6.69	1.84	3.51	1.85	6.73	1.76	2.32
Methionine	1.82	1.37	0.42	0.80	0.55	2.00	0.52	0.68
Isoleucine	1.91	1.44	0.85	1.62	1.12	4.07	0.81	1.06
Leucine	3.21	2.42	1.48	2.83	1.18	4.29	1.30	1.71
Tyrosine	4.86	3.66	0.10	0.19	0.84	3.06	3.15	4.14
Phenyl-alanine	4.11	3.10	0.20	0.38	0.58	2.11	0.81	1.07
β-Alanine	1.08	0.81	3.08	5.88	0.53	1.93	1.99	2.62
γ-Amino-butyric acid	0.33	0.25	0.55	1.05	–		0.25	0.33
Ornithine	0.65	0.49	0.08	0.15	–		0.09	0.12
Ethanol-amine	1.77	1.33	2.13	4.07	–		0.10	0.13
Lysine	8.85	6.67	2.48	4.73	1.24	4.51	2.08	2.74
Histidine	2.62	1.97	2.03	3.88	2.50	9.07	2.52	3.32
Tryptophan	6.31	4.76	+		0.65	2.36	+	
Arginine	5.16	3.90	1.61	3.08	2.94	10.69	4.19	5.51
Total	132.66		52.37		27.49		76.00	

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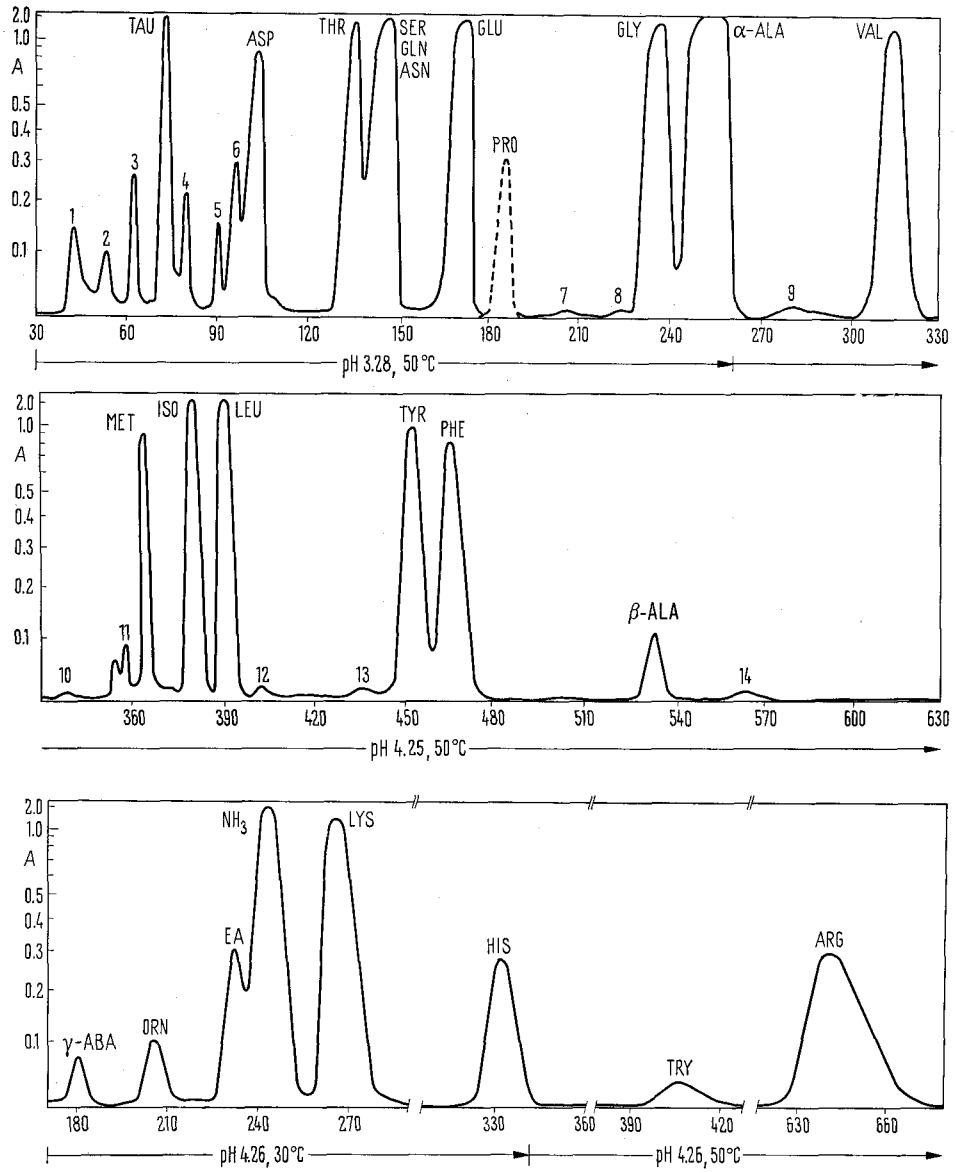

Fig. 1. Chromatographic pattern (automatic amino acid analyzer) of free ninhydrin-reacting components in the adult tsetse fly *G. palpalis* at the time of hatching (0.165 g wet weight/2 ml). Ordinate: Absorption (A) at 440 nm for proline and at 570 nm for all other components. Abscissa: effluent vol. in ml. With exceptions of glutamine (GLN), asparagin (ASN), γ-aminobutyric acid (γ-ABA), ethanolamine (EA) and ammonia (NH_3), all abbreviations refer to the first 3 letters of the corresponding amino acids. Fractions containing phosphate esters, peptides and other derivatives are numbered according to their order of elution from the column.

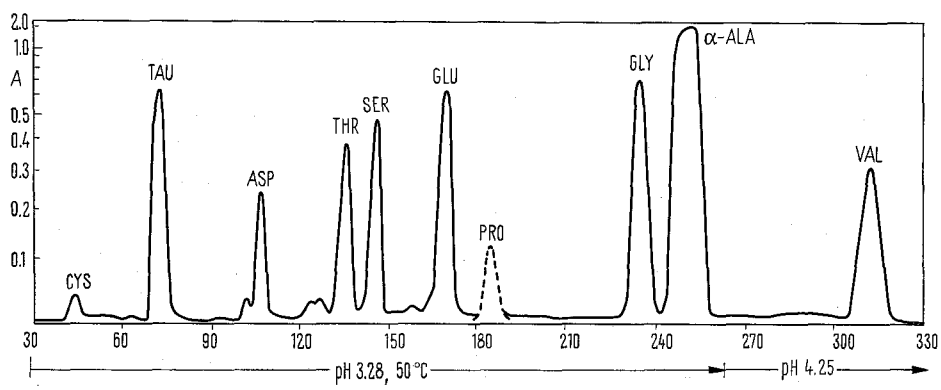

Fig. 2. Chromatographic pattern of the methanol extract (0.066 g/4 ml) in adult *G. palpalis* after acid hydrolysis (6 *N* HCl at 120 °C for 16 h). Only fractions in the first 330 ml effluent vol. are shown. Cysteic acid (CYS) produced from unknown precursor(s) was eluted ahead of taurine. For further explanations, see text in Figure 1.

this point. In any case, the present study has demonstrated that the predominant amino acid derivatives in *G. palpalis* are acidic in nature.

It would be of interest to compare the profiles of amino acids and related compounds in the tsetse fly with those of other dipterous insects which have been investigated by comparable methods. Together with the present results, available data of 1-day-old adult flies of *Culex pipiens*[5] and *D. melanogaster*[6] as well as newly hatched adult flies of *Phormia regina* (unpublished data by amino acid analyzer) are presented in the Table. Since at such early stages the patterns in both sexes are quite similar, the data of adult males and females in the last 3 species were pooled. Alanine has by far the highest concentration among all amino acids in the tsetse fly. Compared to *Drosophila, Culex* and *Phormia*, its contents of aspartic acid, glutamic acid and tryptophan are also remarkably high. On the other hand, the relative concentration of proline amounts to only $^1/_9$–$^1/_3$ of that in the other 3 insects. Since the endogenous pool of this amino acid is low, for fulfilling its function as energy reserve a large part of it must be derived from the ingested blood[9].

Zusammenfassung. Mittels Ionenaustausch-Chromatographie konnten 37 freie Ninhydrin-positive Stoffe in adulten Tsetse-Fliegen, *Glossina palpalis*, aufgetrennt werden. Die meisten Derivate der Aminosäuren wurden vor der Asparaginsäure aus der Harzsäule eluiert, was auf ihre saure Natur hindeutet.

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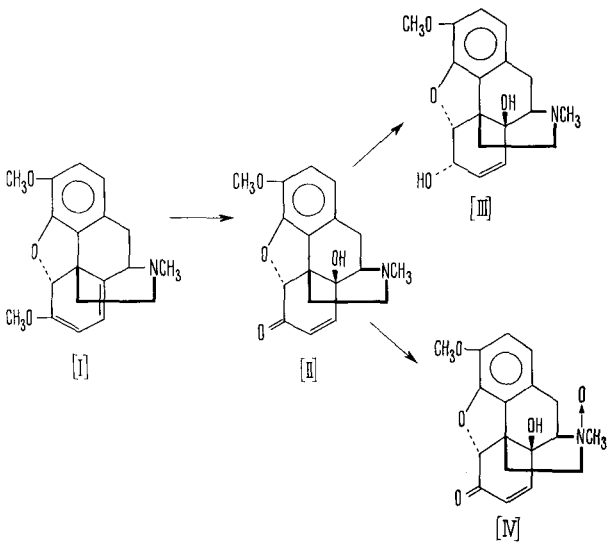
Mikrobielle Umwandlung von Thebain

Die mikrobielle Umwandlung von Alkaloiden, bei denen das Grundgerüst des Substrates weitgehend erhalten bleibt, ist erst in den letzten 10 Jahren untersucht worden[1,2]. Am häufigsten wurden Morphinane, Indol- und Steroidalkaloide eingesetzt. Eingehende Untersuchungen zur Umwandlung von Alkaloiden der Morphin-Reihe sind im Arbeitskreis von Tsuda in Japan durchgeführt worden[3–8]. Im Rahmen eines umfangreichen Screening-Programms hat man 1700 verschiedene Bakterien- und Pilzstämme auf ihre Fähigkeit, Thebain umzuwandeln, getestet. Insgesamt liessen sich 120 «Thebain umwandelnde Stämme» selektieren; meist handelt es sich um Basidiomyceten. Beim Einsatz des holzzerstörenden Pilzes *Trametes sanguinea* (L EX FR) Lloyd liessen sich nach Zugabe von Thebain zur Fermentationslösung 2 Hauptprodukte isolieren, nämlich 14-β-Hydroxycodeinon (II) und 14-β-Hydroxycodein (III). Die Ausbeuten von (II) und (III) nach Zugabe von Thebain war abhängig von der eingesetzten Nährlösung. Folgende Sequenz der Umwandlung konnte gesichert werden:

Thebain (I) → 14-β-Hydroxycodeinon (II) →
14-β-Hydroxycodein (III).

Die mikrobielle Umwandlung verschiedener 6,14-*endo*-Äthenotetrahydrothebaine ist kürzlich von Mitscher et al.[9] beschrieben worden. *Cunninghamella-echinulata*-(NRRL-A-11498)-Kulturen sind in der Lage, u. a. die Methylgruppe am Piperidino-Stickstoff abzuspalten. Entalkylierungen in der Morphin-Reihe durch mikrobielle Enzyme waren bislang nicht bekannt.

Wir untersuchten die Umwandlung von Thebain mittels holzzerstörender Pilze der Gattung *Trametes* europäischer Herkunft. Insgesamt wurden 35 Stämme verschiedener *Trametes*-Arten geprüft. Die Kultivation wurde in einer 4prozentigen Malzextraktlösung (NL 1) durchgeführt. Die meisten Stämme waren in der Lage, das Substrat (I) umzuwandeln, wobei neben einer Hauptkomponente A verschiedentlich noch mehrere Produkte



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