Phytochemistry, 1979, Vol. 18, pp. 1807–1810. © Pergamon Press Ltd. Printed in England. 0031-9422/79/1101–1807 $02.00/0

NON-PROTEIN AMINO ACIDS OF *ACACIA* SPECIES AND THEIR EFFECT ON THE FEEDING OF THE ACRIDIDS *ANACRIDIUM MELANORHODON* AND *LOCUSTA MIGRATORIA*

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(*Received* 19 *February* 1979)

Key Word Index—*Acacia*; Leguminosae; *Anacridium melanorhodon*; *Locusta migratoria*; Acrididea; non-protein amino acids; insect feeding.

Abstract—The leaves of *Acacia* species have been found to contain homoarginine, pipecolic acid and 4-hydroxypipecolic acid. The nymphs of the tree locust *Anacridium melanorhodon*, which feed on the leaves of *Acacia* species, were not inhibited from feeding on palatable media containing concentrations of these amino acids equivalent to, or greater than, those found in the leaves. The graminivorous *Locusta migratoria* was more sensitive to these compounds, inhibitory effects being observed at concentrations comparable to those found in the leaves. The inhibitory effects of mixtures of homoarginine and pipecolic acid were additive in *A. melanorhodon* but not in *L. migratoria*. Three of the non-protein amino acids found in the seeds of *Acacia* species, 2,3-diaminopropionic acid, 2-amino-3-acetylaminopropionic acid and 2-amino-3-oxalylaminopropionic acid, were more effective inhibitors of feeding in *Anacridium* than were the leaf amino acids.

INTRODUCTION

It has been suggested that one of the roles of secondary compounds in plants may be to protect these plants against potential predators [1–3]. Certain of the non-protein amino acids accumulated in legume seeds are toxic to the larvae of various seed-eating beetles [3, 4], and to the leaf-eating larvae of *Prodenia eridania* and *Manduca sexta* [5, 6]. Navon and Bernays [7] have also demonstrated that 0.1 M concentrations of many non-protein amino acids inhibit feeding in the acridids *Locusta migratoria migratorioides* (R. & F.) and *Chortoicetes terminifera* (Walker) which are specialist feeders, but are less effective as feeding inhibitors in the polyphagous species *Schistocerca americana gregaria* (Dirsh.) hitherto known as *Schistocerca gregaria* (Forskål).

Another species of acridid, *Anacridium melanorhodon arabafrum* (Dirsh.) which occurs in the Sahel region of Africa, has been reported to feed extensively on species of *Acacia* particularly in the dry season [8]. Plantations of *A. senegal*, grown in Sudan for the commercial production of 'gum arabic' are especially vulnerable to attack by this locust. The defoliation of the trees causes a reduction in gum production for up to two seasons. The species which are host plants of this tree locust are *A. flava*, *A. nubica*, *A. seyal*, *A. tortilis*, *A. albida*, *A. senegal*, *A. mellifera* and *A. laeta* [8]. The non-protein amino acid composition of seeds of these species has been fully investigated and two distinct patterns of amino acids have been found [9]. The first pattern, found in seeds of *A. flava*, *A. nubica*, *A. seyal* and *A. tortilis*, has *N*-acetyldjenkolic acid as the major non-protein amino acid, together with lower concentrations of djenkolic acid, pipecolic acid and 4-hydroxypipecolic acid. The second pattern, found in seeds of *A. senegal*, *A. mellifera*, *A. laeta* and *A. albida*, characteristically includes 4-hydroxypipecolic acid, albizziine, 2,3-diaminopropionic acid (DAPA), 2-amino-3-acetylaminopropionic acid (ADAP), 2-amino-3-oxalylaminopropionic acid (ODAP), 2,4-diaminobutyric acid (DABA) and 2-amino-4-oxalylaminobutyric acid (ODAB). In view of these interspecific differences in seed chemistry, the non-protein amino acids in the leaves of these *Acacia* species have been investigated, and the effect of both leaf and seed non-protein amino acids on the feeding of *Anacridium melanorhodon* and *Locusta migratoria* has been studied.

RESULTS AND DISCUSSION

Leaves of all 21 *Acacia* species analysed contained homoarginine, pipecolic acid and 4-hydroxypipecolic acid as their principal non-protein amino acids. Concentrations of the individual amino acids varied from 0.01 to 0.2% fresh weight. Values for the mature leaves of *A. albida* and *A. tortilis* are shown in Table 1.

Table 2 shows the feeding response of *A. melanorhodon* nymphs to these three non-protein amino acids offered individually on glass fibre filter papers impregnated with sucrose at 5.3% dry weight. Each insect had the choice of such a treated disc and a disc with sucrose only. No inhibitory effect on feeding was produced by concentrations of 0.5% of each amino acid, a slight inhibition was produced by 1.0% concentrations and complete inhibition was produced by 5.0% of homoarginine and pipecolic acid (4-hydroxypipecolic acid was not available for testing at this concentration).

Navon and Bernays [7] tested the effect of pipecolic acid and homoarginine on the feeding of *Locusta migratoria* at concentrations of 0.1 and 0.01 M (approximately 10 and 1%, respectively) and observed inhibition at both concentrations with both amino acids. Further tests reported here with male 5th instar nymphs of *Locusta* (Table 2) using concentrations of homoargi-

Table 1. Amino acids in mature leaves of *A. tortilis* and *A. albida* (μg/g fresh leaf material (±10%))

	Homoarginine	Pipecolic acid	4-Hydroxypipecolic acid
Acacia tortilis	270 (0.03%)	100 (0.01%)	1500 (0.15%)
Acacia albida	175 (0.02%)	220 (0.02%)	610 (0.06%)

nine and pipecolic acid at 0.005, 0.0025 and 0.001 M (approximately 0.5, 0.25 and 0.1%, respectively) showed that 0.5% homoarginine completely inhibited feeding while 0.1% caused a slight inhibition. Pipecolic acid had a slight inhibitory effect on feeding at concentrations of 0.5 and 0.25% but no effect at 0.1%.

In these feeding experiments with *L. migratoria* we observed a greater sensitivity to homoarginine than that reported by Navon and Bernays. This difference may reflect the lower level of sucrose used in our experiments (3.7 mg/disc) compared with the sucrose level (5.5 mg/disc) used by Navon and Bernays [7].

The specialist feeder *L. migratoria* is considerably more sensitive than is the polyphagous species *A. melanorhodon* to the concentrations of homoarginine and pipecolic acid which are found in leaves of *Acacia* species.

Table 3. The effect of mixtures of homoarginine and pipecolic acid offered in choice tests to *L. migratoria* and *A. melanorhodon* nymphs

% Concentration of each amino acid on disc	*A. melanorhodon*	*L. migratoria*
2	+++	
1	++	+++
0.5	0	+++
0.25	0	+
0.1	0	+

Mixtures of homoarginine and pipecolic acid were offered to both *L. migratoria* and *A. melanorhodon* in choice tests. Table 3 shows the feeding inhibition caused by mixtures of 2% homoarginine and 2% pipecolic acid, decreasing to 0.1% homoarginine and 0.1% pipecolic acid. Complete inhibition of feeding of *A. melanorhodon* occurred at 2% concentration whereas that of *L. migratoria* occurred at 0.5% concentration.

Table 4 shows the non-protein amino acids which are found in the seeds of *Acacia* species whose leaves are

Table 2. Anti-feeding effects of amino acids offered to nymphs of *A. melanorhodon* and *L. migratoria* in choice tests on sucrose discs

	Insects	Amino acids as % of disc wts 0.1%	0.25%	0.5%	1.0%	2.0%	5.0%	10.0%
Amino acids which occur in leaves of *Acacia* sp.								
	A. melanorhodon							
Homoarginine				0	+	+++	+++	
Pipecolic acid				0	+	+	+++	
4-Hydroxypipecolic acid				0	0			
	L. migratoria							
Homoarginine		+	++	+++				
Pipecolic acid		0	+	+				
Amino acids which occur in seeds of *Acacia* sp.								
	A. melanorhodon							
Djenkolic acid				0	0		0	
N-Acetyldjenkolic acid				0	0		0	
Albizziine				0	0		++	
2,4-Diaminobutyric acid (DABA)				0	0		++	
2-Amino-4-oxalylaminobutyric acid (ODAB)				0	0		++	
2,3-Diaminopropionic acid (DAPA)				+	+		+++	
2-Amino-3-acetylaminopropionic acid (ADAP)				+	+++		+++	
2-Amino-3-oxalylaminopropionic acid (ODAP)				++	+++		+++	
	*L. migratoria**							
Djenkolic acid					++			++
Albizziine					+++			+++
2,4-Diaminobutyric acid (DABA)					+			+++
2-Amino-4-oxalylaminobutyric acid (ODAB)					0			+++
2,3-Diaminopropionic acid (DAPA)					++			++
2-Amino-3-oxalylaminopropionic acid (ODAP)					+			+++

Key: 0 = no effect on feeding; + = 30–60% inhibition of feeding; ++ = 61–90% inhibition; +++ = 91–100% inhibition.
* Data previously presented by Navon and Bernays [7].

Table 4. Non-protein amino acids in seeds of some species of African *Acacias**

		N-Acetyldjenkolic acid	Djenkolic acid	Pipecolic acid	4-Hydroxypipecolic acid	*S*-Carboxyethylcysteine	*S*-Carboxyisopropylcysteine	Albizziine	2,3-Diaminopropionic acid	2-Amino-3-acetylamino-propionic acid	2-Amino-3-oxalylamino-propionic acid	2,4-Diaminobutyric acid	2-Amino-4-oxalylamino-butyric acid
Bentham's Series Gummiferae and Vassal's subgenus Acacia	*Acacia nubica* Benth.	+ + +		+	+ +								
	Acacia seyal Delile var. *seyal*	+ + +		+	+ +								
	Acacia tortilis (Forsk.) Hayne ssp. *heterocantha* (Burch.) Brenan	+ + +	+ +	+	+ +								
Bentham's Series Vulgares and Vassal's subgenus Aculeiferum sect. Aculeiferum	*Acacia laeta* R.Br. ex Benth.		+	+	+ +	+ +	+	+ +	+	+ + +	+ +		
	Acacia mellifera (Vahl) Benth. ssp. *detinens* (Burch.) Brenan				+ +	+ + +	+	+	+ +	+ + +	+ +	+	+
	Acacia senegal (L.) Willd.			+	+ +	+ +	+	+ +	+	+ + +	+ +		+
	Acacia albida Delile				+			+ + +	+	+ +	+ +		

* Modified from Evans *et al.* [9].
+ + +, Strong; + +, medium; +, weak.

eaten by *A. melanorhodon*. There are two distinct patterns of amino acids in these seeds correlating with the morphological division of the genus according to Bentham [12] and Vassal [13]. Table 2 shows the feeding response of *A. melanorhodon* nymphs to these individual amino acids offered in choice tests on sucrose discs. Three amino acids, DAPA, ADAP and ODAP were inhibitory to feeding at all concentrations tested. Complete inhibition occurred at 1% concentration with ADAP and ODAP, and at 5% concentration with DAPA. Two amino acids (djenkolic acid and *N*-acetyldjenkolic acid) did not inhibit feeding at concentrations up to 5% disc weight. *A. melanorhodon* was not significantly inhibited in feeding by the four non-protein amino acids (*N*-acetyldjenkolic acid, djenkolic acid, pipecolic acid and 4-hydroxypipecolic acid) present in seeds of *A. nubica*, *A. seyal* and *A. tortilis*, although the non-protein amino acids in the seeds of *A. laeta*, *A. mellifera*, *A. senegal* and *A. albida* (albizziine, DAPA, ADAP, ODAP, DABA and ODAB) did inhibit the feeding of the insects. The amino acids which have the greatest inhibitory effect on the feeding of *A. melanorhodon* nymphs are DAPA and its acetyl and oxalyl derivatives, which are known to have toxic effects in mammals, birds and other insects [4, 14–16]. Locusts appear able to discriminate against potentially toxic substances more effectively than many other insects (Bernays, E. A., personal communication).

These experiments have shown that the polyphagous *A. melanorhodon*, which feeds on *Acacia* leaves, shows a greater tolerance towards the non-protein amino acids which occur in these leaves than does *L. migratoria*. The seed amino acids DAPA, ODAP, ADAP, DABA and ODAB produced comparable inhibition in both insect species.

EXPERIMENTAL

Insects. All insects were reared at the Centre for Overseas Pest Research, London. A minimum of 20 *Anacridium melanorhodon arabafrum* (Dirsh.) nymphs as mixed 3rd to 6th instar nymphs were used for each experiment. A minimum of 10 male fifth instar nymphs of *Locusta migratoria migratorioides* (R. and F.), selected as described by Bernays and Chapman [10], were used for each experiment.

Feeding procedures. These were essentially those of ref. [11]. The amino acids to be tested were added in a sucrose soln (9.25 g/l.) to glass fibre discs (Whatman GF/A, 4.25 cm dia, weight *ca* 70 mg) which were acceptable as food to the locusts when treated with sucrose soln alone. The volume of soln required to saturate each disc was 400 µl. L-Amino acids were tested at 0.5, 1.0 and 5.0% of disc weight. The discs were air-dried after the amino acid and sucrose solns had been applied. All tests conducted were 'choice' tests in which each insect was offered a control disc of sucrose alone and a test disc of amino acid and sucrose, fastened on separate pins in a clear plastic box 27 × 15 × 10 cm high. Each box was shielded from its neighbour so that insects in adjacent boxes did not disturb each other. All experiments lasted for 20 hr. The amount of each disc eaten was measured by area (total area of disc = 14.2 cm^2) using a portable electronic area meter (Li-cor, model Li-3000).

Determination of amino acids in leaves. The quantitative estimation of leaf amino acids on paper chromatograms was based on the method of ref. [17]. Leaf material (1 g) was ground with 1 g sand and shaken with 5 ml 70% EtOH for 24 hr. The extract was centrifuged and the supernatant passed through a column (5 × 1 cm) of Dowex 50W-X8 (H^+ form). After washing with H_2O, the amino acids were displaced from the column with 20 ml 2 M NH_4OH. The ammoniacal soln was evapd to dryness, and the residue redissolved in 2.5 ml 70% EtOH. 25 µl of this extract were spotted on to a 2-D paper chromatogram, run in (1) *n*-BuOH–HOAc–H_2O (12:3:5), and (2) PhOH–H_2O (4:1 w/v) in the presence of the vapour of aq. NH_3. The amino acids were detected on the paper by spraying with 0.05% ninhydrin in MeOH. The spots were cut out, and the colour developed fully by the addition of 5 ml 2% ninhydrin in EtOH, heated in boiling water for 15 min, cooled, then diluted with 5 ml 50% EtOH. The resulting colour was measured at 570 nm within 1 hr. Standard curves were prepared for each amino acid, following an identical procedure to that used for the leaf extracts.

Acknowledgements—We are grateful to Dr. E. A. Bernays and Professor R. F. Chapman for their help and use of facilities at the Centre for Overseas Pest Research, and to Mr. A. Antonio for rearing the insects. We thank the National Environment Research Council (U.K.) for financial support.

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