Biochem. J. (1965) **97**, 104

Associations of Amino Acids and Related Compounds in the Seeds of Forty-Seven Species of *Vicia*: their Taxonomic and Nutritional Significance

By E. A. BELL and A. S. L. TIRIMANNA*
Department of Biochemistry, King's College, London, W.C. 2

(*Received* 8 *February* 1965)

1. The genus *Vicia* may be subdivided into groups of species characterized by associations of ninhydrin-positive compounds occurring in high concentrations in their seeds. Despite these subdivisions the overall distribution pattern emphasizes the unity of the genus and the possible value of such studies in establishing degrees of relationship between species within the genus. 2. Canavanine is a major constituent in the seeds of 17 species. 3. γ-Hydroxyarginine, an amino acid that has not been found in other plant genera, occurs in 18 species. 4. γ-Hydroxyornithine, a new natural amino acid, is found in two species. 5. A new naturally occurring ureido amino acid tentatively identified as 'γ-hydroxycitrulline' is found in two species. 6. High concentrations of $\alpha\beta$-diaminopropionic acid are found in seed of *V. baicalensis* and of $\alpha\gamma$-diaminobutyric acid in seed of *V. aurantica*. 7. The neurotoxic amino acid β-cyanoalanine and its γ-glutamyl peptide are found together in high concentrations in the seeds of 16 species of this agriculturally important genus. 8. Seven unidentified ninhydrin-positive compounds occur in high concentration (about 1% of the dry weight or more) in the seed of various species. Details of their R_F values, ionic mobilities and colour reactions are given. 9. The total concentration of extractable ninhydrin-positive compounds varies little between seeds of different species and these compounds may, as has been suggested for those in *Lathyrus*, constitute a readily available form of storage nitrogen. 10. The nature and distribution, as opposed to the total concentration, of the amino acids and related compounds accumulated in the seeds of *Vicia* are different from those accumulated in the seeds of the related genus, *Lathyrus*. One particularly interesting difference is the accumulation of C_6 guanidino amino acids (arginine and γ-hydroxyarginine) by *Vicia* and the accumulation of C_7 guanidino amino acids (homoarginine, γ-hydroxyhomoarginine and the related amino acid lathyrine) by *Lathyrus*. Such differences afford a method for distinguishing between species of these genera.

The distribution of the free amino acids and related compounds that occur in the seeds of *Lathyrus* has been described previously (Bell, 1962*a*, 1964*a*). On the basis of this distribution the genus may be broadly subdivided into groups of species that are characterized, not by the presence or absence of arbitrary concentrations of single amino acids or other ninhydrin-positive compounds, but rather by the presence or absence of groups of associated ninhydrin-positive compounds that show themselves as characteristic patterns of spots on paper after the chromatography and ionophoresis of seed extracts. With the exception of arginine, glutamic acid and aspartic acid, which were found in varying amounts in all the seeds, the 11 compounds that occurred in highest concentration and formed the characteristic associations were not 'protein' amino acids. One of them was β-(γ-glutamylamino)propionitrile (Schilling & Strong, 1954), a second was lathyrine (Bell, 1961), an amino acid of unknown structure, and a third was a new natural amino acid, tentatively identified from R_F values as homoarginine. The remaining eight compounds were unidentified.

High concentrations (frequently more than 1% of the dry weight) of one or more of these compounds were found in the seeds of all but four of the species examined, and it was suggested that 'the most likely explanation of the associations found in the various groups is that the compounds con-

* Present address: Tea Research Institute of Ceylon, Talawakele, Ceylon.

cerned represent a final storage product together with its precursor or precursors'.

Since this survey was made the structure of lathyrine has been established as β-(2-aminopyrimidin-4-yl)alanine (Bell & Foster, 1962), the presence of homoarginine has been confirmed by isolation (Bell, 1962*b*), three of the 'unknowns' have been identified unequivocally as αγ-diaminobutyric acid (Ressler, Redstone & Erenberg, 1961), γ-hydroxyhomoarginine (Bell, 1964*b*) and α-amino-β-oxalylaminopropionic acid (Adiga, Rao & Sarma, 1963; Murti, Seshadri & Venkitasubramanian, 1964) and a fourth tentatively as α-amino-γ-oxalylaminobutyric acid (Bell, 1964*c*).

The fact that homoarginine, γ-hydroxyhomoarginine and lathyrine, which form a characteristic association in the seeds of one subgenus of *Lathyrus*, are structurally similar added weight to the original hypothesis that they might be related biosynthetically. It has now been shown (Bell & Przybylska, 1965) that the hydroxyamino acid is an intermediate in the biosynthesis of lathyrine from homoarginine, thereby confirming the 'end-product precursors' hypothesis in one group of species at least. While the work on *Lathyrus* is being continued a further investigation has been made of the ninhydrin-positive compounds in the seeds of 47 species of the closely related genus *Vicia*. The objects of this investigation were first to determine whether similar associations of amino acids and related compounds occurred in the seeds of other genera, and secondly to determine whether differences, in kind or in distribution, of ninhydrin-positive compounds accumulated in the seeds of their species might provide biochemical criteria for separating two genera that are closely related morphologically.

The results of this investigation are contained in the present paper.

EXPERIMENTAL

Source of seeds. Seeds were supplied by over 40 British and European botanical gardens; seeds of the same species were frequently obtained from several of them. Separate analyses of each species were made, wherever possible, with seed from two or more sources. Of the results given in this paper only those for *V. fulgens* Barr. (Kew) and *V. baicalensis* Fedtsch. (Moscow) are based on the analysis of seed from a single source.

Preparation of seed extracts. Finely ground seed (200mg.) of each species was shaken with ethanol (1ml.) and water (1ml.) for 30min. in an automatic flask shaker. After standing for a further 17hr. at room temperature the suspension was centrifuged and the supernatant used for subsequent analyses. A second series of extracts was prepared in the same way with 0·1N-HCl in place of water.

Comparison of extraction methods. Acid extraction offered no advantage over extraction with aq. 50% (v/v) ethanol. In acid solution hydrolysis of β-cyanoalanine occurred. In the acid extracts the concentration of V.A_1 appeared higher and the concentrations of V.A_3 lower than in the neutral extracts. This may indicate that V.A_1 is a hydrolysis product of V.A_3. The distribution shown in Table 1 is based on the neutral extracts.

Chromatography. One-dimensional chromatograms were prepared by the descending technique on Whatman no. 1 paper, with 0·1ml. of extract. Solvents used were: 1, butan-1-ol–acetic acid–water (12:3:5, by vol.); 2, phenol–water (4:1, w/v) in the presence of the vapour of aq. NH_3 (sp.gr.0·88); 3, butan-1-ol–pyridine–water (1:1:1, by vol.); 4, lutidine (mixed 2,4- and 2,5-isomers)–water (11:5, v/v); 5, ethyl methyl ketone–propionic acid–water (2:1:2, by vol.). The first four solvents were prepared according to Smith (1960) and the last according to Gerritsen, Waisman, Lipton & Strong (1962). Two-dimensional chromatograms were prepared on Whatman no. 1 paper with 0·25ml. of each extract. They were developed with solvents 1 and 2.

Ionophoresis. Ionophoresis was conducted on Whatman no. 1 paper in the following buffer solutions: formic acid (98–100%)–acetic acid–water (33:148:1819, by vol.), pH1·9; acetic acid–pyridine–water (5:0·5:95, by vol.), pH3·6; acetic acid–pyridine–water (0·2:5:95, by vol.), pH6·5; 0·01M-Na_2CO_3, pH11·6. With the three buffers of lower pH a horizontal-ionophoresis method essentially that of Gross (1961) was used, a potential difference of approx. 60v/cm. being applied for 30min. At pH11·6 a hanging-strip method was used, 5v/cm. being applied for 17hr.

Development of colours. Chromatography and ionophoresis papers were dipped in ninhydrin reagent [0·2% (w/v) ninhydrin in 95% (v/v) acetone], the modified Ehrlich reagent 1% (w/v) *p*-dimethylaminobenzaldehyde in acetone–10N-HCl (9:1, v/v) (Smith, 1953), the modified Sakaguchi's reagent 0·1% (v/v) 8-hydroxyquinoline in acetone followed by 0·2% (v/v) bromine in 0·5N-NaOH (Jepson & Smith, 1953), or sprayed with Fearon's sodium pentacyanoammonioferrate reagent at pH7 (Bell, 1958). The periodate–acetylacetone reagent described by Schwartz (1958) for the identification of serine, δ-hydroxylysine and ethanolamine was used as a confirmatory test for γ-hydroxyornithine.

RESULTS

The distribution of the major concentrations of ninhydrin-positive compounds detected in 50% (v/v)-ethanol extracts of seeds of 47 species of *Vicia* are given in Table 1. All the identified compounds occuring in high concentrations are amino acids or derivatives: the R_F values (Table 2) and the ionic mobilities and colour reactions (Table 3) of the unidentified compounds, designated V.A_1, V.A_3 etc., suggest that they too are amino acids or related compounds of low molecular weight.

The species listed in Table 1 have been arranged in four groups. The individual members of each of the first three groups are related by a common association of ninhydrin-positive compounds that occur in high concentrations (relative to the other ninhydrin-positive compounds present) and show themselves as characteristic patterns after paper ionophoresis (Fig. 1) or chromatography.

Table 1. *Ninhydrin-positive compounds present in seed extracts of* Vicia *species*

Glutamic acid and aspartic acid were found in all species; high concentrations of proline were found in species 1, 36 and 42, of serine in species 4 and 11, of an amino acid tentatively identified as γ-hydroxycitrulline in species 23 and 25, of γ-hydroxyornithine in species 24 and 25, of αβ-diaminopropionic acid in species 28, of αγ-diaminobutyric acid in species 45 and of an unidentified basic compound (V.B_3) in species 29, 30 and 31. The letters V.A_1, V.A_3 etc. represent unidentified compounds; Arg, arginine; γ-OH-Arg, γ-hydroxyarginine; Asp(NH_2), asparagine; Homo-Ser, homoserine; Can, canavanine, β-CN-Ala, β-cyanoalanine; Glu-β-CN-Ala, γ-glutamyl-β-cyanoalanine (formerly V.A_2); Pip, pipecolic acid; T, trace; +, concn. approx. 1% of the dry weight; ++ and +++, proportionately greater concns.

Group no.	Species no.	Name	Arg	γ-OH-Arg	Asp-(NH_2)	Homo-Ser	Can	β-CN-Ala	Glu-β-CN-Ala	Pip	V.A_1	V.A_3	V.A_4	V.N	V.B_1	V.B_2
1	1	*V. articulata* Hormen.	T	.	T	.	++	.	.	.	.	.	.	.	.	.
	2	*V. silvatica* L.	+	.	T	.	++	.	.	.	.	.	.	.	.	.
	3	*V. ervilia* Willd.	T	.	T	.	+	.	.	.	.	.	.	.	.	.
	4	*V. tennuisima* Bieb.	++	.	+	.	++	.	.	.	.	.	.	++	.	.
	5	*V. cassubica* L.	T	.	.	T	++	.	.	.	+	++	.	T	++	.
	6	*V. disperma* D.C.	+	.	T	T	+++	.	.	.	.	+	.	+	+	.
	7	*V. atropurpurea* Desf.	T	.	T	(T)	++	.	.	.	+	+	.	T	+	.
	8	*V. benghalensis* L.	T	.	T	(T)	+++	.	.	.	.	+	.	+	+	.
	9	*V. dalmatica* Kern.	T	.	+	(T)	+++	.	.	.	.	+	.	++	T	.
	10	*V. selloi* Vog.	+	.	+	.	+++	.	.	.	+	++	.	++	T	.
	11	*V. villosa* Roth.	T	.	+	(T)	++	.	.	.	.	+	.	T	T	.
	12	*V. cracca* L.	T	.	T	(T)	++	.	.	.	+	++	.	++	.	.
	13	*V. dasycarpa* Ten.	+	.	.	(T)	+++	.	.	.	+	++	.	++	.	.
	14	*V. tennuifolia* Roth.	++	.	+	(T)	+++	.	.	.	.	++	.	+	.	.
	15	*V. hirsuta* Gray	T	.	T	.	++	.	.	.	.	+	.	.	.	.
	16	*V. calcarata* Desf.	T	.	T	.	++	.	.	.	+	++	.	+	.	.
	17	*V. tetrasperma* Moench.	+	.	T	.	++	.	.	.	.	T	.	+	.	.
2	18	*V. pannonica* Crantz	++	.	.	.	.	.	.	.	+	++	.	.	.	.
	19	*V. lutea* L.	+	.	.	.	.	.	.	.	.	++	.	.	.	.
	20	*V. orobus* D.C.	++	.	+	.	.	.	.	.	+	++	.	T	.	.
	21	*V. bithinica* L.	++	.	T	.	.	.	.	.	+	++	.	T	.	.
	22	*V. hyrcanica* Fisch.	+++	.	T	.	.	.	.	.	.	++	.	T	.	.
	23	*V. fulgens* Barr.	+	.	.	.	.	.	.	.	.	++	.	T	.	.
	24	*V. onobrychoides* L.	+	+	T	.	.	.	.	.	+	++	.	.	.	.
	25	*V. unijuga* Braun	T	++	.	.	.	.	.	T	+	++	.	.	.	.
	26	*V. narbonensis* L.	++	+	T	.	.	.	.	.	+	++	.	.	.	.
	27	*V. dumetorum* L.	T	+	.	.	.	.	.	.	T	++	.	.	.	.
	28	*V. baicalensis* Fedtsch.	+	+	++	.	.	.	.	.	.	++	.	.	.	.

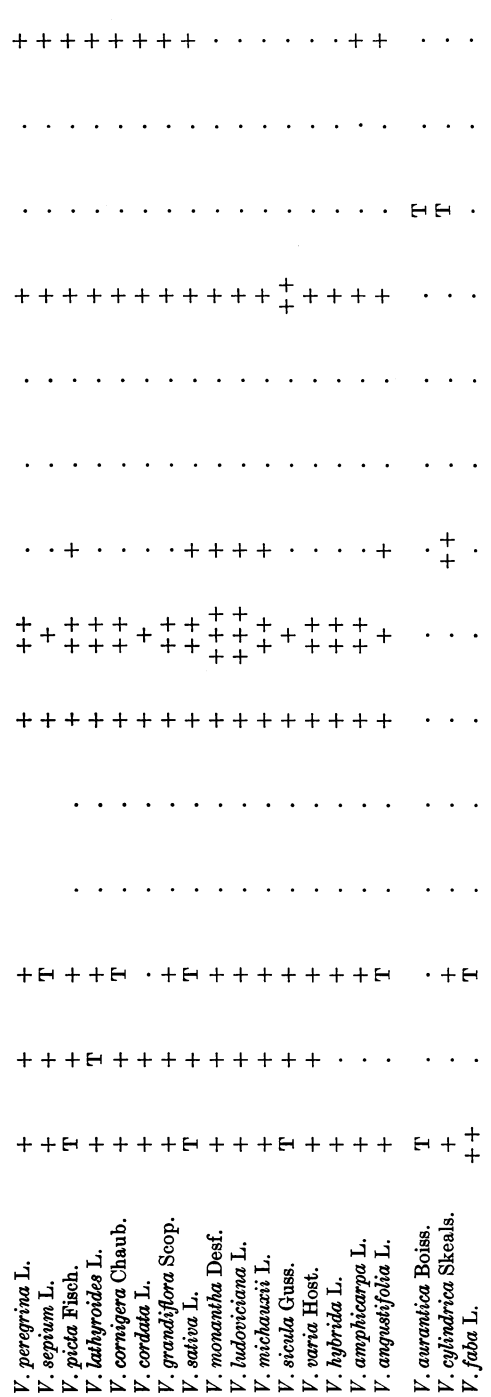


Table 2. *Approximate* R_F ($\times 100$) *values of some less common or unidentified ninhydrin-positive compounds present in* Vicia *seeds*

Solvents 1–5 are defined in the Experimental section.

	$100 \times R_F$				
Solvent ...	1	2	3	4	5
Ninhydrin-positive compound					
γ-Hydroxyarginine	15	76	15	7	32
Homoserine	27	53	30	25	42
Canavanine	15	59	20	10	34
β-Cyanoalanine	32	65	40	39	54
γ-Glutamyl-β-cyanoalanine	16	27	27	36	43
V.A_1	10	6	7	3	39
V.A_3	41	43	27	10	48
V.A_4	12	20	—	—	—
V.N	31	75	43	31	50
V.B_1	31	50	22	13	41
γ-Hydroxyornithine	10	60	19	10	36
'γ-Hydroxycitrulline'	15	52	18	10	35
αγ-Diaminobutyric acid	14	54	22	8	35
αβ-Diaminopropionic acid	14	56	22	10	33

In addition to glutamic acid and aspartic acid, which are present throughout the genus, all species of group 1 contain canavanine, all in group 3 β-cyanoalanine, γ-glutamyl-β-cyanoalanine and V.A_4, and all in group 2 arginine or derivatives (in higher concentration than in species of the other groups) and V.A_3. Group 2 is the least clearly defined of the first three groups, as arginine and V.A_3 are not unique to it. Within this group, however, a transition can be seen from those species that closely resemble the V.A_3-containing species of group 1 (and differ only in accumulating arginine in place of canavanine) to those which, like 13 species of group 3, accumulate γ-hydroxyarginine (Bell & Tirimanna, 1964).

Group 4 contains three species that do not fall naturally into the other groups. Of these *V. cylindrica* and *V. faba* do not contain significant concentrations of any of the 'non-protein' amino acids or related compounds, and *V. aurantica* contains a high concentration of αγ-diaminobutyric acid and its 'pattern' is like that of *Lathyrus aurantius*, *L. luteus* and *L. laevigatus* subspecies *aureus* (Bell, 1962*a*) rather than that of any other species of *Vicia*.

High concentrations of proline are found in *V. articulata*, *V. sativa* and *V. hybrida*, of serine in *V. tennuisina* and *V. villosa*, and of γ-hydroxyornithine in *V. onobrychoides* and *V. unijuga*. A ninhydrin-positive ureido compound is found in *V. fulgens* and *V. unijuga*. This compound, which

Table 3. *Ionic mobilities and colour reactions of ninhydrin-positive compounds present in* Vicia *seeds*

Details are given in the Experimental section.

Ninhydrin-positive compound	Group no.	Ionic mobilities	Characteristic colour reactions
γ-Hydroxyarginine	2 and 3	Positively charged at pH 6·5, 3·6 and 1·9; negatively charged at pH 11·5, moves with arginine	Scarlet with Sakaguchi's reagent
Homoserine	1	Uncharged at pH 3·6; positively charged at pH 1·9, moves with threonine	
Canavanine	1	Positively charged at pH 3·6 and 1·9, moves slightly slower than arginine; negatively charged at pH 11·5, moves faster than arginine	Magenta with sodium pentacyanoammonioferrate
β-Cyanoalanine	3	Uncharged at pH 3·6 and 6·5; positively charged at pH 1·9, moves faster than phosphothreonine and slower than threonine	Green with ninhydrin
γ-Glutamyl-β-cyanoalanine	3	Negatively charged at pH 3·6, moves faster than aspartic acid; positively charged at pH 1·9, moves slower than aspartic acid	
γ-Hydroxyornithine	2	Positively charged at pH 6·5, 3·6 and 1·9, moves slightly slower than ornithine at pH 1·9 and moves noticeably so at pH 3·6 and 6·5; negatively charged at pH 11·5, moves markedly faster than ornithine	Grey–brown when treated successively with ninhydrin and Ehrlich's reagents; yellow with periodate–acetylacetone reagent
'γ-Hydroxycitrulline'	3	Positively charged at pH 1·9, moves slightly slower than citrulline and homocitrulline; negatively charged at pH 11·5, moves with homocitrulline but slightly slower than citrulline	Yellow with Ehrlich's reagent; brown–yellow when treated successively with ninhydrin and Ehrlich's reagent, unlike yellow and rose–red given by citrulline and homocitrulline under same conditions
αγ-Diaminobutyric acid	4 (one species only)	Positively charged at pH 3·6 and 1·9, moves slower than ornithine at pH 1·9 but faster at pH 3·6	Brown–purple with ninhydrin
αβ-Diaminopropionic acid	2 (one species only)	Positively charged at pH 3·6 and 1·9, moves faster than αγ-diaminobutyric acid (but slower than ornithine at pH 1·9)	Dark blue when treated successively with ninhydrin and Ehrlich's reagent
V.A_1	1 and 2	Negatively charged at pH 3·6, moves faster than aspartic acid but slightly slower than γ-glutamyl-β-cyanoalanine	
V.A_3	1 and 2	Negatively charged at pH 3·6, moves faster than aspartic acid, but slower than V.A_1; carries small positive charge at pH 1·9	
V.A_4	3	Negatively charged at pH 3·6, moves slightly faster than γ-glutamyl-β-cyanoalanine	
V.N	1, 2 and 4	An ampholyte uncharged at pH 6·5	
V.B_1	1	Positively charged at pH 3·6, moves slower than arginine	Brown with ninhydrin
V.B_2	3	Positively charged at pH 3·6, moves slightly slower than arginine	Orange with Sakaguchi's reagent
V.B_3	3	Carries small positive charge at pH 3·6	Orange with Sakaguchi's reagent

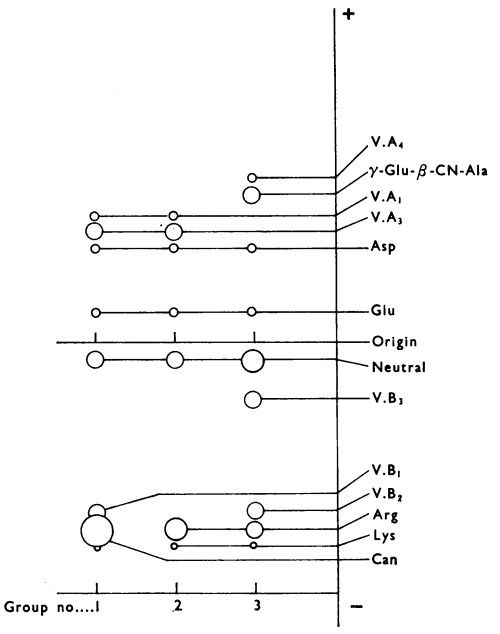


Fig. 1. Relative mobilities of ninhydrin-positive compounds in extracts of species in groups 1–3, when subjected to ionophoresis at pH 3·6. Details are given in the Experimental section. γ-Glu-β-CN-Ala, γ-Glutamyl-β-cyanoalanine; Neutral, unresolved neutral amino acids; Can, canavanine.

accompanies γ-hydroxyarginine in both species and γ-hydroxyornithine in *V. unijuga*, has not been completely characterized; on alkaline hydrolysis, however, it yields γ-hydroxyornithine and ammonia and is tentatively identified as a new amino acid, γ-hydroxycitrulline; the R_F values of this compound are close to those predictable for γ-hydroxycitrulline.

High concentrations of αβ-diaminopropionic acid, an amino acid previously identified in the free state in two species of *Mimosa* (Gmelin, Strauss & Hasenmaier, 1959) and its β-oxalyl derivative in *Lathyrus* (Adiga *et al.* 1963; Murti *et al.* 1964), are present in *V. baicalensis*. High concentrations of an unidentified ninhydrin-positive guanidino compound, $V.B_3$, occur in *V. peregrina*, *V. sepia* and *V. picta*.

The method used in Table 1 to indicate the concentrations of individual compounds is of necessity very approximate. The symbol + represents a concentration of about 1% of the dry weight judged from the intensity of spot given with ninhydrin and assuming a molecular weight of 150 where this is unknown.

To attempt a more quantitative representation would, however, serve no useful purpose as absolute and relative concentrations of these compounds vary with conditions of growth and ripening. These

Table 4. *Variations in the concentration of ninhydrin-positive compounds in the seeds of a single species* (V. unijuga) *from different sources*

T, Trace; +, concn. approx. 1% of the dry weight; ++, +++ and ++++, proportionately greater concns.

Source	γ-Hydroxy-arginine	γ-Hydroxy-ornithine	'γ-Hydroxy-citrulline'
Leipzig	++++	++	++
Palermo	+++	+	+
Munich	++	T	—
Wrocław	++	T	T
Copenhagen	++	T	T
Kew	++	T	—

variations in fact emphasize the advantage of using associated groups of compounds, if present, rather than an arbitrary concentration (usually the minimum concentration detectable by the analytical procedure employed) of a single compound as a criterion of relationship between species. Variation, in this case between seeds of the same species from different sources, is illustrated in Table 4, which shows differences in the concentrations of the three hydroxyamino acids found in seeds of *V. unijuga* grown in different areas. 'γ-Hydroxycitrulline' is most probably present in the seed from Munich and Kew but was not detected by the standard procedures.

DISCUSSION

The studies completed on *Lathyrus* and briefly described in the introduction to this paper have confirmed that the ninhydrin-positive compounds associated together in the seeds of at least one group of species of that genus must consist of a final product together with its precursors. It also seems probable that the difference between those species which accumulate lathyrine and those which only accumulate homoarginine in their seeds is the absence from the second group of enzymes capable of hydroxylating and cyclizing the guanidino amino acid.

While the significance of the other associations in *Lathyrus* and the relationships of the various subgenera to one another was (and is) still under investigation it was decided to make a similar investigation of the genus *Vicia*. The results of this investigation are summarized in Table 1 and show that the seeds of all of the 47 species of *Vicia* examined contain high concentrations of one or more ninhydrin-positive compounds extractable with 50% (v/v) ethanol. The simplest subdivision of the genus is into species with, and species without, canavanine. In the seeds of three species, canavanine is the only 'non-protein' amino acid or unidentified ninhydrin-

positive compound that occurs in high concentration; this suggests that it may be the end product of a short biosynthetic pathway, which proceeds without an appreciable accumulation of intermediates. In the seeds of 13 other species canavanine is accompanied by high concentrations of the unidentified acidic compound V.A_3, the compounds designated V.A_1, V.B_1 and V.N also being present in some of them. The syntheses of canavanine and V.A_3 appear to be unrelated, however, as V.A_3 is absent from four species that contain canavanine and present in the 11 species of group 2 that contain no canavanine. It may possibly represent the end product of a second biosynthetic pathway.

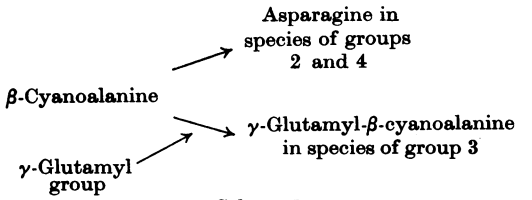
In addition to V.A_3, accumulations of arginine and in five instances γ-hydroxyarginine are found in species of group 2. In three of these species other hydroxyamino acids, probably derivatives or precursors of γ-hydroxyarginine, are found.

In group 3 a well-defined association of compounds (β-cyanoalanine, γ-glutamyl-β-cyanoalanine and V.A_4) occurs. By growing species representative of groups 2 and 3 and *V. faba* in an atmosphere containing $H^{14}CN$ it has been possible to demonstrate (Fowden & Bell, 1965) that β-cyanoalanine is in fact synthesized by seedlings of species other than those of group 3. In the species of group 3, however, β-cyanoalanine accumulates in the free state or as its glutamyl peptide, whereas in the others it appears to be largely hydrolysed to asparagine (Scheme 1).

The occurrence of $\alpha\gamma$-diaminobutyric acid in *V. aurantica* raises the question of the true identity of this species. This amino acid was present in the two samples of seed (from Munich and Lausanne) that were available, and their amino acid 'patterns' were unlike those of any other species of *Vicia* examined but were identical with those of three *Lathyrus* species. *V. baicalensis* is unique among the species of these two genera that have been analysed in containing high concentrations of free $\alpha\beta$-diaminopropionic acid.

When the findings of the present survey are compared with those of the earlier survey of *Lathyrus*, interesting similarities and even more interesting differences between the two genera are apparent. Qualitative differences are found both within and between the genera, yet the total concentration of extractable ninhydrin-positive material present in the seeds of all species (*Lathyrus* and *Vicia*) remains very constant. In considering the role of the compounds in *Lathyrus* it was suggested that 'although the total free non-protein amino acid nitrogen may be very much less than the protein nitrogen stored in a seed, it may nevertheless constitute a small highly concentrated reserve immediately available to the embryo on germination'. This hypothesis may equally well explain the occurrence of the free amino acids and other ninhydrin-positive compounds in the seeds of *Vicia*, although the restriction to 'non-protein' amino acids can no longer be made as arginine itself is found in high concentration in several species. As in *Lathyrus* the distribution of certain compounds suggests that they may be alternative storage products. High concentrations of canavanine in group 1 are, for example, replaced by high concentrations of arginine or arginine plus γ-hydroxyarginine in groups 2 and 3, and high concentrations of V.A_3 and associated compounds in groups 1 and 2 being replaced by the cyano compounds and V.A_4 in group 3. Though the evolution of different metabolic pathways or the predominance of different alternative but coexistent pathways in different species makes the division shown in Table 1 possible, the species of the different subgenera are nevertheless clearly related in terms of the overall distribution pattern, even as they are in terms of morphology. The occurrence of V.A_3, for example, relates groups 1 and 2, and γ-hydroxyarginine relates groups 2 and 3, just as the occurrence of homoarginine and α-amino-β-oxalylaminopropionic acid relates different groups in *Lathyrus*. Even the most dissimilar examples within each genus can be seen as the end products of a gradual stepwise divergence of biochemical characteristics traceable through intermediate forms rather than as unrelated species.

No such relationship is apparent between the two genera themselves, however. Arginine is the only amino acid found in high concentrations in the seeds of species from both genera, and if the anomalous *V. aurantica* is disregarded there is no 'nonprotein' amino acid or derivative common to both. Canavanine, γ-hydroxyarginine, 'γ-hydroxycitrulline', γ-hydroxyornithine, β-cyanoalanine, γ-glutamyl-β-cyanoalanine, six unidentified ninhydrin-positive compounds and in one species $\alpha\beta$-diaminopropionic acid are found in *Vicia*; whereas homoarginine, γ-hydroxyhomoarginine, lathyrine, $\alpha\gamma$-diaminobutyric acid, α-amino-β-oxalylaminopropionic acid, 'α-amino-γ-oxalylaminobutyric acid', β-(γ-glutamylamino)propionitrile and three unidentified ninhydrin-positive compounds (different from those of *Vicia*) are found in *Lathyrus*. The accumulation of C_6 guanidino compounds or cana-



Scheme 1.

vanine by *Vicia* and of C_7 guanidino compounds or $\alpha\gamma$-diaminobutyric acid by *Lathyrus* is perhaps a distinction of particular interest.

These findings suggest that the study of the distribution and metabolism of 'non-protein' amino acids and related compounds in plants may help, not only in establishing phylogenetic relationships between species within a genus, but also in defining the genus itself.

In addition to its possible phylogenetic significance, this study has revealed the presence of high concentrations (about 1% of the dry weight and more) of the neurotoxin β-cyanoalanine and its γ-glutamyl derivative, not only in the seeds of *V. sativa* from which they were originally isolated (Ressler, 1962; Ressler, Nigam, Giza & Nelson, 1963), but also in the seeds of 15 other species. These findings would seem to be of particular interest in this agriculturally important genus.

The authors thank Mr W. C. Mackenzie for help in obtaining seed.

REFERENCES

Adiga, P. R., Rao, S. L. N. & Sarma, P. S. (1963). *Curr. Sci.* **32**, 153.
Bell, E. A. (1958). *Biochem. J.* **70**, 617.
Bell, E. A. (1961). *Biochim. biophys. Acta*, **47**, 602.
Bell, E. A. (1962*a*). *Biochem. J.* **83**, 225.
Bell, E. A. (1962*b*). *Biochem. J.* **85**, 91.
Bell, E. A. (1964*a*). *Nature, Lond.*, **203**, 378.
Bell, E. A. (1964*b*). *Biochem. J.* **91**, 358.
Bell, E. A. (1964*c*). *Abstr. 1st Meet. Fed. Eur. biochem. Soc., Lond.*, A68, p. 53.
Bell, E. A. & Foster, R. G. (1962). *Nature, Lond.*, **194**, 91.
Bell, E. A. & Przybylska, J. (1965). *Biochem. J.* **94**, 35P.
Bell, E. A. & Tirimanna, A. S. L. (1964). *Biochem. J.* **91**, 356.
Fowden, L. & Bell, E. A. (1965). *Nature, Lond.*, **206**, 110.
Gerritsen, T., Waisman, H. A., Lipton, S. H. & Strong, F. M. (1962). *Arch. Biochem. Biophys.* **97**, 34.
Gmelin, R., Strauss, G. & Hasenmaier, G. (1959). *Hoppe-Seyl. Z.* **314**, 28.
Gross, D. (1961). *J. Chromat.* **5**, 194.
Jepson, J. B. & Smith, I. (1953). *Nature, Lond.*, **172**, 1100.
Murti, V. V. S., Seshadri, T. R. & Venkitasubramanian, T. A. (1964). *Phytochemistry*, **3**, 73.
Ressler, C. (1962). *J. biol. Chem.* **237**, 733.
Ressler, C., Nigam, S. N., Giza, Y.-H. & Nelson, J. (1963). *J. Amer. chem. Soc.* **85**, 3311.
Ressler, C., Redstone, P. A. & Erenberg, R. H. (1961). *Science*, **134**, 188.
Schilling, E. D. & Strong, F. M. (1954). *J. Amer. chem. Soc.* **76**, 2848.
Schwartz, D. P. (1958). *Analyt. Chem.* **30**, 1855.
Smith, I. (1953). *Nature, Lond.*, **171**, 43.
Smith, I. (1960). *Chromatographic and Electrophoretic Techniques*, vol. 1, p. 84. London: William Heinemann (Medical Books) Ltd.