Tabelle der Analysen in %

Präparat		C	H	N	O	S	O-Methyl	C-Methyl	C-Äthyl	Acetyl
I	ber.	68,72	7,34	7,29			16,14	3,91		
$C_{22}H_{28}N_2O_4$ . . .	gef.	68,85	7,37	7,55			15,28	3,63	negativ	
I-Acetylderivat .	ber.	67,58	7,09	6,57	18,76			(2) 7,04		10,10
$C_{24}H_{30}N_2O_5$ . . .	gef.	67,53	7,08	6,50	18,61			7,16		10,40
III	ber.	73,59	8,03	8,58	9,81		9,50	4,60		
$C_{20}H_{26}N_2O_2$ . . .	gef.	73,38	8,07	8,58	9,58		9,77	3,22 4,72	negativ	
IV	ber.	67,47	6,71	5,83		6,67		3,12		
$C_{27}H_{32}N_2O_4S$ . .	gef.	67,69	6,79	5,73		6,43		2,30±0,24	negativ	
V	ber.	74,54	8,53	7,90			(2) 17,50	(2) 8,46		
$C_{22}H_{30}N_2O_2$ . . .	gef.	73,82	8,29	7,85			17,17 (O-Alk als O-Me)	8,23	negativ	
VI	ber.	77,88	7,84	9,08				4,86		
$C_{20}H_{24}N_2O$ . . .	gef.	77,75	7,91	9,16				2,2	negativ	
VIII	ber.	77,38	8,44	9,03	5,15			4,83		
$C_{20}H_{26}N_2O$ . . .	gef.	77,34	8,55	9,07	5,31			4,47	positiv	
«β-Isomeres» . .	ber.	77,38	8,44	9,03			9,98	4,84		
$C_{20}H_{26}N_2O$ . . .	gef.	77,42	8,51	9,03			10,24	1,87	negativ	

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Summary

Voacristine is identical with voacangarine. Its degradation products include iboxygaine and ibogaine. Structures for iboxygaine and its internal quaternary tosylate salt are briefly discussed.

Free Amino Acids of the Hemolymph of *Anacridium aegyptium* L. (Orthoptera)

In *Schistocerca gregaria* Forsk. the tegument of the brown hoppers contains a redox pigment, insectorubin, which is present in very small quantities in the green ones (Goodwin[1]). The same pigment is mainly responsible for the colour of the nymphs of the grasshopper *Anacridium aegyptium* L. (Colombo, Franco, and Moccellin[2]). In *A. aegyptium* the ground colour appears to be genetically determined[2], in the locust *S. gregaria* the pigmentation is related to the phase of which the determinism is rather complex (Hunter-Jones[3]).

Locust insectorubin is indistinguishable from the brown pigment of the eye of *Drosophila melanogaster* (wild type) and *Ephestia kühniella* (Goodwin and Srisukh[4]), in which it is formed from tryptophan (Beadle[5]). However the locust insectoburin contains, according to Goodwin and Srisukh[4], a pyrrole nucleus and tryptophan may be not the only precursor.

The aim of our work was to discover: (a) if there are any significant differences between green and brown hoppers of *A. aegyptium* in the free amino acids of the blood; (b) if tryptophan supplied to 4th instar hoppers has any effects on pigments of next instar and on the free amino acids content in the hemolymph.

The blood of insects contains large quantity of free amino acids: in Orthoptera the only quantitative determination, so far as we know, has been made by Duchâteau, Florkin, and Sarlet[6]. They analysed the free amino acids of the blood of 5th instar hoppers of *Locusta migratoria migratorioides* R. F., by the microbiological method.

Material and Methods. The blood was taken from brown and green 5th instar hoppers, from male and female adults, and from hoppers supplied with tryptophan, of *Anacridium aegyptium* L. of a laboratory strain kept at 28–30°C at moderate crowding and fed with privet.

Tryptophan was given to a group of 4th instar brown hoppers for 7 days through the privet leaves wetted with a 0·2% solution of tryptophan in diluted ethanol and allowed to dry.

Blood was collected by cutting off the hind leg. At least 6 animals were used for each group in order to get 0·5–1·0 ml of fluid.

The free amino acids from deproteinized blood were determined by the use of the fluorodinitrobenzene (FDNB) method of Sanger[7] and the paper chromatographic system of Levy[8]. The ether-soluble 2,4-dinitro-

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Free aminoacids content of the hemolymph of *Anacridium aegyptium* L.

Aminoacids	*mM* per 100 ml					Percentages				
	5th instar hoppers			Adults		5th instar hoppers			Adults	
	Brown	Green	Brown Tryptophan fed	Females	Males	Brown	Green	Brown Trypto-phan fed	Fe-males	Males
Glutamic acid .	0·654 ± 0·058	0·274 ± 0·006	0·562	0·031 ± 0·005	0·491 ± 0·007	24·71	24·86	27·16	3·27	34·04
Glycine	0·579 ± 0·019	0·135 ± 0·021	0·330	0·284 ± 0·039	0·409 ± 0·028	21·79	12·25	15·94	29·95	28·36
Proline	0·371 ± 0·032	0·172 ± 0·030	0·415	0·199 ± 0·048	0·185 ± 0·022	13·96	15·60	20·05	20·99	12·82
Alanine . . .	0·232 ± 0·008	0·104 ± 0·005	0·209	0·088 ± 0·005	0·075 ± 0·004	8·73	9·43	10·10	9·28	5·20
Aspartic acid .	0·233 ± 0·024	0·041 ± 0·005	0·107	0·040 ± 0·006	0·079 ± 0·003	8·76	3·72	5·17	4·21	5·47
Valine	0·198 ± 0·009	0·098 ± 0·010	0·121	0·062 ± 0·010	0·053 ± 0·007	7·45	8·89	5·84	6·54	3·67
Lysine	0·093 ± 0·011	0·034	—	0·027 ± 0·001	0·033	3.50	3·08	—	2·84	2·28
Threonine . . .	0·083 ± 0·003	0·109 ± 0·011	0·151	0·047 ± 0·007	0·041 ± 0·005	3·12	9·89	7·29	4·95	2·84
Leucines . . .	0·101 ± 0·023	0·068	0·095	0·074 ± 0·009	0·032	3·70	6·17	4·59	7·80	2·21
Tyrosine . . .	0·076 ± 0·010	0·028	0·079	0·073 ± 0·013	0·026	2·86	2·54	3·81	7·70	1·80
Phenylalanine .	0·037 ± 0·007	0·039	—	0·023	0·018	1·39	3·53	—	2·42	1·24
Arginine . . .	+	+	+	+	+					
Histidine . . .	+	+	+	+	+					
Tryptophan . .	+	+	+	+	+					
Total	2·657	1·102	2·069	0·948	1·442	99·97	99·96	99·95	99·95	99·93

phenyl-amino acids (DNP-aminoacids) were chromatographed on paper by the method of BISERTE and OSTEUX[9].

Since the DNP-aspartic and -glutamic acids gave a single spot, this was eluted and rechromatographed using a phosphate buffer (2·5 *M*) which gave complete separation.

The DNP-amino acids left in the aqueous layer (ε-DNP-arginine and Imidazol-DNP-histidine) were separated with monodimensional chromatography by the method of BLACKBURN and LOWTHER[10].

Suitable controls were carried out with known DNP-amino acids in the same way until the identity was unequivocal.

The spots were eluted from the paper with 1% $NaHCO_3$ solution (15 min at 60°C); quantitative determination was made with a spectrophotometer (Hilger-Uvispeck) at 360 mμ (385 mμ for the DNP-proline).

Results. Results are reported in the Table. Figures are averages of three determinations and they are expressed in millimoles (*mM*) for 100 ml of blood. In the right half of the Table, the same data are reported as percentages of each amino acid on the total *mM* found in each sample.

Fourteen amino acids were found (leucine and isoleucine were not distinguished, leucines in the Table). Three: glutamic acid, proline, and glycine, were present in larger quantity than the others and represent more than 50% of the total molarity. Tryptophan, histidine, and arginine were detectable on the chromatograms, but in so small a quantity that their DNP-derivatives could not be read on the spectrophotometer.

Our results are in agreement with those obtained with microbiological method by DUCHÂTEAU, FLORKIN, and SARLET[6].

The results shown in the Table indicate that there is not a striking difference in the free amino acids of the blood between different coloured hoppers; glycine and aspartic acid appear to be in less quantity, threonine, leucines, and phenylalanine in larger amount in the green hoppers than in the brown ones. It is difficult to relate these findings to the insectoburin formation; it is likely that the difference in pigmentation is mainly due to a different metabolism of the epidermic cells.

In the tryptophan-fed hoppers, neither increased tryptophan nor tryptophan metabolism products (kynurenines, anthranilic acids etc.) were found; no effects were observed on pigmentation. The free amino acids content of the blood did not show any great difference from the untreated brown hoppers; while phenylalanine and lysine were absent proline and threonine appear to be increased, glycine and aspartic acid decreased. The changes are in some way similar to that found in the green hoppers.

The percentage composition of the blood free amino acids is strikingly different between adults and hoppers, and in the adults between males and females. These differences are obviously related to different metabolism; however the lack of knowledge on this subject in Orthoptera does not allow any inference to be made before further researches are carried out.

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Istituti di Chimica Farmaceutica e di Zoologia dell'Università di Padova, June 18, 1959.

Riassunto

Sono stati determinati 14 aminoacidi liberi nell'emolinfa: di essi acido glutamico, glicina e prolina rappresentano insieme il 50% e più del contenuto totale. Le ninfe verdi contengono meno glicina e acido aspartico e più treonina e fenilalanina in confronto alle ninfe brune. Gli individui adulti mostrano in contenuto relativo di aminoacidi diverso dalle ninfe e fra i due sessi. Somministrazione di triptofano a ninfe brune non ha dato variazioni negli aminoacidi liberi, non aumento dell'aminoacido stesso, né presenza di prodotti del suo metabolismo nel sangue.

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