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COMPARATIVE STUDIES ON THE AMINO ACID POOLS OF THREE LATHYRUS SPECIES

BY

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Abstract

The amino acid pools of different organs of *Lathyrus niger*, *L. maritimus* and *L. sylvestris*, have been studied at four developmental stages. The occurrence of rare non-protein amino acids is the most characteristic feature of each species. They all contain pipecolic acid but in *L. maritimus* it is present in high concentrations and this species also synthesizes 5-hydroxypipecolic acid. *L. maritimus* contains abundant γ-methylglutamic acid, which was not found in the other two species. Homoserine and α,γ-diaminobutyric acid are characteristic of *L. sylvestris*. Homoarginine occurs only in traces in *L. maritimus*, despite the fact that it is the precursor of lathyrine, a compound which is prominent in all the organs of this species. Lathyrine accumulates in the wintering organs of *L. niger*. Using radioactive homoarginine as tracer, it could be demonstrated that it was effectively catabolized in the leaves of *L. niger* but served as a precursor of lathyrine in the fruits. The seedlings of *L. sylvestris*, which do not contain homoarginine, catabolized it only slightly. This species has lost the ability to form lathyrine and homoarginine and was unable to catabolize lathyrine. Lathyrine was catabolized in young seedlings of *L. niger* except in the white underground part of the stem. Some evolutionary relationships deduced from the metabolism of homoarginine, lathyrine, homoserine, α,γ-diaminobutyric acid, pipecolic acid and γ-methylglutamic acid are discussed.

I. Introduction

About thirty years after the establishment of nitrogen as an element the first amino acid was isolated. In the year 1806, VAUQUELIN & ROBIQUET succeeded in crystallizing a nitrogen-containing substance from the juice of *Asparagus sativus*. This compound was later named asparagine after the plant in which it was discovered. After this, some further amino acids were isolated from animal material but half a century passed before any other amino acids were found in the plant kingdom. Then aspartic and glutamic acids were isolated from legumin and wheat gluten (RITTHAUSEN 1866, 1868, 1869).

Towards the end of the nineteenth century several of the other known amino acids in plants were detected in legumes. In addition some new amino acids not previously found in other organisms were first isolated from plants, for example, glutamine from cucumber (SCHULZE & BARBIERI 1877), and phenylalanine and arginine from *Lupinus* (SCHULZE & BARBIERI 1881 and SCHULZE & STEIGER 1886, 1887). Many amino acids earlier isolated from animal organisms were also found in plants, e. g. leucine, tyrosine, histidine, lysine, valine, isoleucine, alanine, cystine and proline in etiolated seedlings of *Vicia* and/or *Lupinus* (GORYP-BESANEZ 1874; SCHULZE & BARBIERI 1881, 1883; SCHULZE 1893, 1899; SCHULZE & WINTERSTEIN 1905 and WINTERSTEIN & PANTANELLI 1905).

Before the Second World War some non-protein amino acids were found. Of those typical of some leguminous species, trigonelline (JAHNS 1885, 1887), 3,4-dihydroxyphenylalanine (GUGGENHEIM 1913 and TORQUATI 1913 a and b), canavanine (KITAGAWA & TOMIYAMA 1929), abrine (GHATAK & KAUL 1932), djenkolic acid (VAN VEEN & HYMAN 1935), mimosine (RENZ 1936 and MASCRÉ

1937) and willardine (RENZ 1936) deserve mention. Also from the seeds of *Lathyrus sativus* two fractions were isolated (DILLING 1920) and a substance crystallized (ALTON & CHOPRA 1922) which caused neurological symptoms in experimental animals similar to those long known to be caused by seeds of some *Lathyrus* species when used as food (HIPPOCRATES cit. SELEY 1957).

The rapid development of analytical methods in biochemistry after the Second World War provided entirely new possibilities for analysing the chemical constituents of plants and clarifying their metabolism. The most important new techniques were the use of radioisotopes to indicate biosynthetic pathways, chromatography to determine the metabolites produced and new preparation and purification methods for enzymes. This also made it possible to identify the compounds causing the symptoms of the group of diseases known as lathyrism. As by-products of these mostly medically orientated studies many new amino acids and peptides have been isolated from species of the genera *Lathyrus* and *Vicia*. The occurrence of these compounds has even been used to aid in clarifying the relationships between these species (BELL 1962 b, 1964 c, 1965; BELL & FOWDEN 1964; BELL & TIRIMANNA 1965 and TSCHIERSCH & HANELT 1967). Little is known about the physiological significance and occurrence of these rare amino acids in the different parts of the plants. Few comprehensive studies have been made on the amino acid pools of plants and many investigators have been content with the mere isolation of a new compound (cf. STEWARD & DURZAN 1965). The present work is intended as a basic study of comparative physiology and chemical evolution in the genus *Lathyrus*.

The aim of the study has been to compare the amino acid pools of chemically and morphologically different species of *Lathyrus* (*L. niger*, *L. maritimus* and *L. sylvestris*) belonging to different subgroups (TAUBERT 1891 and ASCHERSON & GRAEBNER 1906—1910). The following points have been studied: 1. the differences in the constituents of the amino acid pools of each species in different parts of the plant during development; 2. the changes in the amino acid pools occurring within each species in different stages of development (seedlings, budding, flowering and fruiting plants); 3. comparison between corresponding parts of these three plants; and 4. a study of the reason for the differences in the amino acid composition between and within the species.

II. Previous studies concerning the amino acid pool in the tribe *Vicieae*

In the family Papilionaceae the genus *Lathyrus* belongs to the tribe *Vicieae*, which consists of five genera: *Vicia*, *Lathyrus*, *Pisum*, *Cicer* and *Lens*. All these genera include economically important species and consequently a great deal

is known about the chemical constituents of these plants. Since the Papilionaceae family is one of the largest angiosperm families, I shall restrict this review mainly to the literature concerning the non-protein amino acids in the tribe *Vicieae*.

A. Lathyrogenic compounds

Studies on the amino acid constituents of species of the genus *Lathyrus* received a strong impetus when a peptide, β-(γ-glutamyl)aminopropionitrile, was isolated from the seeds of *Lathyrus odoratus* and *L. pusillus* (DUPUY & LEE 1954, 1956; MCKAY et al. 1954 and SCHILLING & STRONG 1954, 1955) and shown to be the compound that caused the skeletal deformations in experimental animals known as osteolathyrism. This compound has later been detected in the seeds of *L. hirsutus* and *L. tuberosus* also (BELL 1964 c and PRZYBYLSKA & RYMOWICZ 1965) and in the seedlings of *L. sylvestris* and *L. odoratus* (RESSLER 1964). Neither this so-called *Lathyrus* factor nor its precursor β-aminopropionitrile was found in the seeds of the economically important *L. sativus* (STRONG 1956 and GANAPATHY et al. 1958).

Another disease, caused by some compounds isolated later (α,γ-diaminobutyric acid, α-amino-β-oxalylaminopropionic acid, β-cyano-L-alanine, γ-glutamyl-β-cyano-L-alanine) which occur in several *Lathyrus* and *Vicia* species (RESSLER et al. 1961; ADIGA et al. 1962, 1963; RESSLER 1962, 1964; MURTI et al. 1964; RAO et al. 1964 and TSCHIERSCH & HANELT 1967), is named neurolathyrism (cf. SELEY 1957). In the light of these studies the reason for the somewhat contradictory previous results (cf. ANDERSON et al. 1924—1925; STOCKMAN 1929; ZAGAMI 1931; GEIGER et al. 1933 and MCCARRISON & KRISHNAN 1934) was understood to be due to the fact that seeds of different *Lathyrus* species do not all contain lathyrogenic compounds and also that some experimental animals are more tolerant to these substances than others.

α,γ-Diaminobutyric acid was first isolated from hydrolysates of some antibiotics of the polymyxin group (CATCH & JONES 1948, CATCH et al. 1949 and HAUSMANN & CRAIG 1954). This compound occurs in three related *Lathyrus* species in every part of the plant: *L. sylvestris*, *L. latifolius* and *L. heterophyllus* (cf. also PRZYBYLSKA & RYMOWICZ 1965 and SIMOLA 1966). BELL (1962 b, 1964 c) has reported that seeds of twelve species of *Lathyrus* contain this substance, but other studies do not confirm his results. This compound is also characteristic of some liliaceous species (FOWDEN & BRYANT 1958, 1959). Aspartic-β-semialdehyde and β-cyanoalanine are alternative precursors of α,γ-diaminobutyric acid (cf. FOWDEN 1965).

The α- and γ-oxalyl derivatives of α,γ-diaminobutyric acid have been detected in the seeds of ten European *Lathyrus* species (e.g. *L. sylvestris*) belonging mainly to the Eulathyrus section (BELL 1964 b; BELL & O'DONOVAN 1966 and PRZYBYLSKA & RYMOWICZ 1965). The toxicity of these compounds is unknown. PRZYBYLSKA & RYMOWICZ (op.cit.) found α-amino-γ-oxalylaminobutyric acid in the roots but not in the seeds of *L. tuberosus* and they were unable to detect α-oxalylamino-γ-aminobutyric acid in this species.

α,β-Diaminopropionic acid has not been found in the free state in *Lathyrus* species, but its neurotoxic β-oxalyl derivative has been isolated from the seeds of *Lathyrus sativus* (ADIGA et al. 1962, 1963; MURTI et al. 1964 and RAO et al. 1964). This compound has also been detected in the seeds of twenty other species of this genus (BELL 1964 b and BELL & O'DONOVAN 1966). Most of these species belong to the subgroups *Eulathyrus*,

Clymenum and *Cicercula*. Some of the results, however, are somewhat contradictory. α-Amino-β-oxalylaminopropionic acid occurs in small quantities in *L. sylvestris* but in rather large amounts, especially in the roots and mature seeds, in species of the section *Clymenum* (PRZYBYLSKA & RYMOWICZ 1965). Its concentration also increases during the germination of *L. sativus* (MALATHI et al. 1967). The precursor of α-amino-β-oxalylaminopropionic acid, α,β-diaminopropionic acid, has been found in hydrolysates of viomycin, an antibiotic synthesized by some *Streptomyces* (HASKELL et al. 1952). This amino acid also occurs in the free state in seeds of *Mimosa palmeri* (GMELIN et al. 1959).

β-Cyano-L-alanine is especially characteristic of the genus *Vicia*, occurring in the subgroups *Vicia* and *Atossa*, but it is also present in smaller quantities in the seeds of seven *Lathyrus* species (e.g. *L. sylvestris* and *L. latifolius*), either in free form or bound as a γ-glutamyl peptide (RESSLER 1962, 1964; RESSLER et al. 1963 and TSCHIERSCH &HANELT 1967). These two compounds also occur in small quantities in many food and forage plants such as lentils, soyabean, lima bean and alfalfa. They have not been found in subsequent investigations on *Lathyrus* species (BELL 1964 c, 1965 and PRZYBYLSKA & RYMOWICZ 1965). β-Cyanoalanine has been suggested (RESSLER et al. 1963 and RESSLER 1964) to be the biosynthetic intermediate in a newly recognized pathway for the biosynthesis of asparagine from cyanide in plants. Serine or phosphoserine has been assumed to be the acceptor of cyanate and β-cyanoalanine is formed in the reaction. This com-

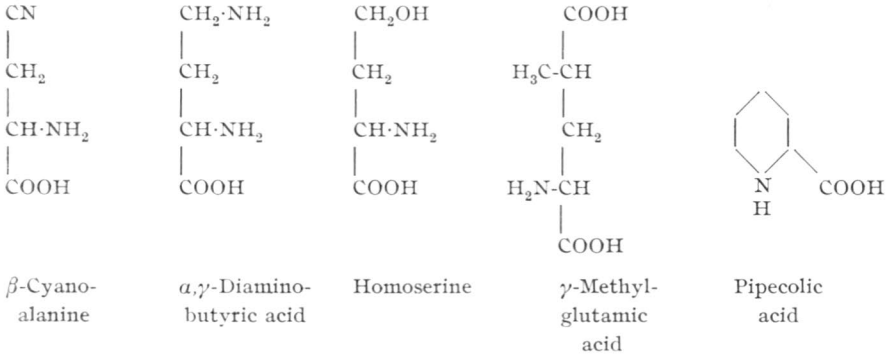

FIG. 1. Structural formulae of some amino acids characteristic of *Lathyrus* and *Vicia* species.

pound is considered to be a labile metabolite with a high turnover rate (FOWDEN et al. 1964). It is almost exclusively converted into either a γ-glutamyl peptide or asparagine, depending on the species concerned. *Lathyrus odoratus* illustrates a marked synthesis of asparagine (FOWDEN 1965). The binding of cyanide in amino acids provides a way of eliminating the highly toxic free cyanide and a new method for the fixation of inorganic nitrogen, which is not limited to the leguminous species (BLUMENTHAL - GOLDSMIDT et al. 1963 and TSCHIERSCH 1964 a and b). However, it is uncertain whether this pathway has any physiological significance in natural conditions.

B. Non-lathyrogenic compounds

As a result of researches centred around lathyrism, several new amino acids which are not lathyrogenic have been discovered in the genus *Lathyrus* (cf. Fig. 1). They are lathyrine, homoarginine, γ-hydroxyhomoarginine, and γ-hydroxynorvaline (BELL 1961, 1962 c, 1963 a, 1964 a; BELL & FOSTER 1962 and FOWDEN 1966). Only the last-named compound, which up to now has been detected within the genus *Lathyrus* only in the seeds of *L. odoratus* (FOWDEN 1966), occurs outside the genus *Lathyrus*. It is a common constituent of seeds among species of the genus *Astragalus* (DUNNILL & FOWDEN 1967).

Lathyrine has been isolated from the seeds of *L. tingitanus* (BELL 1961; BELL & FOSTER 1962 and NOWACKI & PRZYBYLSKA 1961). This compound has also been called tingitanine (NOWACKI & PRZYBYLSKA op.cit.). Lathyrine has been found in the seeds of 13 out of the 50 European *Lathyrus* species studied (BELL & FOWDEN 1964). It also occurs in the seeds or leaves of most of the endemic North and South American species studied (SIMOLA 1966). This compound is found only in the fruits or underground parts in most of the European species synthesizing it (cf. PRZYBYLSKA & RYMOWICZ 1965 and SIMOLA 1966). Only in *L. maritimus, L. tingitanus* and *L. sphaericus* has this amino acid a wider occurrence.

Homoarginine is characteristic of the genus *Lathyrus*. It has been found in the free state in the seeds of most European *Lathyrus* species studied (BELL 1962 a and b and RAO et al. 1963). According to the work of PRZYBYLSKA and RYMOWICZ (1965), this compound is absent from the vegetative parts of plants and is later synthesized in the seeds of representatives of most subgroups. Homoarginine was not found in species of the *Eulathyrus* section except *L. tingitanus*. Lysine is apparently an intermediate in the synthesis of homoarginine and lathyrine in *L. tingitanus* (NOWACKI & NOWACKA 1963). Owing to the long duration of their experiment, there may also have been some secondary labelling. It has also been established that in this plant the synthesis of lathyrine is restricted to the roots (BELL & PRZYBYLSKA 1965) and homoarginine is only hydroxylated in the seeds (BELL 1964 a).

Homoarginine inhibits the growth of some microorganisms, but its effect is competitively reversed by arginine (VOLCANI & SNELL 1948; WALKER 1955 b and BOLLARD 1966). Homoarginine and lathyrine promote pollen tube growth in *Lathyrus niger*, a species which naturally contains these amino acids. In most of the other *Vicieae* species studied they had, if anything, a very slight inhibitory effect (SIMOLA 1967). It is concluded that non-protein amino acid might influence hybridization between species.

γ-Hydroxyhomoarginine has been found in nature in the seeds of four *Lathyrus* species (e.g. *L. aphaca, L. tingitanus*). The occurrence of this compound is limited to this part of the plant (BELL 1963 b, 1964 a and PRZYBYLSKA & RYMOWICZ 1965).

It has also recently been shown that the seeds of *L. maritimus* and *L. aphaca* contain γ-methylglutamic acid (PRZYBYLSKA & STRONG 1968). This amino acid was first found

in the leaves of a fern, *Phyllitis scolopendrium* (VIRTANEN & BERG 1955). This and some other related compounds also occur in several liliaceous species and in *Polygala vulgaris* (Polygalaceae) (ZACHARIUS et al. 1954; WICKSON & TOWERS 1956; FOWDEN & STEWARD 1957 a and b and JIRÁČEK et al. 1962). γ-Methyleneglutamic acid and γ-methyleneglutamine are also known to occur in groundnut plants, peas and bean seedlings (DONE & FOWDEN 1952 and HYDE 1954).

C. Chemotaxonomy in the genus *Lathyrus*

The Eurasiatic species of *Lathyrus* have been classified in tofive groups on the basis of the uncommon amino acids occurring in the seeds (BELL 1962 b, 1964 c, 1965 and BELL & FOWDEN 1964).

1. Species containing α,γ-diaminobutyric acid, α-amino-β-oxalylaminopropionic acid, α-oxalylamino-γ-aminobutyric acid and α-amino-γ-oxalylaminobutyric acid (e.g. *L. sylvestris*, *L. latifolius*).

2. Species containing homoarginine and α-amino-β-oxalylaminopropionic acid (e.g. *L. ochrus* and *L. sativus*).

3. Species containing homoarginine, lathyrine and some unidentified compounds (e.g. *L. maritimus* and *L. niger*).

4. Species containing abundant homoarginine and an unidentified neutral compound also present in group 3 (e.g. *L. vernus* and *L. montanus*).

5. Species containing homoarginine and/or β-(γ-glutamyl)aminopropionitrile (e.g. *L. odoratus* and *L. hirsutus*).

This classification is based on analyses of the amino acid pool of only one part of a plant and is not linked up with the morphology and cytology of the genus and some mistakes appear to have been made in the identification of the seed samples (cf. also BRUNSBERG 1965).

There are also some comparative studies on the variation of the amino acid pool in different *Lathyrus* species (PRZYBYLSKA & RYMOWICZ 1965 and SIMOLA 1966). The occurrence of α,γ-diaminobutyric acid, α-amino-β-oxalylaminopropionic acid and α-amino-γ-oxalylaminobutyric acid is mainly confined to some species of the subgroup *Eulathyrus*, the occurrence of lathyrine to the subgroups *Aphaca*, *Orobastrum* and *Orobus*. The occurrence of lathyrine in *Lathyrus* species is apparently a very primitive feature, because morphologically very dissimilar endemic species of North and South America contain this amino acid (cf. SIMOLA 1966, 1968). The applicability of morphological taxonomy in the genus *Lathyrus* to phylogenetic considerations has up to now been very limited, owing to the frequent tendency to overrate certain characteristics of the flowers. Because the classification of ASCHERSON & GRAEBNER (1906—1910) has been used in many floras and in chemotaxonomic studies (PECKET 1959, 1960, BRUNSBERG 1965 and PRZYBYLSKA & RYMOWICZ 1965) reference is made to these subgroups in the present study.

D. Chemotaxonomy in the genus *Vicia*

A chemotaxonomic classification has also been made for the Eurasian species of *Vicia* (BELL & TIRIMANNA 1965 and TSCHIERSCH & HANELT 1967). From this it emerges that species containing canavanine are also morphologically primitive. They therefore show the basis of morphological and chemical evolution within the genus and the tribe *Vicieae*. *Vicia* is the only genus in this tribe that contains this amino acid, although it is common in many other genera of the family Papilionaceae (KITAGAWA & TOMIYAMA 1929;

ACKERMANN & APPEL 1939; FEARON & BELL 1955; BELL 1958, 1960; TSCHIERSCH 1959, 1961; BIRDSONG et al. 1960 and TURNER & HARBORNE 1967). The species not containing any rare non-protein amino acids are morphologically highly evolved (TSCHIERSCH & HANELT 1967) and the commonest crop plants of the genus are members of this group. The ability to synthesize canavanine is a primitive feature within the Papilionaceae but a highly evolved characteristic in Leguminosales (cf. TSCHIERSCH & HANELT 1967 and TURNER & HARBORNE 1967).

Canavanine can inhibit the growth of several microorganisms (HOROWITZ & SRB 1948; WALKER 1955 a and WIAME et al. 1962) as well as plant organs (BONNER 1949; STEWARD, POLLARD, PATCHETT & WITKOP 1958 and SAMBORSKI & FORSYTH 1960), and even the pollen tube growth of some representatives of the tribe *Vicieae* (SIMOLA 1967), apparently by serving as an antimetabolite of arginine. Canavanine in some concentrations is known to stimulate pollen tube growth in *Vicia unijuga*, in which this amino acid occurs naturally.

Several other rare amino acids have been detected in the seeds of some *Vicia* species. γ-Hydroxyornithine has apparently been found only in the seeds of *Vicia sativa* (BELL & TIRIMANNA 1964), but γ-hydroxyarginine has been detected not only in many species of *Vicia* (BELL & TIRIMANNA 1963 a and b, 1964, 1965 and TSCHIERSCH & HANELT 1967) but also in some invertebrates, *Polycheira rufescens* and *Anthophopleura japonica* (FUJITA 1959 and MAKISUMA 1961, cit. MEISTER 1965).

The natural occurrence of β-cyanoalanine and its γ-glutamyl peptide is almost exclusively confined to the seeds of the subgroup *Vicia* (TSCHIERSCH & HANELT 1967). According to some older reports (GUGGENHEIM 1913 and TORQUATI 1913 a and b) *Vicia faba* contains 3,4-dihydroxyphenylalanine. This compound was also detected in the seeds of *Mucuna* (MILLER 1920 and DAMODARAN & RAMASWAMY 1937).

α-Aminoadipic acid has been found in some *Vicia* and *Pisum* species. This compound is fairly widespread in higher and lower plants (BLASS & MACHEBOEUF 1946; BERG et al. 1954; HATANAKA & VIRTANEN 1962; LAWRENCE & GRANT 1963 and BENT & MORTON 1964). It is a constituent of corn protein, a precursor of lysine in some fungi (WINDSOR 1951 and ROTHSTEIN 1965) and a precursor of pipecolic acid in *Acacia* and *Aspergillus* (FOWDEN 1960 and ASPEN & MEISTER 1962).

E. Compounds common to the genera *Lathyrus*, *Vicia* and *Pisum*

Two of the biosynthetic pathways concerning the metabolism of rare amino acids are common to the genera *Lathyrus*, *Vicia* and *Pisum*. They are the abilities to synthesize homoserine and pipecolic acid (MORRISON 1952; VIRTANEN et al. 1953; MIETTINEN 1955; PRZYBYLSKA 1963, 1964; PRZYBYLSKA & RYMOWICZ 1965 and BELL & TIRIMANNA 1965). The other two genera of this tribe, *Lens* and *Cicer*, do not contain these amino acids (PRZYBYLSKA & NOWACKI 1961) as far as is known. However, these two compounds are also fairly common in the amino acid pools of representatives of other genera.

L-Homoserine was first found in the free state in the roots of *Trifolium pratense* (HUNT 1951) and in seedlings and fruits of *Pisum sativum* (MIETTINEN et al. 1953; VIRTANEN et al. 1953 and HYDE 1954). It also occurs in *Pisum* as the corresponding lactone (MIETTINEN et al. 1953) and as O-acetylhomoserine (GROBBELAAR & STEWARD 1958). In some *Lathyrus* species an O-oxalyl derivative of this compound is found (PRZYBYLSKA & RYMOWICZ 1965). Homoserine is one of the main components in the bleeding sap of *Pisum* (BRENNAN et al. 1964; PATE & WALLACE 1964 and WALLACE & PATE 1965).

Homoserine is synthesized in every part, except the mature seeds, of most European *Lathyrus* species (PRZYBYLSKA 1963, 1964 and PRZYBYLSKA & RYMOWICZ 1965). The occurrence of O-oxalylhomoserine is more sporadic. These compounds were not detected in the subgroups *Nissolia*, *Aphaca* and *Orobastrum*. Homoserine has been detected chromatographically in taxonomically dissimilar genera (e.g. *Trifolium*, *Astragalus*, *Acacia*, *Malus*, *Peperomia*, *Coleus*, *Zea*, *Taxus* and *Asplenium*) (BERG et al. 1954 and DUNNILL & FOWDEN 1967).

The synthesis of homoserine has been studied in microorganisms and in *Pisum sativum*. Glutamate, β-aspartyl phosphate and γ-aminobutyrate can serve as precursors of this compound, which is an intermediate in the metabolism of threonine, methionine and isoleucine (TEAS et al. 1948; TEAS 1950; BLACK & WRIGHT 1953, 1955; COHEN et al. 1954; NISMAN et al. 1954; WORK 1955; SASAOKA 1958; SASAOKA & INAGAKI 1960 and DATTA & GEST 1965).

Pipecolic acid occurs in the free state in the amino acid pools of several plants. It has been found in many leguminous genera (e.g. *Pisum*, *Vicia*, *Lathyrus*, *Phaseolus*, *Trifolium* and *Lupinus*) as well as in some other dicotylenous (e.g. *Malus*, *Solanum*, *Urtica* and *Vaccinium*) and monocotyledonous genera (*Hordeum* and *Musa*) and some ferns, mosses and fungi (HULME & ARTHINGTON 1950; MORRISON 1952, 1953; ZACHARIUS et al. 1952; MIETTINEN et al. 1953; GROBBELAAR & STEWARD 1954; HYDE 1954; MCKEE et al. 1955; PLESHKOV & FOWDEN 1959; FREIBERG & STEWARD 1960 and STEWARD et al. 1960). The transformation of lysine to pipecolic acid has been demonstrated in fungi (SCHWEET et al. 1954 and ASPEN & MEISTER 1962) and in several legumes, e.g. *Pisum* and *Acacia* (LOWY 1953; GROBBELAAR & STEWARD 1954; MEISTER et al. 1957; FOWDEN 1960 and HYLIN 1964).

F. Compounds found especially in the genus *Pisum*

There is much information on the chemical constituents of *Pisum*, because species of this genus are generally used in physiological and biochemical experiments. Several aspects of the amino acid metabolism of *Pisum sativum* have also been studied, such as changes in the amino acid pool during germination of seeds and development of pods, amino acid uptake by roots and transport of nitrogen compounds (BERG et al. 1954; MCKEE et al. 1955; VALLE & VIRTANEN 1960; LAWRENCE & GRANT 1963; BRENNAN et al. 1964 and PATE & WALLACE 1964). Here, it will be enough to refer briefly to the rare amino acids found within the tribe *Vicieae* only in the genus *Pisum* and also in genera of other tribes. Some physiologically or biochemically interesting results are also mentioned.

α-Aminobutyric acid has been detected in the free state and as a peptide in *Pisum* (CHRISTIANSEN & THIMANN 1950; HUNT 1951; AUCLAIR & MALTAIS 1952 and VIRTANEN & MIETTINEN 1953). This compound was first detected in edestin and yeast proteins (ABDERHALDEN & BAHN 1937). It is rather a common compound in the plant kingdom, occurring in both microbes and higher plants (DENT et al. 1947; WORK 1949; HUNT 1951 and MCKEE 1962).

γ-Glutamylalanine and glutathione occur in high concentrations in young pea plants (VIRTANEN & BERG 1954 and MIETTINEN 1955). γ-Glutamylalanine is synthesized especially in the roots of this plant (MIETTINEN 1959). Indole-3-acetyl-D,L-aspartic acid is another compound typical of pea roots (ANDREAE & GOOD 1955; KLÄMBT 1960 and ANDREAE et al. 1961). The latter compound has also been detected in tomato seedlings (ROW et al. 1961). Baikian (4,5-dehydropiperidine-2-carboxylic acid) has been identified

chromatographically in *Pisum* and in some other legumes (KING et al. 1950; HYDE 1954 and HEGARTHY 1957).

Aminoethanol has been found in the seeds and pods of *Pisum sativum* (HYDE 1954). It occurs in relatively high concentrations in the stems of this species but in other parts only sporadically (MIETTINEN 1955). It has also been detected in *Acacia* and in *Musa* (VIRTANEN & KARI 1955 a and STEWARD et al. 1960). Cadaverine and agmatine occur sporadically in *Pisum* (HYDE 1954 and MIETTINEN 1955). Potassium-deficient red clover and barley accumulate putrescine and agmatine, which are decarboxylation products of ornithine and arginine respectively (SMITH & RICHARDS 1962). Trigonelline was first detected in the seeds of *Trigonella* (JAHNS 1885, 1887). This compound has also been found in some other leguminous genera (*Pisum* and *Phaseolus*) as well as in species of many other families (SCHULZE & TRIER 1911; WEEVERS 1933; ACKERMANN & APPEL 1939 and MCKEE et al. 1955). In the green pea nicotinic acid is an effective precursor of trigonelline (ZEIJLEMAKER 1953).

III. The amino acid pools of different organs at different stages of development

A. Material and methods

1. Collection and storage of material

Lathyrus (*sect. Eulathyrus*) *sylvestris* L. and *L.* (*sect. Orobus*) *niger* (L.) Bernh. were grown in the Botanical Garden of the University of Helsinki in the same humus soil. The *L.* (*sect. Orobastrum*) *maritimus* (L.) Bigel. (*L. japonicus* Willd.) material was collected from its natural environment, in sandy soil on the sea shore in Kallvikudde near Helsinki. The samples of each plant species were collected at corresponding stages of development on sunny days between 12 and 14 hrs to avoid any possible diurnal variation in the amino acid pool (cf. RABSON & STEWARD 1962). Some meteorological data for the date and time of year at which the material was collected are presented in Table 4.

In June 1963, samples of leaves of *L. niger* were collected under natural conditions in the Botanical Garden for two days at intervals of six hours. The light intensity was measured with a photometer (Eel Lightmaster) and the temperature was also taken. In the middle of June the day is nearly 19 hours long in Helsinki. The differences in temperature between day and night were relatively small.

It was sometimes difficult to decide the corresponding parts of the three species owing to their rather dissimilar size and mode of growth. For example, the average length of the shoot of flowering *L. sylvestris* may be 120 cm and that of *L. maritimus* only 30 cm. Therefore a big plant has been studied more accurately because there are more internodes per shoot. The leaves were classified as full-sized or young on the basis of their fresh weight. The weight of young leaves was usually less than 0.5 g, but in seedlings of *L. niger* under 0.1 g. The fresh weight per 100 seeds was determined and used as a criterion of the stage of development. Some details of the material are given in Tables 1—3.

The size of samples varied with the part of the plant, e.g. from 2 to 10 g of rootstocks, and usually from 2.0 to 10 g of leaves but only from 10 to 50 mg of pistils and anthers. Samples were generally collected in duplicate. Only very small samples were not divided into two small ones. The dry weight was usually estimated from 0.5, 1 or 2 g of material, depending on the plant material available. The samples for amino acid analyses were placed in 94 per cent ethanol (about 100 ml per 10 g fresh weight) and stored at +4°C until required.

2. *Preparation of amino acid concentrates*

Extraction: The ethanol containing the greatest part of free amino acids was decanted from the samples for amino acid analyses and the plant material was homogenized in 70 % ethanol (about 75 ml per 10 g) and left to stand overnight at room temperature. The homogenate was filtered and the extracts combined.

Ion exchange: Prior to chromatography and electrophoresis the samples were passed through a cation exchange column (0.9 × 12 cm) in H^+-form (Ionentauscher I, Merck,) The ethanolic eluate was stored for determination of free sugars and evaporated to dryness at 60°C. The column was washed with about 50 ml of distilled water and the amino acids were removed with 50 ml of 2 N ammonium hydroxide (Merck) (cf. STEWARD et al. 1960). The column was washed with 10 ml of distilled water. The basic eluates were combined and evaporated to dryness on a warm plate in a fume cupboard at 50°C.

Storage of samples: The residue was dissolved in a known amount of water (usually in 1/10 or 1/5 of the fresh weight of the samples). Dissolution is not quite quantitative, but larger amounts of water would have increased the aliquot to be pipetted onto the starting point for chromatography. The vessels used for evaporation were rinsed with toluol (Merck p.a.) in order to transfer the amino acid solution as quantitatively as possible into the small bottles in which the samples were stored under toluol in −15°C until they were chromatographed.

3. *Electrophoresis and chromatography of amino acids*

Paper electrophoresis: A preliminary run was made (Whatman No. 1; buffer: pyridine, acetic acid, water, 1:10:200, pH 3.6; BELL 1962 b) in order to find convenient aliquots for chromatography and to identify the most acid (α-amino-γ-oxalylaminobutyric acid) and the most basic amino acids (β-aminopropionitrile, α,γ-diaminobutyric acid and homoarginine) characteristic of *Lathyrus* species. The voltage gradient was 8V/cm and the running time 3 hours.

Chromatography: The amino acids were separated by ascending two-directional paper chromatography (Whatman No. 1, sheet size 20 × 20 cm) in a chromatoframe set for 12 sheets (Plouvier, Servall) or on thin-layer sheets 20 × 20 cm in size coated with cellulose (MN 300 G, Merck) (cf. STAHL 1962). The paper chromatography proved to be more practical and repeatable and was therefore used for most separations. Thin layer chromatography may in some cases give sharper separation, especially if there are a large number of amino acid components of low concentration in the same analysis.

The convenient amount of the solution to pipette per sheet varied according to the species and the part of the plant. For fresh leaves of *L. sylvestris*, for example, it represents about 25 mg (2.5 μl of the final solution), for those of *L. maritimus* 50 mg (5 μl) and of *L. niger* 100 mg (10 μl). The analyses were performed in duplicate and 2—3 sheets of each sample were chromatographed. One sheet was run with a double amount of amino acid solution for special reactions, which are often less sensitive than the ninhydrin reaction.

The first solvent, phenolic water (4:1), pH 4.2, was used without an ammonia atmosphere. This solvent retained its usefulness longer without ammonia. Most amino acids had Rf-values nearly corresponding to those mentioned by STEWARD et al. (1955) but the separation of arginine and homoarginine was somewhat better. Phenolic water (4:1) containing ammonia (pH 5.5) was also tried, because it is a standard solvent system and had been used in several previous investigations (cf. STEWARD et al. op.cit. and STEWARD

& POLLARD 1962). The chromatograms were left to dry in a fume cupboard overnight, after which any remaining phenol was removed in a ventilated oven at 60°C for 45 minutes.

As the second solvent the upper phase of an n-butanol-acetic acid -water mixture (4:1:5) was mostly used. The sheets were dried in the same way as after the first run. The following solvent combinations were also used for more accurate identification of amino acids:

1) n-butanol, ethylmethylketone, 1 N ammonia, water (5:3:1:1); n-butanol, acetic acid, water (see above) (WOLFE 1957).

2) Phenol, water (4:1); n-butanol, ethylmethylketone, dicyclohexylamine, water (10:10:2:5) (BOWDEN 1959).

3) Phenol, water (3:1); n-propanol, water (4:1) (TSCHIERSCH & HANELT 1967).

4) Phenol, water (4:1); PEPP solvent mixture (HANES et al. 1961); for separation of homoarginine from arginine. Chemicals of Merck (p.a.) were used except for dicyclohexylamine (puriss.), which was produced by Fluka.

For more accurate identification of the different components of the amino acid pools, some samples from each species were first analysed by one-way paper electrophoresis as explained above and then by ascending chromatography with phenol water solution or ethylmethylketone, propionic acid, water (2:1:2) (PRZYBYLSKA & RYMOWICZ 1965).

Some compounds were also identified by electrophoresis after two-dimensional chromatography. The spot areas of several unstained chromatograms were located with the aid of a stained chromatogram and by staining some other less important parts of the chromatograms. The spots were dissolved in water, concentrated and analysed. The electrophoretic mobility was compared with those of known reference compounds.

Ion-exchange chromatography: In order to confirm the identification of some constituents of the amino acid pool of *L. maritimus*, some representative samples and several reference substances were analysed, using a Hitachi Perkin-Elmer 034 automatic amino acid analyser. In this procedure the amino acids are analysed as their zinc complexes (a slight modification of the method described by ARIKAWA 1967 and in the standard methods for protein amino acids[1]).

4. Development of chromatograms

Ninhydrin reagent was used for all amino acids and peptides. The chromatograms were usually developed by dipping them in a fresh solution containing 100 mg ninhydrin, 10 ml acetic acid and 2 ml 2,4,6-collidine in 50 ml of acetone. The sheets were then dried for 6 min. at 80°C. Many amino acids acquire characteristic colours: lathyrine orange-red, asparagine and proline yellow, aspartic acid and β-alanine blue (cf. STAHL 1962).

The following special reagents were used for estimation of amino acids of different chemical nature:

Sakaguchi's reaction, as modified by JEPSON & SMITH (1953), was used for estimation of arginine and homoarginine. Chromatograms were dipped in a 0.1 per cent solution of 8-hydroxyquinoline in acetone. After drying in air they were sprayed with fresh sodium hypobromate solution (about 2 per cent bromide in 0.5 N NaOH).

Isatin reagent as modified by KNAUT (1964) was used for identification of proline, pipecolic acids, phenylalanine and tyrosine especially. The chromatograms were dipped

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[1]) The standard methods for protein amino acids. Accelerated amino acid analyses with the ligand system. Hitachi LTD 1967.

in a solution containing 0.2 per cent isatine, 4 per cent acetic acid and 0.06 per cent cadmium chloride in acetone. The chromatograms were heated at 90°C for 10 min. All protein amino acids react and give characteristic colours. Modifications described by McKEE et al. (1955) and SAIFER and ORESKES (1956) were also tried.

Ehrlich's reagent was used for estimation of citrulline, homocitrulline, tryptophane, tryptamine and urea. Chromatograms were sprayed with 1 per cent solution of 4-dimethylaminobenzaldehyde in 96 per cent ethanol. The chromatograms were then placed in an atmosphere saturated with hydrochloric acid vapour for 3—5 min. Indole compounds give a pink colour, ureides a yellow one. The reaction is not quite specific (cf. STEWARD et al. 1960). The procedure is described in a manual edited by Merck. This contains several other special staining methods for amino acids, many of which were also tested in this work. All chemicals used were produced by Merck.

5. Hydrolysis of peptides

Some amino acid concentrates containing a wide range of unknown ninhydrin-positive substances, usually in low concentrations, were hydrolysed in 6 N HCl for 6 hours at 138°C in an evacuated sealed tube (KIVIRIKKO 1963). The hydrolysate was then diluted with distilled water and neutralized with 0.5 N NaOH. The solution was desalted on a cation exchange column and chromatographed as described above (p. 13—14).

6. Reference compounds

The amino acids were identified with the help of co-chromatography and co-electrophoresis and with various colour reagents, and their mobilities compared to the Rf-values of known compounds (cf. CONSDEN et al. 1944; DENT 1948; MIETTINEN 1955; STEWARD et al. 1955; CRAMER 1958; SMITH 1958; BOWDEN 1959; LINSKENS 1959; PALMENTIER & VANDERHAEGHE 1960 and STEWARD & POLLARD 1962).

The reference substances used are listed below. For the identification of very rare amino acids, samples of which were not available, amino acid concentrates were made from the following species and chromatographed: seeds of *Vicia sativa* L., *V. sepium* L., *Lathyrus odoratus* L., *L. sativus* L., *L. tingitanus* L. and seeds of several *Astragalus* species. The amino acid pools of these species are known (BELL 1964 c; DUNNILL & FOWDEN 1967 and TSCHIERSCH & HANELT 1967).

List of reference substances used

Agmatine sulphate, H.L.R[6]
DL-Alanine, Difco Laboratories
DL-α-Aminoadipic acid, Mann[1]
DL-α-Amino-n-butyric acid, Eastman Kodak Company, Rochester, N.Y.
Aminoethanol, Schering Kahlbaum AG., Berlin
γ-Aminolaevulinic acid hydrochloride, Fluka[2]
β-Aminopropionitrile, K & K. Laboratories Inc.
L-Arginine, B.D.H.[3]
L-Asparagine, Merck[4]
L-Aspartic acid, Fluka
Betaine HCl, L. Light & Co. Ltd, Colnbook
L-Canavanine sulphate, Mann
DL-Citrulline, Fluka
DL-Cysteic acid, Fluka
L-α,γ-Diaminobutyric acid dihydrochloride, Fluka
α,β-Diaminopimelic acid, N.B.C.[5]

DL-α,β-Diaminopropionic acid monohydrochloride, Fluka
L-Glutamine, H.L.R.
L-Glutamic acid, Merck
L-Glutamic acid γ-methyl ester, Fluka
Glycine, Merck
Histamine dihydrochloride, Merck
L-Histidine monohydrochloride, Merck
L-Homocitrulline, Mann
DL-Homocysteine, Fluka
L-Homoarginine, N.B.C.
DL-Homoserine, Mann
L-Hydroxyproline, Fluka
L-Leucine, General Biochemicals Inc.
L-Lysine monohydrochloride, Fluka
L-Methionine, Fluka
DL-Methionine sulphoxide, Sigma
L-Ornithine monohydrochloride, N.B.C.
DL-β-Phenylalanine, B.D.H.
DL-Pipecolic acid hydrochloride, Sigma
L-Proline, Fluka
Putrescine dihydrochloride, H.L.R.
L-Serine, Fluka
Spermidine, Fluka
Taurine, Fluka
L-Threonine, Fluka
L-Tryptophane, N.B.C.
Tyramine, H.L.R.
Tyrosine, Merck
β-Ureidopropionic acid, Ega-Chemie KG Keppler & Reif.
DL-Valine, Merck

1. Mann Research Laboratories Inc.
2. Fluka AG. Buchs. SE.
3. The British Drug Houses Ltd.
4. E. Merck AG., Darmstadt
5. Nutritional Biochemicals Corporation
6. F. Hoffmann — La Roche & Co. AG

7. *Isolation of lathyrine*

Seeds of *L. maritimus* (125 g, collected near Porvoo) were ground with a grain mill (Casella) and extracted overnight with 250 ml of diethylether (Merck p.a.) at room temperature and after this with 150 ml of diethylether. The suspension was filtered and the meal was extracted overnight with 250 ml of 70 per cent ethanol and after this for two hours with 125 ml of 70 per cent ethanol. The filtrates were combined and passed through a cation exchange column in H^+-form. The fractions were eluted with 0.5 N ammonium hydroxide solution. The extinction of the fractions was measured at 300 mμ, where the absorption maximum of lathyrine lies (cf. BELL & FOSTER 1962), using a Beckman DB spectrophotometer. The fractions containing the bulk of the lathyrine were combined and evaporated to dryness on a warm plate. A paper electropherogram of the residue was run and it appeared that the sample was not yet quite pure. The residue was dissolved in a small amount of hot distilled water. The solution was allowed to crystallize at $+4°C$. Lathyrine is much less water-soluble than many other amino acids (BELL 1962 b). The crystals were centrifuged and rinsed with cold distilled water. After this they were dissolved and recrystallized in the manner described above. The sample was shown by electrophoresis to consist of chromatographically pure lathyrine. The uncrystallized part

of the lathyrine solution was ion-exchanged again and crystallization was repeated. About 100 mg of crystalline lathyrine was recovered.

The purity of the isolated sample of lathyrine was verified by infrared spectroscopic analyses (Beckman IR 8) and the spectrum compared with that reported in the literature (NOWACKI & PRZYBYLSKA 1961), with which it was in good accord. Thirteen milligrams of pure lathyrine was sent for tritiation to the New England Nuclear Corporation, where such tritiation had already been carried out earlier (RAMACHANDRAN & RAO 1964). The specific activity of the sample was 1.28 millicuries/mg. However, a large part of the sample was decomposed during the tritiation. Further purification was carried out by the author. The sample was divided into two portions, which were purified separately. It was dissolved in hot distilled water but the greater part remained undissolved. The solution was filtered and passed through a ion-exchange column as described above. The fractions containing lathyrine were combined and also the fraction before and after them The fractions were evaporated to dryness and their radioactivity measured with a Geiger-Müller tube with thin endwindow connected to a counter (FRIESEKE HOEFNER). About 2/3rds of the activity was in the lathyrine fractions. Electropherograms were also run from each lathyrine-containing fraction and it could be demonstrated that lathyrine was the only amino acid in the fractions, but only about 1/7 of the labelling was in the lathyrine band. The bulk remained near the start. Fractions containing lathyrine in large quantities were combined and further purification was carried out using zone paper electrophoresis. A part of the electropherogram was stained with ninhydrin for localization of lathyrine. Measurement of the radioactivities of the different zones showed that about 1/4th of the label was in the lathyrine. When lathyrine was dissolved from the paper with hot distilled water and the solution was evaporated to dryness, its activity was only 540 counts/min.

8. Semiquantitative and colorimetric estimation of amino acids

The size of the spot areas and the intensity of the colour of the ninhydrin stained chromatograms were compared to a wide concentration range of reference amino acids chromatographed with the solvent combinations and staining reagents used in the assays. The amount of each amino acid was estimated on scale ranging from 1 to 10 (rarely 15 and 20) in order to facilitate presentation of the results in the form diagrams. The scale was calibrated by running chromatograms of corresponding reference substances (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 50 and 100 μg). The amounts have been expressed as absolute quantities in μg/g fresh weight and the estimated mean values have been marked under each column in the diagrams.

In some cases the amount of the amino acids was also estimated colorimetrically from the intensity of the ninhydrin-positive spots. The chromatograms were dipped in a 0.25 per cent solution of ninhydrin in acetone. They were left to dry in darkness for 30 min. After this they were heated for 6 min. at 80°C. The stained spot areas were cut out and the colour extracted for 15 min. in 75 per cent ethanol (3 parts absolute alcohol, 1 part saturated aqueous solution of $CuSO_4$; filtered) (LEWIS & CONZALVES 1960) and the extinction was measured at 570 mμ. The lathyrine spot does not dissolve well in this solvent but of a wide range of solvents tested by the author, that consisting of 50 per cent ethanol containing 0.05 per cent $CuSO_4$ and 0.5 N HCl proved to be the best one. The extract has a very clear absorption maximum at 390 mμ.

In view of the time that would be consumed in making quantitative colorimetric analyses of a wide range of substances occurring in very dissimilar concentrations, I have

mostly contented myself with rougher semiquantitative estimations of the amino acids and noted only their relative amounts, but I have also endeavoured to give enough data for semiquantitative comparisons.

9. Presentation of results

The results are presented in the form of diagrams (Figs. 3—6). The separate parts of the plants, in all the developmental stages studied, have been grouped together in order to reveal any clear trends within an organ during development and to facilitate an overall comparison between plant organs. Where an organ has been analysed in several parts, the lower and usually older part has been marked in the diagram above the younger one. The developmental stages have been presented in their natural sequence from top to bottom. The amino acids have been grouped as protein amino acids, non-protein common intermediate products, rare non-protein amino acids and peptides typical of the *Lathyrus* species and unknown compounds which usually occur only in traces. The most characteristic amount of each amino acid in μg/g fresh weight has been marked under the column of each amino acid. Owing to fact that the convenient amount to pipette at the start point on chromatograms varies with the species (cf. p. 13), the absolute quantities, corresponding to the same values in the scale (from 1 to 10) in the diagrams, are not the same when different species are compared.

B. Results and discussion

1. General comparative remarks

The three species studied have rather similar patterns of protein amino acids. Alanine, valine, leucine-isoleucine, aspartic acid, glutamic acid and serine with γ-aminobutyric acid and asparagine form the main part of the pool. Proline occurs in higher concentrations in *L. maritimus* than generally in the other two species. *L. maritimus* and *L. niger* have greater similarities with each other than with *L. sylvestris*. They both synthesize lathyrine, but in *L. niger* this compound accumulates in the underground parts and fruits, whilst in *L. maritimus* it is present in all plant organs. *L. maritimus*, however, contains γ-methylglutamic acid and 5-hydroxypipecolic acid and some peptides which neither *L. maritimus* nor *L. sylvestris* possess. *L. sylvestris*, on the other hand, synthesizes homoserine and α,γ-diaminobutyric acid. *L. maritimus* does not contain these compounds and *L. niger* only homoserine in some plant organs. Both in different developmental stages and in plant organs *L. sylvestris* and *L. maritimus* show rather little variation in the amino acid pool. No clear diurnal endogenous rhythm could be detected in the amino acid pool of leaves of *L. niger*. However, the analytical method used is too insensitive to reveal small variations. Only the amounts of asparagine and leucine were a little higher at night than during the daytime. The relative differences in temperature and also in light between day and night were rather small.

In the following representation the occurrence of each constituent in the amino acid pool is described in greater detail. The amino acids have been grouped, wherever possible, on the basis of connections in biosynthetic pathways (cf. Fig. 2).

2. Alanine and serine

Alanine (No. 1 in the diagrams) is one of the main components in the amino acid pool of *L. niger* and *L. maritimus*. In *L. sylvestris* this amino acid also occurs constantly, but not usually in such high concentrations as in the two former species. It is also a predominent compound in pods of *Pisum sativum* (McKee et al. 1955). The concentration of alanine is somewhat lower in the underground part of the vertical stem and in the anthers of *L. niger* as well as in the pericarps and older seeds of *L. maritimus* than on average in these species. The older seeds and pericarps of *L. sylvestris*, however, contain this amino acid in relatively large amounts in comparison to the underground parts of young seedlings.

Serine (No. 4) occurs quite constantly in moderate concentrations in the amino acid pool of every plant organ. Its concentration is very low in older seeds, as in those of *Pisum* (McKee et al. 1955) and in rootstocks of *L. sylvestris* and *L. niger*. This compound does not accumulate in any particular part of the plant. Its concentration in the leaves of *L. niger* is somewhat higher than in the stems. Serine is a precursor of glycine, tryptophane and cysteine (cf. Nair & Vaidyanathan 1964 and Fowden 1965). These three protein amino acids are lacking from the amino acid pool of *Lathyrus* species and from the vegetative parts of *Pisum* or occur in low concentrations (cf. Miettinen 1955 and Przybylska & Rymowicz 1965).

Aminoethanol (No. 29) is the decarboxylation product of serine. It occurs sporadically in relatively small quantities in the three species studied. This compound is somewhat more common in the floral parts of *L. maritimus* than in other plant organs. It occurs in considerable amounts in several organs of *Pisum sativum* and also in *Musa* (Hyde 1954; Miettinen 1955 and Steward et al. 1960).

3. Valine and leucine-isoleucine

Valine (No. 2) and leucine-isoleucine (No. 3) have very parallel distribution patterns within the plants studied as well as in pods of *Pisum* (McKee et al. 1955). They occur constantly in relatively large amounts. The concentrations of these two amino acids are low in the underground parts of young *L. sylvestris*, like the concentration of alanine, and increase during later developmental stages; valine is lacking from the underground parts during the seedling stage.

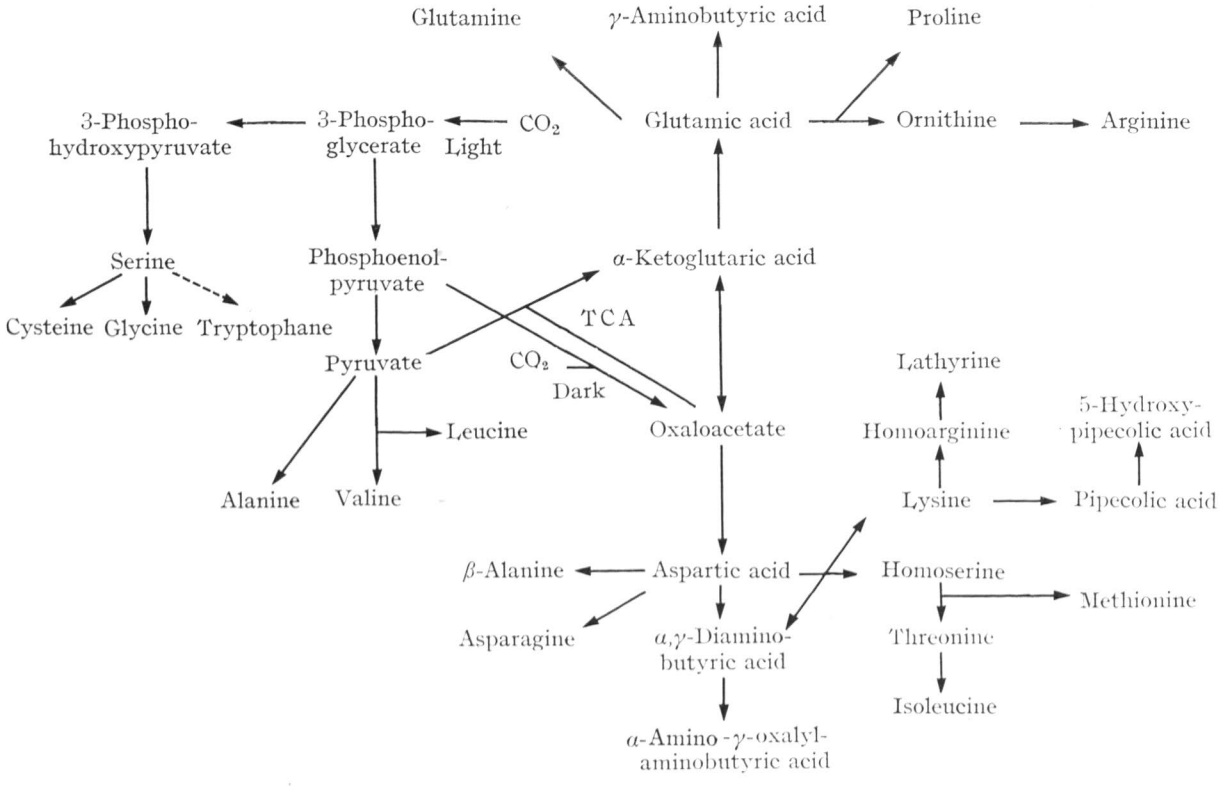

FIG. 2. An overall scheme showing the main biosynthetic relationships of several protein amino acids and non-protein amino acids characteristic of *Lathyrus* species. Not all successive reactions are marked. TCA = tricarboxylic acid cycle. For details see FOWDEN (1965).

In *L. niger* the amounts of valine and the leucines increase in the rootstocks and underground part of the vertical stem during the middle stages and decrease during the fruiting stage in the shoot. The concentration of valine and the leucines is low or they are altogether lacking from many floral parts of *L. sylvestris* and *L. niger*. In *L. maritimus*, on the contrary, the amount of leucines is high but valine is absent from the stamens and pistils and also from the pericarps, although both are abundant in the peduncles of *L. sylvestris* during both the bud and flower stages and also in the flowers themselves. PRZYBYLSKA & RYMOWICZ (1965) report that *Lathyrus* species contain both leucine and isoleucine, but in their study, as in the method used routinely in this work, valine overlaps methionine. Ionexchange chromatography showed that the rootstocks of *L. maritimus* contain no methionine but equal quantities of leucine and isoleucine.

4. Amino acids derived from aspartic acid

Aspartic acid (No. 6) and asparagine (No. 7) occur very constantly in medium concentrations in *Lathyrus* species. The amount of asparagine is high, especially in the underground parts of *L. maritimus* and *L. niger*, but it is not usually found in the pericarps of these plants or in some floral parts of *L. sylvestris*. It has been detected that asparagine is also lacking from the seeds of *Pisum sativum* after the middle stage of their development (MCKEE et al. 1955). The variation in the quantity of asparagine in some samples of the stems of *L. sylvestris* may be due to the relatively low night temperature (cf. Tables 3—4 and Fig. 5), which is known to have stronger effects on the amino acid pool (especially on the amount of glutamine, glutamic acid and asparagine) of *Mentha piperita* than changes in the photoperiods (RABSON & STEWARD 1962). Asparagine and aspartic acid are two of the main constituents in the root and petiole bleeding sap of *Pisum* (BRENNAN et al. 1964 and PATE & WALLACE 1964). Asparagine is the most prominent free amino acid in this plant and in the tubers of some potato strains (MIETTINEN 1955 and STEWARD & DURZAN 1965) and one of the main amino acids in many other species (SCHWAB 1936; VICKERY & PUCHER 1943; STEWARD et al. 1954 and WILDING et al. 1960). The amount of this amino acid increases during germination and in etiolated plants (cf. VENEKAMP 1955). Aspartic acid and asparagine serve as good nitrogen sources for some embryo cultures (RIKER & GUTSCHE 1948; SPOERL 1948 and RIJVEN 1956)

The equilibrium between glutamic and aspartic acids and their amines has been studied in detail in *Mentha piperita* by CRANE and STEWARD (1962 a and b). Both long-day conditions and a high potassium concentration in the nutrient solution tend to promote the shift from amides towards amino acids. Both short-

day conditions and a high calcium concentration tend to promote the shift from amino acids to amides. Under short-day conditions asparagine accumulated during the night, glutamine during the day (CRANE & STEWARD 1962 a).

β-Alanine (No. 17) is formed from aspartic acid by decarboxylation in the seeds of a number of legumes (AMBE & SOHONIE 1959). This compound is a common constituent of the amino acid pools of all three *Lathyrus* species studied and does not show any uniform localization in the plants. It occurs in rather low concentrations, which presumably explains why it has not been detected earlier in *Lathyrus* species. β-Alanine accumulates in the leaves, floral parts, seeds and tops of the shoots of *L. maritimus*, and in the stems, floral parts and pericarps of *L. niger*. In *L. sylvesteris* this compound is rarer in the shoot of the fruiting plant than in the shoot at earlier developmental stages. It also occurs in low concentrations in many organs of the pea (CHRISTIANSEN & THIMANN 1950; AUCLAIR & MALTAIS 1952; HYDE 1954; MCKEE et al. 1955; MIETTINEN 1955 and LAWRENCE & GRANT 1963).

β-Alanine is one of the most ubiquitously distributed amino acids in plants. It occurs in the free state in both mono- and dicotyledons and microorganisms (DENT et al. 1947; FOWDEN 1951; SYNGE 1951; CLOSE 1960 and MORRIS et al. 1961). β-Alanine is synthesized in most organisms, because it is a part of pantothenic acid and coenzyme A. This amino acid is also formed from β-ureidopropionic acid, which is an intermediate in the catabolism of uracil (BATT & EXTON 1956; FRITZSON 1957; FRITZSON & PIHL 1957; EVANS & AXELROD 1961; BARNES & NAYLOR 1962 and TSAI & AXELROD 1965). β-Alanine is thus obviously not of any great chemotaxonomic significance.

α,γ-Diaminobutyric (No. 19) acid occurs only in *L. sylvestris* of the species studied, but is found in all the organs of the plant and in every developmental stage. In the leaves its concentration is somewhat higher in flowering plants than in other stages. This amino acid is found in especially high concentrations in the rootstocks, peduncles, buds and seeds. *Lathyrus* species which contain α,γ-diaminobutyric acid also contain homoserine (cf. PRZYBYLSKA & RYMOWICZ 1965) Aspartic-β-semialdehyde is apparently a precursor of these two amino acids and azetidine-2-carboxylic acid (cf. FOWDEN 1965). The last-named compound is typical of some liliaceous species which also contain α,γ-diaminobutyric acid (FOWDEN & BRYANT 1958). α-Amino-γ-oxalylaminobutyric acid (No. 20) was detected in high concentrations in the flowers and fruits more often than in the other parts of *L. sylvestris*.

β-Aminopropionitrile (No. 21) and its γ-glutamyl derivative are rather common in *L. sylvestris*. These compounds occur only sporadically in *L. niger*. The shoot of *L. maritimus* contains this amino acid in considerable quantities relatively constantly but does not contain its γ-glutamyl derivative. In *L. sylvestris* there is a shift towards synthesis of glutamyl derivative. RESSLER (1964) has

likewise found β-(γ-glutamyl)aminopropionitrile in the seedlings of this plant. It has been suggested that β-cyanoalanine might be the precursor of both β-aminopropionitrile and α,γ-diaminobutyric acid (RESSLER 1962).

In my studies homoserine (No. 23) was found in *Lathyrus sylvestris* at every stage of development and in every part of the plant. It is one of the main constituents of the amino acid pool of this species. Homoserine was also detected in smaller quantities in the aerial shoot of *L. niger*. The seeds of *Pisum sativum* contain only a little homoserine, but during germination the amount increases two hundred fold in the embryos (BERG et al. 1954 and LAWRENCE & GRANT 1963). This compound is a precursor of threonine, methionine and isoleucine (NAYLOR et al. 1958). Homoserine dehydrogenase has been found in *Pisum* both in decotylized seedlings and in cotyledons, and the pathways of homoserine biosynthesis is similar to that in yeast (SASAOKA & INAGAKI 1960 and SASAOKA 1961). The homoserine dehydrogenase isolated from *Rhodospirillium rubrum* is subject to feed-back inhibition by threonine (DATTA & GEST 1965). Both methionine and isoleucine, which are the biosynthetic end-products, can relieve this inhibition. They can also stimulate the reverse reaction, the formation of aspartic-β-semialdehyde from homoserine. Homoserine does not generally accumulate in plants, although they synthesize threonine (NAYLOR et al. op.cit.). Threonine (No. 5) is found only sporadically in *L. maritimus* and it is altogether lacking from the pool of *L. sylvestris*, although its precursor homoserine is a common constituent in the last-named species. In *L. niger* threonine occurs constantly in moderate concentrations in the stems and leaves at all except the earliest stages. This compound is found in the pistils and stamens of both *L. maritimus* and *L. niger*. Threonine and homoserine have similar distribution patterns in the developing pods of the pea (MCKEE et al. 1955).

5. Amino acids derived from glutamic acid

The amount of glutamic acid (No. 8) is very constant in the *Lathyrus* species studied (cf. Figs. 3—6). The occurrence of glutamine (No. 9) is more variable. Glutamine is lacking from the underground parts and fruits of *L. sylvestris* but occurs sporadically in other parts. In *L. niger* it is not found in the floral parts or pericarps but is rather common in the underground parts, stems and seeds. In *L. maritimus* glutamine is mostly concentrated in the rootstocks, stems, stamens and pistils. Glutamine and asparagine are characteristic of non-growing potato and carrot tissues, but are lacking or occur in low concentrations in the amino acid pool of actively growing tissue cultures (STEWARD, THOMPSON & POLLARD 1958). It has been established that species containing glutamic acid in high

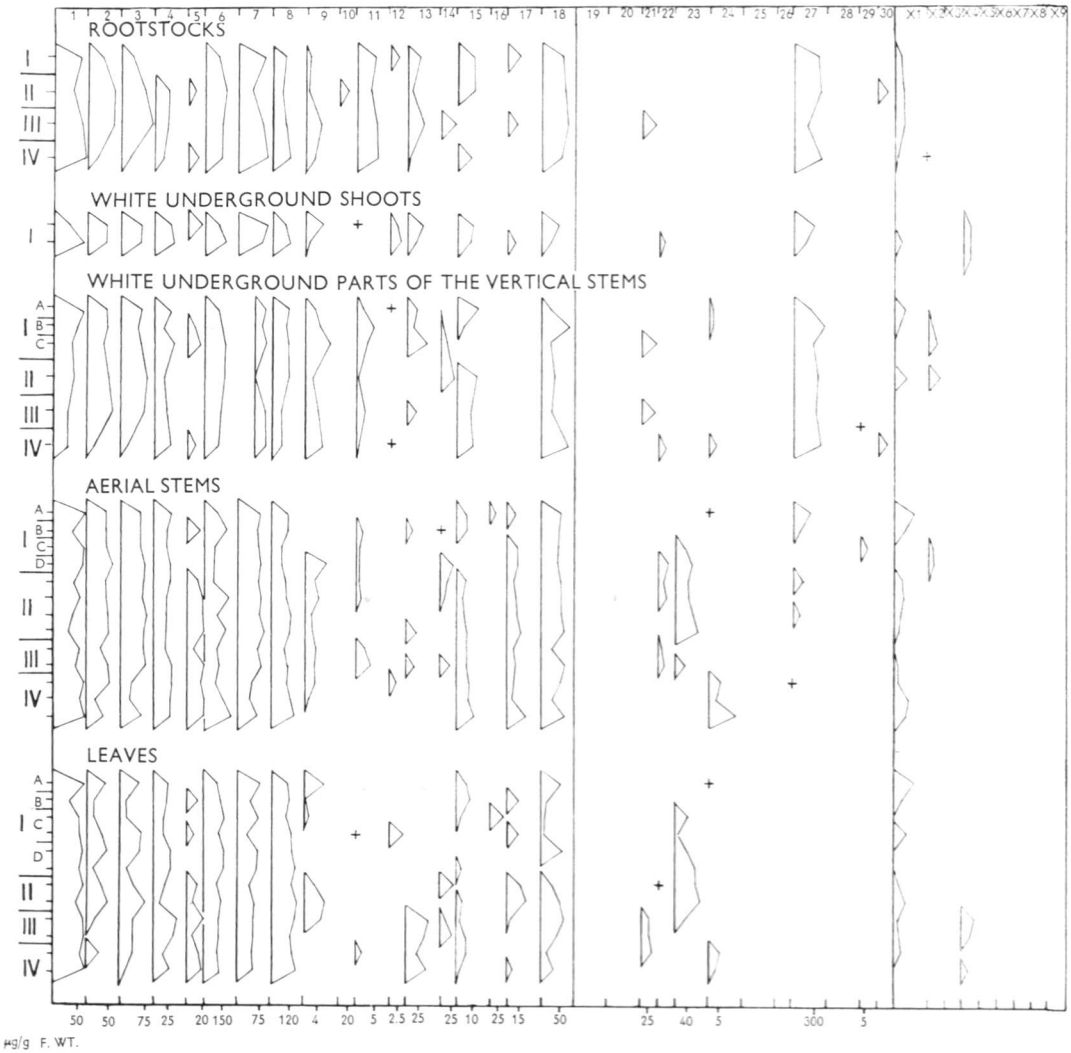

FIG. 3. Diagram showing the amino acid constituents of vegetative organs of *L. niger* at different stages of development (I A-D = seedlings, II = budding, III = flowering, IV = fruiting plants). Description of the material in Table 1, p. 60. The order of amino acids is indicated in Figs. 4 and 8 (cf. also p. 18).

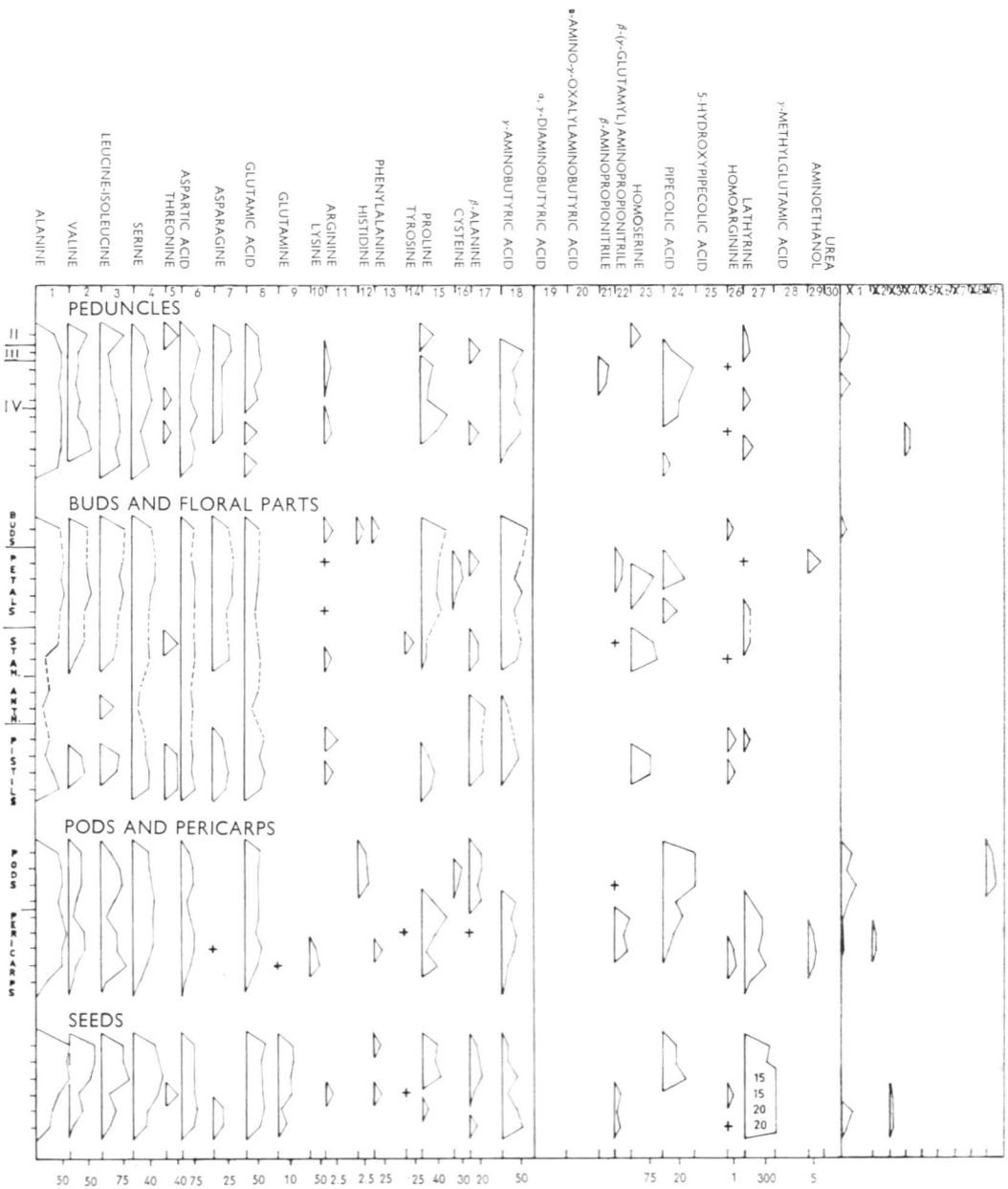

FIG. 4. Diagram showing the amino acid constituents of reproductive organs of *L. niger* at different stages of development (cf. Fig. 3).

concentrations are usually able to tolerate more ammonia than other species, because they are able to bind it in the form of glutamine (SCHWAB 1936 and LISS 1958). Glutamine is also a superior nitrogen source for the in vitro growth of embryos of several plant species (RIJVEN 1952, 1955, 1956, 1958 and NORSTOG 1961). Glutamic acid is the precursor of γ-aminobutyric acid, ornithine, arginine and proline.

γ-Aminobutyric acid (No. 18) is a widespread constituent of all three species studied in the present work. It is fairly evenly distributed among the various organs of these species but is absent from the pericarps of *L. maritimus*. PRZYBYLSKA & RYMOWICZ (1965) have found this compound in every *Lathyrus* species studied, but it is lacking from the mature seeds of several species, including those of *L. maritimus* and *L. sylvestris*. This amino acid has been found in several organs of many legumes (HYDE 1954; MCKEE et al. 1955; MIETTINEN 1955; BUTLER & BATHURST 1958 and LAWRENCE & GRANT 1963). It is interesting that γ-aminobutyric acid in bound form is only to be found in substantial amounts in the root nodules of some leguminous species, especially those of *Trifolium repens* (BUTLER & BATHURST op.cit.).

It has been found that γ-aminobutyric acid often accumulates in ageing leaves (FREIBERG & STEWARD 1960; WEINBERGER & GODIN 1964 and KHAVKIN 1964). This corresponds well to the observation that the activity of glutamic acid decarboxylase also increases during their senescence (WEINBERGER & CLENDENNING 1952 and WEINBERGER & GODIN op. cit.). In the present work such a tendency was detected in the stems and leaves of *L. sylvestris*. The old leaves of *L. maritimus*, on the contrary, do not contain more γ-aminobutyric acid than the younger ones. The amount of this amino acid also increases in some rapidly growing tissues (THOMPSON et al. 1953), which indicates that this compound is an intermediary rather than an end-product.

γ-Aminobutyric acid has a very wide distribution in the plant kingdom. Its total absence from the amino acid pool of a species is, moreover, rare. It is found in bacteria, fungi and algae (ACKERMAN 1910; WORK 1949; BENT & MORTON 1950; REED 1950; FOWDEN 1951; CLOSE 1960 and LEWIS & CONZALVES 1960, 1962 a and b) in gymnosperms and in several angiosperm genera (cf. DENT et al. 1947; HULME & ARTHINGTON 1950; WESTALL 1950; SYNGE 1951; THOMPSON et al. 1951, 1953; LINKO 1960; STEWARD et al. 1960; CRANE & STEWARD 1962 a and b and GAGNON 1966).

The formation of γ-aminobutyric acid from glutamic acid as a result of decarboxylation has been demonstrated with the aid of enzyme preparations obtained from microbes and from higher plants (SCHALES et al. 1946). However, γ-aminobutyric acid is not always a decarboxylation end product. It is synthesized in *Pseudomonas* through succinate and succinic semialdehyde (BACHRACH 1960). It has been established in carrot tissue that γ-aminobutyric acid is rapidly converted to glutamic acid and glutamine (STEWARD, BIDWELL & YEMM

1958) and in peanut mitochondria to succinate (DIXON & FOWDEN 1961).

γ-Methylglutamic acid (No. 28) occurs only in *L. maritimus* of the three species studied. This compound is one of the main constituents in this species. It occurs in rather large quantities in most organs and at all developmental stages. This amino acid likewise occurs in the seeds of *L. aphaca* (PRZYBYLSKA & STRONG 1968) but not in the other species studied. In *L. aphaca* it also occurs in the vegetative parts (SIMOLA, unpublished data). γ-Methylglutamic acid has the erythro-L configuration in the genera *Phyllitis* and *Lathyrus* (BLAKE & FOWDEN 1967 and PRZYBYLSKA & STRONG 1968). Threo-γ-methyl-L-glutamic acid, however, is one of the four isomers of γ-methylglutamic acid, the substrate of glutamine synthetase in the pea (KAGAN & MEISTER 1966).

Arginine (No. 11) was usually present in low concentrations or was lacking from the vegetative parts of *Lathyrus* species. This compound was only detected in any substantial quantity in some floral parts and rootstocks. Its quantity in pea seedlings depends on the organ and its stage of development (MIETTINEN 1955). The developing fruits of *Pisum* contain only traces of arginine (MCKEE et al. 1955). Arginine is commonly found in growing tissues and in the anthers of *Lilium* its amount increases during mitosis (STEWARD et al. 1954 and NASATIR et al. 1961). On the other hand, this compound is more abundant in non-growing potato tuber and carrot root tissues than in rapidly growing cultures of the corresponding tissues (STEWARD, THOMPSON & POLLARD 1958). Arginine is a storage compound in the underground parts in the families Saxifragaceae and Rosaceae. In some genera of the Papilionaceae the corresponding storage amino acid is proline (REUTER 1957).

Proline (No. 15) is one of the main constituents of the amino acid pool of *L. maritimus*, but does not accumulate in the underground parts. This amino acid is also generally to be found, especially in the floral parts, in all three species studied. It generally occurs in large quantities in pollen grains (AUCLAIR & JAMIESON 1948; VIRTANEN & KARI 1955 b; BRITIKOV et al. 1964 and TUPÝ 1964). It has been established that the free proline found in the pollen of *Amaryllis hybrida* reaches this site by transport from the vegetative organs (BRITIKOV et al. op. cit.) and not local synthesis from glutamic acid, as earlier supposed (VIRTANEN & KARI op. cit.).

The old peduncles of *L. sylvestris* contain large amounts of proline, but in the pericarps and seeds at the same developmental stage it is only present in very small quantities. The pericarps of *Pisum sativum* do not contain proline in the free state and in the young seeds it is only present in traces (MCKEE et al. 1955). The amount of this amino acid is surprisingly small in the fruits of *L. maritimus*, except in their youngest stage. It may be that proline transported from other parts of plants is effectively used in protein synthesis. The amount of many other protein amino acids is also rather small, especially in the pericarps, as compared with that of several non-protein amino acids.

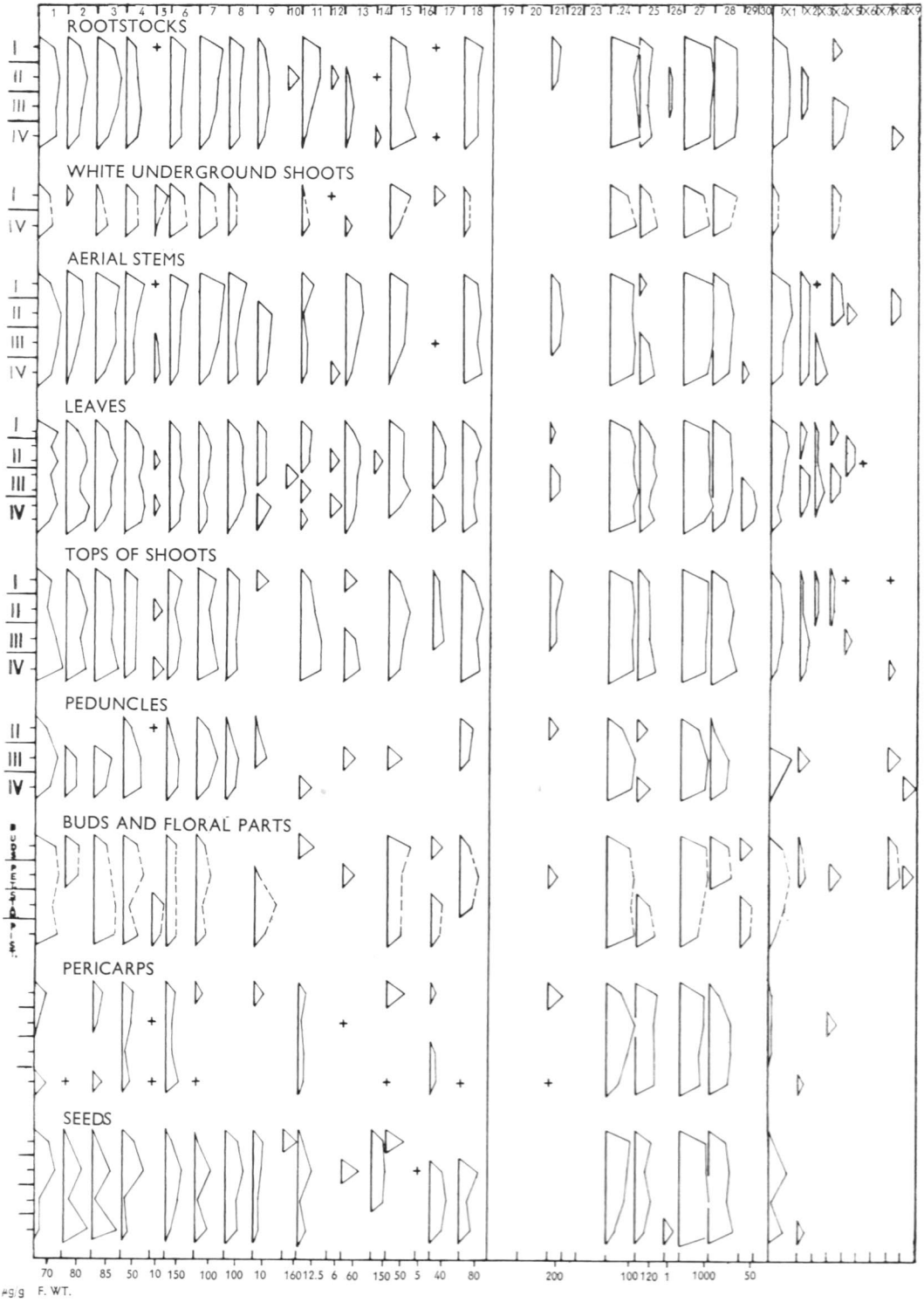

FIG. 5. Diagram showing the amino acid constituents of different organs of *L. maritimus* at different stages of development (cf. Figs. 3-4 and Table 2, p. 61).

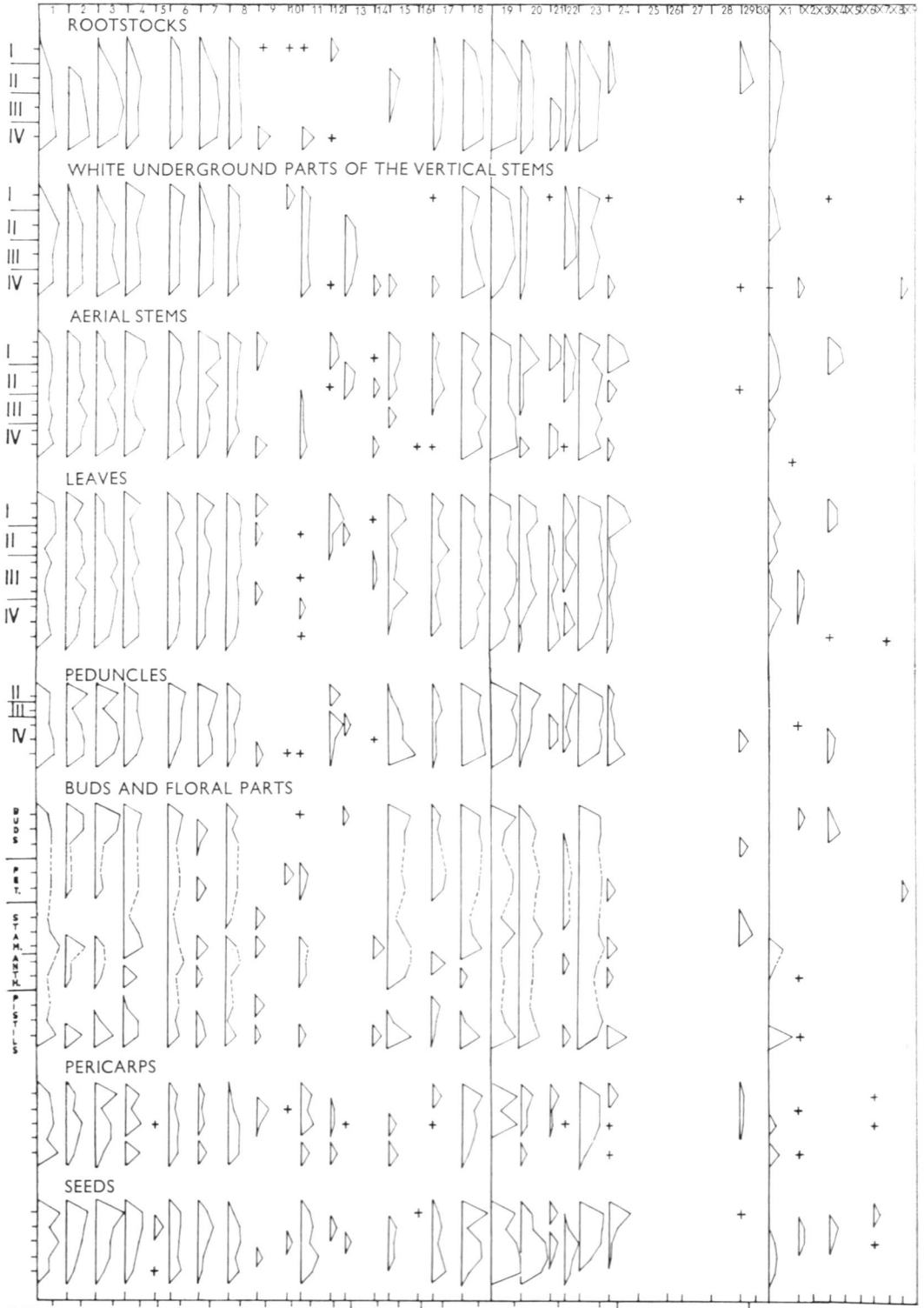

FIG. 6. Diagram showing the amino acid constituents of different organs of *L. sylvestris* at different stages of development (cf. Figs. 3-4 and Table 3, p. 61).

6. Amino acids derived from lysine

Pipecolic acid (No. 24) is constantly present in large amounts (about 100 µg/g fresh weight) in the amino acid pool of *L. maritimus*. The concentration is somewhat lower in the older fruits. In *L. niger* this amino acid is only found in high concentrations in the fruiting plant. The quantity of pipecolic acid is very high in the young pods, which do not contain proline, lathyrine or homoarginine. The pericarps of *Pisum sativum* do not contain pipecolic acid but it occurs in the young seeds in small amounts (McKee et al. 1955). In *L. sylvestris* this compound is present in the young stems and leaves in somewhat higher concentrations than in the other parts, in which it is usually very sparse or entirely lacking.

The synthesis of pipecolic acid via lysine has been established in several legumes (Lowy 1953; Grobbelaar & Steward 1954; Meister et al. 1957; Fowden 1960 and Hylin 1964) and microorganisms (Schweet et al. 1954 and Aspen & Meister 1962). In this as well as in earlier studies (Przybylska & Rymowicz 1965) the amount of lysine in all three species under review has been found to be very small or nil. The enzymatic balance is therefore geared towards pipecolic acid synthesis, especially in *L. maritimus*.

It has been found that in *Neurospora*, seedlings of both green bean (*Phaseolus radiatus*) and pea, and rat liver, enzymes effecting the last steps of proline synthesis also catalyse the production of pipecolic acid (Meister et al. 1957). The occurrence of pipecolic acid is therefore dependent on the substrate specificity of the proline-synthesizing enzymes. *L. maritimus*, which contains more proline than *L. niger* and *L. sylvestris*, also contains more pipecolic acid than these two species. In catabolism, pipecolic acid has been demonstrated to break down via α-aminoadipic acid to glutaric acid (Grobbelaar & Steward 1954 and Rao & Rodwell 1962).

5-Hydroxypipecolic acid (No. 25) was found in the present study only in *L. maritimus*. It occurred in all stages of development and in most parts of the plant, especially in the pericarps. It was not present in the buds and petals and its concentration was low in some stem and leaf samples from young plants. This compound has not hitherto been found in the genus *Lathyrus*.

5-Hydroxypipecolic acid has been isolated from *Acacia* and a palm, *Rhapis excelsa* (*R. flabelliformis*) (Virtanen & Kari 1954, 1955 a), from the pericarps of the date (*Phoenix dactylifera*) and from the genera *Strelitzia*, *Baikiaea* and *Leucaena* (Virtanen & Kari 1955 a; Grobbelaar et al. 1955 and Hegarty 1957). The ability to synthesize this amino acid is clearly polyphyletic but its occurrence can be used as a chemical characteristic within a genus. Whether the Finnish *L. maritimus* is actually a separate chemical race has not yet been investigated. Many of the species which contain 5-hydroxypipecolic acid also synthesize pipecolic acid and/or 4-hydroxypipecolic acid and baikiain. Lysine

likewise functions well as a precursor of 5-hydroxypipecolic acid (HYLIN 1964). A metabolic relationship between 5-hydroxylysine and 5-hydroxypipecolic acid has been established in animal tissues (LINDSTEDT & LINDSTEDT 1959).

Lysine is apparently an intermediate in the synthesis of homoarginine and lathyrine in *L. tingitanus* (NOWACKI & NOWACKA 1963). I have supposed that homoarginine could be formed by a system homologous to the urea cycle: lysine and carbamyl phosphate would form homocitrulline and after condensation with aspartate this would lead to homoarginine and fumaric acid. Homoarginine is then decomposed by an enzyme to lysine and urea or dehydrogenated to lathyrine.

In the present study homoarginine (No. 26) was found sporadically in small quantities in *L. maritimus*, although it is the precursor of lathyrine, which is one of the main constituents of the amino acid pool of this plant. The occurrence of homoarginine was nearly parallel with that of lathyrine in *L. niger* but its amplitude of occurrence in the plant was a little narrower. Homoarginine did not accumulate in the seeds but was rapidly transformed to lathyrine.

In most European *Lathyrus* species homoarginine is present in the mature seeds but not in the vegetative parts (cf. BELL 1964 c and PRZYBYLSKA & RYMOWICZ 1965). It was not found in *L. sylvestris*. This compound is rare in the subgroup *Eulathyrus*. γ-Hydroxyhomoarginine was not detected in the present study. Nor has it been found in these species earlier (BELL 1963 b, 1964 a and c and PRZYBYLSKA & RYMOWICZ op. cit.).

Lathyrine (No. 27) occurs in high concentrations in every part of *L. maritimus*. Otherwise only *L. tingitanus* and *L. sphaericus* have this kind of general distribution of lathyrine (PRZYBYLSKA 1962 a and b and PRZYBYLSKA & RYMOWICZ 1965). However, most North and South American endemic species of *Lathyrus* also contain it in the vegetative parts (SIMOLA 1966).

L. niger represents a species in which lathyrine accumulates in the wintering organs. Lathyrine is found in the rootstocks and in the underground parts of the vertical stem but disappears from the aerial stem. To find out whether light was responsible for the decomposition of lathyrine, the stems of eight budding plants were darkened with aluminium foil for ten centimetres upward from the soil level. After eight days the underground white part of the stem, the darkened part of the stem and ten centimetres above it were analysed separately and also the corresponding parts of untreated plants.

There was no difference in the amount of lathyrine in the darkened part of the stem of *L. niger* compared with the corresponding parts exposed to light. Light is therefore not the direct activator of the enzyme decomposing lathyrine in the stem. The physiological boundary between the different parts of the stem is apparently formed at an early stage of development, when the tissues are differentiating, by a process in which light apparently plays an important role.

Young pods do not contain this compound but when seeds begin to develop they contain lathyrine and apparently induce the pericarps to form this compound. *L. aphaca* and *L. pratensis* have a similar distribution pattern of lathyrine to *L. niger*. In mature seeds of *L. maritimus* and *L. niger* it is one of the main components. Like most species of the *Eulathyrus, Clymenum* and *Cicercula* sections, *L. sylvestris* does not contain this compound.

7. Aromatic amino acids

Tyrosine (No. 14) was found only in trace amounts in the aerial vegetative shoot of *Lathyrus* species in the present study. This compound accumulates in the underground vertical part of the stem of *L. niger* and in the seeds of *L. maritimus*. m-Tyrosine is present in especially large quantities in the latex of *Euphorbia myrsinites* (MOTHES et al. 1964). MÜLLER and SCHÜTTE (1967) have established that m-tyrosine is synthesized via shikimic acid but the pathway branches off after this and phenylalanine is therefore apparently not a direct precursor of tyrosine.

Phenylalanine (No. 13) occurs only sporadically in *L. sylvestris*. It shows a slight concentration in the underground vertical part of the stem of this plant. In *L. niger* this amino acid is especially abundant in the rootstocks and in the leaves of the fruiting plant. In *L. maritimus* phenylalanine occurs constantly in moderate concentrations in the leaves. It is also relatively common in the other vegetative parts of the plant, but very rare in the floral parts and fruits. Phenylalanine and tyrosine are found in low concentrations in the amino acid pools of some other *Lathyrus* species (PRZYBYLSKA & RYMOWICZ 1965) and in the genus *Pisum* (MCKEE et al. 1955 and MIETTINEN 1955).

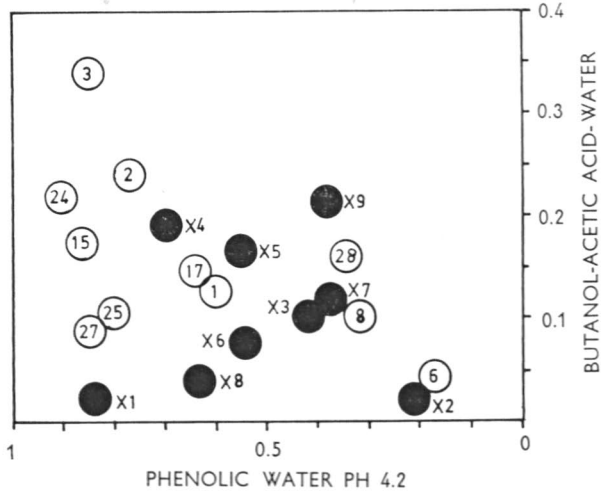

FIG. 7. Map showing the Rf-values of unidentified peptides and amino acids (black circles) and some of the other amino acids characteristic of *Lathyrus* species studied (open circles) (cf. Fig. 8).

8. Unidentified amino acids and peptides

Nine small ninhydrin-positive spots could be detected especially in chromatograms run from samples of *L. maritimus* (cf. Fig. 7). As a results of the sporadic occurrence of these compounds and the small amounts recovered, it was not possible to identify them. The hydrolysis of some amino acid samples showed that at least three of the unknown compounds (X1—3) are peptides. Their colour reactions (usually pink or yellow) are similar to those seen in fingerprint electropherograms of the seed proteins of some *Lathyrus* and *Vicia* species (JACKSON et al. 1967).

Peptide X1 was found in all three species studied. This compound occurs very constantly in *L. maritimus*, especially in the floral parts, but only in traces in the pericarps and it is lacking from the older rootstocks. In *L. niger* this peptide does not occur in the flowers but it is common in the stems and rootstocks. In *L. sylvestris* it likewise accumulates in the rootstocks and stems, except during the fruiting stage.

Peptide X2 is very common in *Lathyrus maritimus* in low concentrations. It occurs constantly in the stems and tops of this species. Peptide X3 is common in the leaves. Other unidentified compounds do not clearly accumulate in any part of the plant or any developmental stage.

IV. Feeding experiments with amino acids

A. Material and methods

1. Experiments with fruiting Lathyrus niger

In order to establish whether lathyrine is synthesized in the fruits of *L. niger* or is only translocated to them at night from the underground parts, and in order to study the biosynthesis of this amino acid, the following experiments were carried out.

Detached shoots of *L. niger* (length about 35 cm) bearing young fruits, which do not contain lathyrine, were used. The leaves were removed from the lower part of the shoots. The stems were washed in running tap water and sterilized in 1 % sodium hypochlorite solution for 1 min. After this they were washed carefully with sterilized distilled water. About 1 cm was removed from the lower end of each shoot. The shoots (5) were put in Erlenmeyer flasks containing 1 l sterile nutrient solution (cf. VIRTANEN et al. 1933). The containers were closed with cotton wool which the stems perforated. The plants were allowed to develop for 11 to 16 days with a photoperiodism of 16 hours light, 8 hours darkness (six daylight lamps, Airam L 40 W-35 H 11). The samples were gathered and treated as described earlier (p. 12—14).

1. a. Ammonium nitrate (1mM) as nitrogen source. Time of experiment 11 days.
   b. Similar experiment, but 30 mg/l aureomycin in order to inhibit growth of microbes (cf. CROMWELL & ROBERTS 1964).
2. Homoarginine (1 mM) as nitrogen source; aureomycin; 11 days.
3. Lysine (1 mM) and carbamylphosphate as nitrogen source (1 mM); aureomycin; 11 days.

4. Homocitrulline (1 mM) and asparagine (1 mM) as nitrogen source; aureomycin; 12 days.

5. No nitrogen source; aureomycin; 11 days.

6. a. Ammonium nitrate (1 mM) as nitrogen source; 50 μc guanidino-^{14}C-homoarginine; aureomycin; amount of solution 500 ml. The experiment was carried out in a big glass container through which an air stream was led and the effluent air was then led through 4 N NaOH in which CO_2 was captured.

b. A similar experiment without radioactive homoarginine. The amino acid composition was determined separately from stems, leaves, peduncles, pericarps and seeds. The results are presented in this order from top to bottom in the diagrams (cf. Fig. 8).

2. *Experiments with seedlings*

In order to establish whether there are any differences between *Lathyrus* species in their ability to catabolize *lathyrine*, the following experiments were carried out. The method was the same as in earlier experiments in which an amino acid in a standard mineral nutrient medium, usually without any other nitrogen source, is translocated in the plant by the transpiration flow. Shoots of young seedlings were used in these studies because lathyrine was shown to disappear from *L. niger* in this stage of development (cf. p. 27). The amount of nutrient solution was usually 50 ml.

1. a. Young shoots of *L. niger* (length 10—15 cm) were used. They transpired standard nutrient solution containing a small quantity of tritiated lathyrine (concentration of ammonium nitrate 1 mM) at a rate of 40 ml in 4 days. Underground parts of the stem, aerial stem and leaves were analysed separately (cf. Fig. 8).

b. Control experiment without lathyrine.

2. a. Young shoots of *L. maritimus* (10—15 cm). Similar experiment as in 1 a with tritiated lathyrine. Transpiration during 4 days 42 ml. Stems and leaves were analysed separately, as in all the following experiments.

b. Control experiment without lathyrine.

3. Young shoots of *L. sylvestris* (length ca. 20 cm); lathyrine (1 mM) as sole nitrogen source. 50 ml of nutrient solution was transpired in 24 hours.

a. After feeding with lathyrine the shoots had to transpire nutrient solution (10 ml) not containing lathyrine. This took about 5 hours.

b. Similar control experiment but without lathyrine.

c. An experiment like 3 a, but the plants were fed, after treatment with lathyrine, for 6 days with a standard nutrient solution of ammonium nitrate (1 mM) as nitrogen source. The upper and lower parts of the stem were analysed separately (cf. Fig. 8).

d. Similar control experiment but without lathyrine.

In order to study the possible pathway of the catabolism of *homoarginine* in *L. sylvestris*, which only sporadically contains this amino acid, the following experiments were carried out.

1. a. Experimental conditions as in 3 c but the nutrient solution contained homoarginine (1 mM) instead of lathyrine.

b. Control experiment without homoarginine.

c. Experimental conditions as described above, but 20 μc of homoarginine instead of unlabelled homoarginine in 25 ml of nutrient solution, 2 days after which the experimental plants were fed with a nutrient solution containing no homoarginine, half of them for two days, the other half for four days (cf. Fig. 8). The experiment was carried out in a big glass container and the CO_2 in the outflowing air was bound with 4 N NaOH, as in the experiments with fruiting *L. niger* (6 a).

The catabolism of *α,γ-diaminobutyric acid* and *homoserine* was studied in young seedlings of *L. maritimus* and *L. niger* in the following experiments.

1. a. *L. maritimus;* α,γ-diaminobutyric acid (2 mM) as the only nitrogen source in 50 ml of nutrient solution; transpiration of the solution took four days; two days in nutrient solution without nitrogen.

b. An experiment similar to 1 a but with homoserine (2 mM) as the only nitrogen source; transpiration of 50 ml of the solution took six days. In this experiment the leaves became yellowish.

c. Control experiment with ammonium nitrate (2 mM) as nitrogen source for five days; two days without nitrogen.

2. a. *L. niger,* α,γ-diaminobutyric acid (1 mM) as the only nitrogen source in 50 ml of nutrient solution; transpiration of the solution took three days. Two days without nitrogen. Brown edges of leaves.

b. An experiment similar to 2 a but with homoserine (1 mM) as the only nitrogen source. Transpiration took two days. Five days without nitrogen.

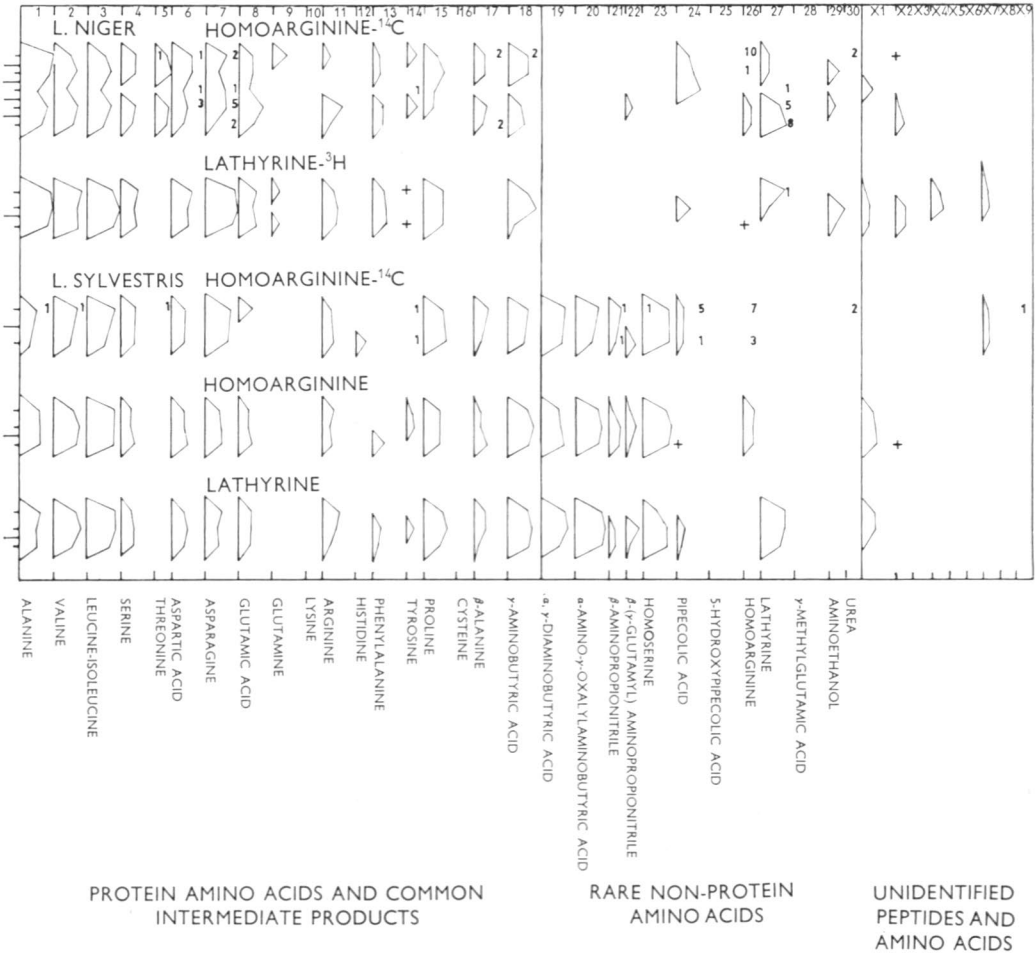

FIG. 8. Diagram showing the results of some feeding experiments, explanations in the text.

Autoradiography: Kodak X-ray films 24 × 24 cm in size were used for autoradiography of chromatograms containing labelled compounds. Development of autoradiograms took from 1 to 5 months, depending on the activity. The films were developed with a developer and a fixative produced by Kodak for this purpose. The intensity of the spots in the autoradiograms was estimated with the naked eye on a scale ranging from 1 to 10 and the number marked in the diagrams (Fig. 8).

B. Results and discussion

It can be seen from the diagrams (Fig. 8) that in *L. niger* lathyrine synthesis occurs in both pericarps and seeds when ammonium nitrate is the sole nitrogen source available. Aureomycin did not inhibit this reaction any more than it does the reaction connected with γ-coniceine synthesis from lysine and α-aminoadipic acid in hemlock (CROMWELL & ROBERTS 1964). Thus the nutrient solution could be kept uncontaminated for rather a long time.

Lathyrine was synthesized with every combination of amino acids used and also in plants fed with nutrient solution not containing nitrogen. Lysine with carbamyl phosphate caused browning of the leaf edges, which suggests that that this species is not very tolerant to extra amounts of lysine. However, the amount of this amino acid was not appreciably increased in these plants.

None of the amino acids tested brought about any marked change in the amino acid pool. It was even difficult to detect the added amino acid in the pool. In plants without any nitrogen source the amino acid pool was nearly similar to that of the other groups. Presumably the nitrogen resources within the shoot itself, which could not be eliminated in an experiment of this kind, were utilized in this case. This indicates that the amino acid pool of this species is very stable and that amino acids added to the nutrient solution in moderate concentrations are easily eliminated without accumulation of any product even when its precursor is fed to the plant. For example, the amount of pipecolic acid did not increase when its precursor lysine was given. The enzymes connected with amino acid metabolism possibly act in suboptimal substrate concentrations and the metabolism of cells also needs a distinct amino acid pool.

In experiments carried out by VALLE & VIRTANEN (1960), barley was unable to assimilate most of the extra amino acids given and so these accumulated in the cells. In the pea most of the amino acids were metabolized. The concentrations of amino acids in the nutrient solution was about the same as in the present study but the observation time was much longer in the experiments of VALLE & VIRTANEN and the amino acids were fed via the roots.

Using autoradiography and counting of the radioactivity of the spots, it could be established that homoarginine is completely decomposed in the leaves

of *L. niger*, even though this amino acid does not normally occur in them. In the stems, most of the labelling remained in the homoarginine. This compound is apparently decomposed by an enzyme, homoarginase, to lysine and urea. The latter compound is then decomposed by urease with release of $^{14}CO_2$, which is derived from the labelled ^{14}C-atom in the guanidino radical. A considerable amount of the $^{14}CO_2$ released in the air could be detected when bound in NaOH. Homoarginine is therefore not a mere by-product of the metabolism. Slight labelling could also be detected in aspartic acid, asparagine, threonine, β-alanine, γ-aminobutyric acid and urea in stem tissues. The same compounds except for homoarginine and urea could be found in the peduncles, but in smaller amounts, because this tissue contains very little cytoplasm.

Labelled lathyrine could be found in both pericarps and seeds and even in the peduncles. This compound was the main labelled compound in the pods but label could also be found in some other amino acids, e. g. aspartic acid and asparagine. This may be due to secondary labelling as a result of photosynthetic CO_2 fixation. The last-named products are really metabolic key substances in this process (CALVIN & BENSON 1949; SMITH et al. 1961; ARONOFF 1962 and HILLER 1964). Slight labelling of β-alanine was also seen, this substance presumably indicating the catabolic pathway of lathyrine (cf. BARNES & NAYLOR 1962 and TSAI & AXELROD 1965).

Lathyrine is apparently mainly synthesized in the seeds, because the pericarps begin to contain this compound after the start of development of the seeds, which contain this amino acid right from the beginning of their development. The seeds apparently also induce the pods to form this compound, or else lathyrine is translocated from the seeds to the pods. The small amount of lathyrine in the peduncles indicates that to a small extent lathyrine is translocated from the fruits to the underground parts of the plant. Small quantities of lathyrine could even be detected in the nutrient solution after the experiment.

In young seedlings of *L. sylvestris* (cf. Fig. 8) homoarginine was relatively effectively catabolized in both stems and leaves. Many amino acids, e. g. pipecolic acid, valine, alanine, threonine, and tyrosine, were labelled as a result of the de novo synthesis of amino acids through fixation of radioactive CO_2 in photosynthesis. *L. sylvestris* thus possesses an enzyme capable of decomposing homoarginine, although this species does not synthesize this amino acid.

In experiments on the metabolism of tritiated lathyrine this compound was catabolized so effectively in the leaves of young seedlings of *L. maritimus* that no products could be detected. In the stems only a small amount of radioactive lathyrine was present. Thus this amino acid is apparently catabolized even in *L. maritimus*, which contains this compound in high concentrations in every

part of the plant, but its synthesis is also very rapid and so there are no great differences in the quantity of lathyrine present during development.

Lathyrine was also effectively catabolized in young seedlings of *L. niger*, except in the white underground parts of the vertical stems. Here, in contrast, lathyrine tended to accumulate and this part of the plant also contains this amino acid naturally. Therefore I suppose that enzymes catabolizing lathyrine are lacking from this part or are not present in the active state. Owing to the small quantity of labelled lathyrine relative to the quantity of endogenous amino acids in this plant, no catabolic products could be detected.

In seedlings of *L. sylvestris* lathyrine was not catabolized when it was their only available source of nitrogen. This species has therefore lost its ability to synthesize enzymes for the catabolism of lathyrine in spite of the fact that it does not synthesize this amino acid in natural conditions or in feeding experiments when supplied with the precursor of this compound.

Homoserine was to some extent converted to threonine in *L. niger*, which contains this compound only in small amounts. Nor did α,γ-diaminobutyric acid accumulate in *L. niger* and *L. maritimus*, which do not naturally possess this compound in their amino acid pools. The metabolic product could not be detected with unlabelled α,γ-diaminobutyric acid, because the amount of any normal constituent in the amino acid pool of *L. niger* did not significantly increase. The quantity of γ-methylglutamic acid was somewhat increased in *L. maritimus* fed with homoserine and α,γ-diaminobutyric acid, which may be due to some transamination reactions (cf. FOWDEN & DONE 1953). The experiments with α,γ-diaminobutyric acid-2-C^{14} have shown that in the animal organism 5 per cent of this compound is excreted as β-alanine-1-C^{14}. The labelling patterns found in tissues suggest that part of the α,γ-diaminobutyric acid is converted to aspartate or some closely related compound (MUSHAHWAR & KOEPPE 1963). Some toxic symptoms could be seen in *L. maritimus* (yellowish leaves, after treatment with homoserine) and in *L. niger* (brown edges of leaflets, after treatment with α,γ-diaminobutyric acid).

V. General discussion

A. Protein and common non-protein amino acids and amides

The prominent protein amino acids which occur in the free state in most parts and in most developmental stages of the *Lathyrus* species studied are: alanine, valine, leucines, serine, aspartic acid, asparagine, glutamic acid and glutamine. γ-Aminobutyric acid is also a very abundant constituent in these species. Most of these compounds are encountered in relatively large quantities

in the amino acid pool in representatives of all classes of the plant kingdom (cf. bacteria, HOLDEN 1962; fungi, MCKILLICAN 1960; CHATTAWAY et al. 1961; algae, LEWIS & CONZALVES 1960, 1962 a and b; mosses, MAASS & CRAIGIE 1964; ferns, ALLSOPP 1948, STEWARD et al. 1954; gymnosperms, STEWARD & DURZAN 1965; dicotyledons, THOMPSON & STEWARD 1952, STEWARD & CRANE 1962 b; monocotyledons, DUVICK 1952; FOWDEN & STEWARD 1957 a and b; FREIBERG & STEWARD 1960; STEWARD et al. 1960 and BIDWELL 1963).

Many of these almost ubiquitous free amino acids are among the early products of photosynthetic carbon dioxide fixation. The most prominent early labelled amino acids in both higher and lower plants are various combinations of the following compounds: aspartic acid, asparagine, glutamic acid, glutamine, serine, alanine, threonine and glycine (CALVIN & BENSON 1949; SMITH et al. 1961; HEBER 1962; BASSHAM et al. 1964; HILLER & WHITTINGHAM 1964 and ONGUN & STOCKING 1965 a and b). Glutamic and aspartic acids are key compounds in the incorporation of ammonium ions into amino acids (VIRTANEN & TARNANEN 1932; MOTHES 1940; VICKERY et al. 1940; MACVICAR & BURRIS 1948; RAUTANEN 1948 and BASSHAM & KIRK 1964).

Comparison of the free protein amino acids of the seeds of genera belonging to the tribe *Vicieae* and of the young seedlings of *Vicia faba*, *Pisum sativum* and the three *Lathyrus* species studied demonstrates that there are striking differences within the tribe and within its genera. Arginine is the predominant amino acid in the seeds of *Vicia faba* (BOULTER & BARBER 1963) and also one of the main amino acids in the seeds of other species of the section *Vicia* (TSCHIERSCH & HANELT 1967). This amino acid is abundant in the seeds of most species in this tribe but a number of species of *Lathyrus* and *Vicia* only contain it in low concentrations (e. g. *L. maritimus*, *V. tetrasperma*) (PRZYBYLSKA & NOWACKI 1961 and TSCHIERSCH & HANELT op. cit.) The investigations carried out by BELL (1964 c), however, have shown that the seeds of most *Lathyrus* species contain only traces of arginine, whereas most of them have high or considerable quantities of homoarginine. *Vicia* and *Lathyrus* species containing only low concentrations of arginine in the seeds often contain large amounts of some rare non-protein amino acids (e. g. canavanine, lathyrine). In respect of other free protein amino acids the species of the tribe *Vicieae* are relatively alike. Aspartic acid-asparagine, glutamic acid-glutamine and alanine are the other main constituents (cf. PRZYBYLSKA & NOWACKI 1961 and TSCHIERSCH & HANELT 1967).

Young seedlings of *Vicia faba* (14 days old) contain only traces of arginine. Histidine is the predominant free amino acid besides glutamic acid (BOULTER & BARBER 1963). The prominant amino acids in young seedlings of *Pisum sativum* are homoserine, glutamic acid, asparagine, γ-aminobutyrate, alanine and serine (BERG et al. 1954; SASAOKA 1958 and LAWRENCE & GRANT 1963).

Histidine is found only in low concentrations and therefore the amino acid pool of *Pisum* more nearly resembles that of *Lathyrus* species (cf. Figs. 3—6) than the pools of *Vicia faba*.

B. Non-protein amino acids characteristic of *Lathyrus* species, with special reference to phylogeny

It is in relation to the occurrence of the non-protein amino acids typical of *Lathyrus* species that the most interesting results were obtained, and the differences between the species studied are most relevant in respect of these compounds. The presence of γ-methylglutamic acid and 5-hydroxypipecolic acid, and a high concentration of lathyrine and pipecolic acid in all developmental stages and in every plant organ are characteristic of *L. maritimus* (cf. Figs. 5 and 11). *L. niger* and *L. sylvestris* do not contain the two first-mentioned compounds. In *L. niger* lathyrine is mainly found in underground parts and fruits. Homoserine and α,γ-diaminobutyric acid are typical of every part of *L. sylvestris* (cf. Figs. 6 and 10).

The differences in the composition of the amino acid pools may be due to combinations of the following possibilities.

1. An amino acid is synthesized in the tissue in which it occurs.
2. An amino acid is translocated into a tissue, but not synthesized in it.
3. An amino acid is effectively catabolized in the tissue.

Therefore the dissimilarities found in amino acid pools are results of differences in the activities of the enzymes concerned with amino acid metabolism, which for their part depend on dissimilarities in the genetic constitution of species and differences in gene activity within a plant.

The synthesis of lathyrine in the fruits of *L. niger* could be demonstrated in numerous experiments. Using radioactive homoarginine it could be shown that this compound is effectively catabolized in the leaves of this plant and also to some extent in the stem (cf. Fig. 8). Only in the pods could radioactive lathyrine be found. In the seeds the label was mostly detected in this amino acid. It seems therefore that homoarginine is dehydrated to lathyrine in both the pods and the seeds, which also contain the latter compound naturally.

There are some dissimilarities between *Lathyrus* species in respect of the site where lathyrine is synthesized. Bell & Przybylska (1965) reported that in *L. tingitanus* the synthesis of lathyrine was limited to the roots, in spite of the fact that in this species the amino acid is present everywhere in the plant (cf. Przybylska & Rymowicz 1965). Recently, however, it has been, established that lathyrine is also formed in the shoot of this plant (Nowacki et al. 1967). The concentration of lathyrine in the rootstocks and fruits of *L. niger* is appar-

ently due to the fact that the vegetative aerial shoot has lost its ability to synthesize lathyrine (cf. Figs. 3—4 and 9). Even when the precursor homoarginine is supplied in excess, this species is unable to form lathyrine in the vegetative parts. Small amounts of lathyrine may be translocated in the stem from the pods to the rootstocks, even though this compound is not usually found in the stem, because small amounts of lathyrine could be detected in the nutrient solution after such a feeding experiment. The translocation of amino acids is known to be a very rapid process (cf. KURSANOV 1963).

Radioactive lathyrine was shown to be effectively catabolized in the young shoots of *L. niger* (cf. Fig. 8), as would be expected in view of the disappearance of this compound from the shoots at this stage of development (Fig. 3). It was not catabolized in the white underground part of the shoot, which also contains lathyrine naturally. This compound is apparently accumulated in it by an active mechanism. The dissimilarities in the amino acid pools of the different parts are therefore due to dissimilarities in the occurrence of the enzymes needed for their synthesis, catabolism or accumulation.

Lathyrine was also catabolized in young shoots of *L. maritimus*, which generally contain large quantities of this amino acid. There is apparently an equilibrium between the synthesis and catabolism of this compound. The feeding experiments showed that *L. sylvestris* has lost the ability to synthesize this amino acid from its precursor homoarginine. Nor can this species catabolize lathyrine but it does to some extent catabolize homoarginine, which it does not contain (cf. Fig. 8). Lathyrine is a common constituent of seeds and shoots of North and South American endemic species of *Lathyrus* (SIMOLA 1966), but is not so common in some morphologically highly evolved Mediterranean species (e. g. *L. ochrus*, *L. sylvestris*) of the subgroups *Clymenum* and *Eulathyrus* (cf. DAVIES 1958; BELL 1964 c and SIMOLA 1966, 1968).

It seems to me that the ability to synthesize lathyrine and its precursor homoarginine is, from the evolutionary standpoint, an old and primitive feature within the genus *Lathyrus* but within the tribe *Vicieae* a new and far-evolved one. For these amino acids, although restricted to the genus *Lathyrus*, have a very wide distribution within this genus. Nearly all *Lathyrus* species can synthesize homoarginine but most Eurasian ones have lost the ability to form lathyrine, and may even have evolved from such species. The functional occurrence of lathyrine (accumulation in storage tissues) is obviously a highly evolved feature, which has developed polyphyletically in morphologically dissimilar Eurasian *Lathyrus* species, such as *L. niger*, *L. aphaca* and *L. pratensis* (cf. PRZYBYLSKA & RYMOWICZ 1965 and SIMOLA 1966). Only a few Eurasian species (*L. maritimus*, *L. tingitanus* and *L. sphaericus*), like many South and North American species, show the same primitive feature that lathyrine is also found in the aerial vegetative parts of the plant.

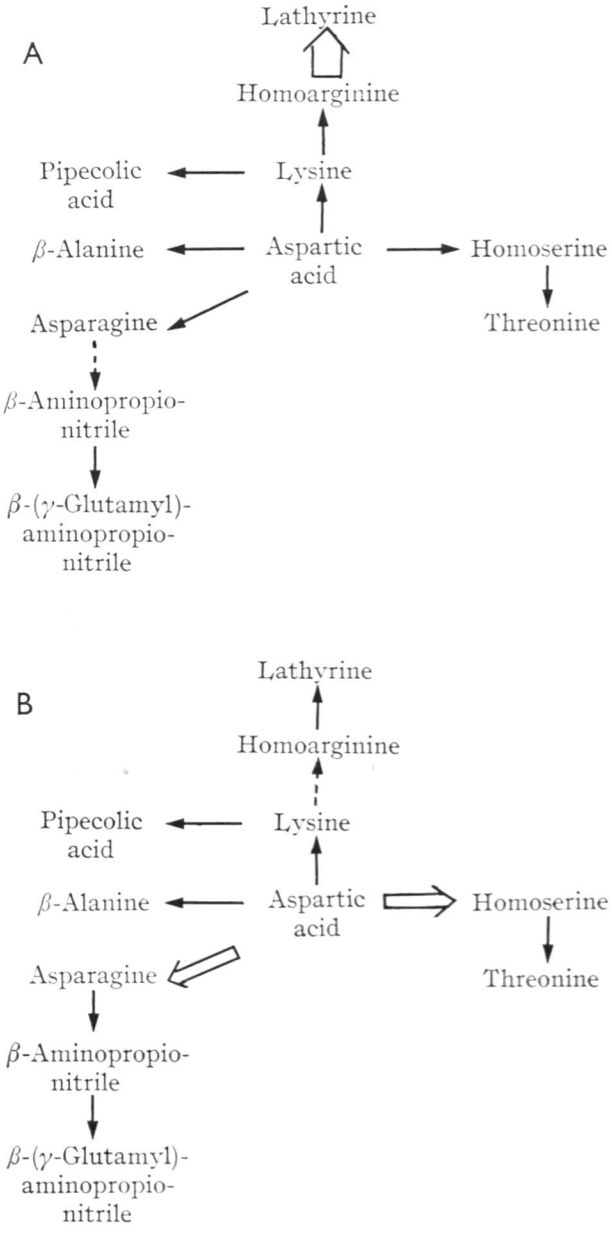

FIG. 9. An overall scheme showing the characteristic biosynthetic pathways of the amino acid pool in *L. niger* (A = fruits, B = leaves and aerial stems).

The ability to synthesize homoarginine has also apparently been eliminated from some Eurasian species of *Lathyrus* (e. g. *L. sylvestris, L. tuberosus* and *L. odoratus*), mostly belonging to the subgroup *Eulathyrus* (cf. PRZYBYLSKA & RYMOWICZ 1965). These species synthesize homoserine in most vegetative parts. This feature is one that these species share with the genera *Pisum* and *Vicia* within the same tribe. Homoserine is rather generally found in the amino acid pool of legumes and of several other plant genera as well (cf. p. 11). The ability to synthesize homoserine has evolved polyphyletically in several taxonomically dissimilar plant groups and this amino acid can be used as a chemotaxonomic character only within certain genera, such as *Lathyrus*. Homoserine occurs in the shoot in most European *Lathyrus* species, but not in the subgroups *Orobastrum, Aphaca* and *Nissolia*, which usually contain lathyrine (PRZYBYLSKA & RYMOWICZ op. cit.).

In feeding experiments *L. niger* and *L. maritimus* do not accumulate homoserine, but the amount of threonine was somewhat increased. The dissimilarities in the free amino acid pool are therefore due to the balance in enzymatic reactions. In *Pisum* and several *Lathyrus* species the enzymatic conversion of homoserine to threonine is slower than the synthesis of homoserine. This leads to accumulation of free homoserine (cf. NAYLOR et al. 1958).

α,γ-Diaminobutyric acid is a characteristic constituent of the amino acid pool of *Lathyrus sylvestris*. The occurrence of this compound within the genus *Lathyrus* is restricted to species which also synthesize homoserine (PRZYBYLSKA & RYMOWICZ 1965). It seems to me that the occurrence of α,γ-diaminobutyric acid is a far-evolved feature which is seen in the genus *Lathyrus* only in some closely related and also morphologically highly evolved species (cf. SIMOLA

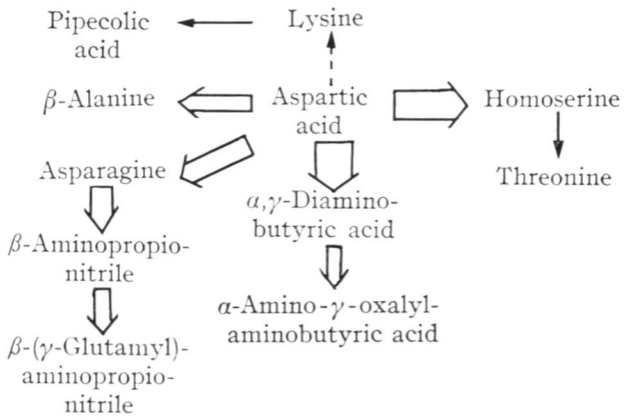

FIG. 10. An overall scheme showing the characteristic biosynthetic pathways of the amino acid pool in *L. sylvestris*. The thickness of the arrows is proportional to the amounts of the products.

1966). This neurotoxic compound has been formed polyphyletically in both higher and lower plants (cf. pp. 6 and 22). It was surprising, however, to find that *Lathyrus* species (*L. niger* and *L. maritimus*) which do not contain this compound could to some extent metabolize it in feeding experiments.

From my feeding experiments it can be concluded that the amino acid pool of the fruiting shoots of *L. niger* is very stable and an amino acid occurring in excess is readily catabolized. Only lysine when given with carbamyl phosphate had harmful effects on the leaves. It is possible that the ability to synthesize homoarginine and lathyrine represents a new way, besides the formation of pipecolic acid, to avoid the toxicity of lysine. In my experiments the tissues of *L. niger* did not accumulate lysine but apparently converted it to pipecolic acid. There is a sharp physiological difference between some monocotyledons and legumes in their ability to make use of an external source of amino acid (cf. VALLE & VIRTANEN 1960).

Pipecolic acid is one of the main amino acids in *L. maritimus* (cf. Figs. 5 and 11). This compound is also found in *L. sylvestris* and *L. niger* but in most of the earlier studies it has not been found in this genus. 5-Hydroxypipecolic acid is a characteristic compound of *L. maritimus*. This compound has not been detected in other *Lathyrus* species. 5-Hydroxypipecolic acid has been evolved polyphyletically in many genera which also contain pipecolic acid, baikian and/or 4-hydroxypipecolic acid (cf. p. 30).

γ-Methylglutamic acid is an abundant compound in *L. maritimus*. Its quantity was somewhat increased in young seedlings fed with α,γ-diaminobutyric acid and homoserine, which may be due to some transamination reactions (cf.

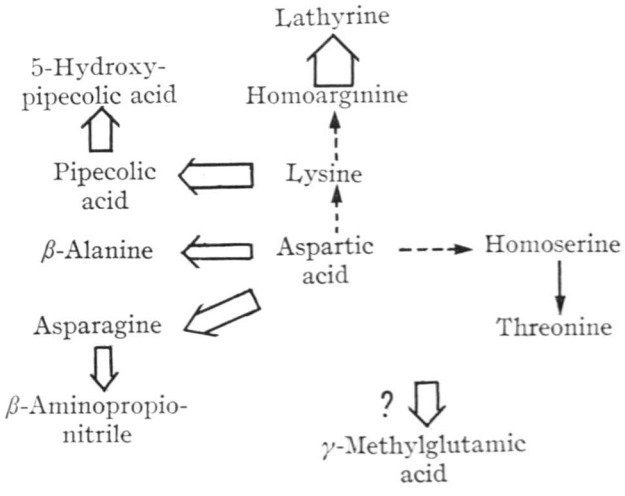

FIG. 11. An overall scheme showing the characteristic biosynthetic pathways of the amino acid pool in *L. maritimus*.

FOWDEN & DONE 1953). The keto acid analogue of γ-methylglutamic acid, α-keto-γ-methylglutamic acid, is an abundant natural constituent of *Lilium regale* (WICKSON & TOWERS 1956). Among *Lathyrus* species γ-methylglutamic acid has been detected only in *L. maritimus* and *L. aphaca* (cf. PRZYBYLSKA & NOWACKI 1961 and PRZYBYLSKA & STRONG 1968). Owing to the fact that in the genus *Lathyrus* γ-methylglutamic acid is limited to these two species, it is obvious that this compound has been evolved polyphyletically within this genus and several times within the pteridophytes (cf. p. 9).

The feeding experiments in the present study demonstrate that a rare amino acid is usually effectively synthesized in the same tissue in which it naturally occurs (lathyrine in *L. niger*). Accumulation of an amino acid as a result of transport is relatively rare. Slight catabolism compared to the biosynthesis of the compound is presumably the reason for the accumulation of lathyrine in seeds. The effective catabolism of a compound which is not found in a certain part of a plant is one reason for its absence (homoarginine in leaves of *L. niger*). Another is the possibility that the tissue has lost the ability to form an enzyme needed for the biosynthesis of this compound although some other parts of the plant can synthesize this amino acid. However, a compound not found in the amino acid pool of a plant may be a labile intermediate in the biosynthesis of a common compound and therefore fail to accumulate in the pool (homoserine in *L. maritimus*).

In view of the fact that some characteristic compound may be lacking from some parts of a species, it is my opinion that a chemotaxonomic classification of a genus should be based on comparison of data from several corresponding plant organs. No far-reaching systematic conclusions can be drawn from the presence or absence of a single compound in one plant organ. Chemotaxonomy is a valuable aid in phylogenetic considerations when it is linked with other characteristics of the genus (cytological, embryological, anatomical, morphological, etc.). It is difficult, however, to determine the direction of chemical evolution. In the first stage, I suppose, when the genera are becoming isolated, the ability to synthesize a new compound should be looked on as a far-evolved feature. Later, the compound may lose its importance, the ends-steps of the long biosynthetic pathways may be lost and genetic material be used for the development of new abilities. Evolution of this kind has taken place in the metabolism of the phenolic compounds. In the most advanced dicotyledons, these compounds have the shortest biosynthetic pathways in the shoot (BATE-SMITH 1962), in which they are without any known physiological role. Similarly, the human organism cannot synthesize the amino acids which have the longest synthetic pathways (DAVIS 1961).

Amino acids, on the other hand, have more important physiological effects on species than do, for example, phenolic compounds. Some protein amino

acids have a strong effect on the morphology of the plant (VIRTANEN & LINKOLA 1946; SANDERS & BUCKHOLDER 1948 and WARIS 1957, 1967). The amino acid pool of a species may also naturally be one of the endogenous factors influencing the morphogenesis of a plant. The centre of both the morphological and the chemical evolution of the genus *Lathyrus* lies in the Mediterranean region (cf. DAVIES 1958 and SIMOLA 1966, 1968).

The rare non-protein amino acids can also form a hybridization barrier between species that have dissimilar amino acid pools by serving as antimetabolites of common protein amino acids. This mostly depends on the specificity of the amino acid-activating enzymes. It has been established, for example, that azetidine-2-carboxylic acid, a normal constituent of many liliaceous species, is not affected by a proline-activating enzyme in these species but is activated by it in some leguminous species not naturally containing this amino acid (PETERSON & FOWDEN 1963).

It was also found that some rare non-protein amino acids (homoarginine, lathyrine, canavanine and α,γ-diaminobutyric acid) characteristic of the *Vicieae* had dissimilar effects on pollen germination and pollen tube growth depending on the natural amino acid composition of the species studied (SIMOLA 1967). This was most clearly seen in *L. niger*, which contains both homoarginine and lathyrine naturally. These compounds promoted the germination and growth of pollen tubes at all concentrations studied in this species. In most other species they had, if anything, a slight inhibitory effect. It has been pointed out that the *Lathyrus* species which hybridize are in fact chemically related (BELL & FOWDEN 1964 and SIMOLA 1966).

On the basis of the present study and the literature referred to, I consider an accurate phytochemical comparison of species at various developmental stages and an experimental clarification of the physiological causes for the differences observed to be of value in the elucidation of the chemical and physiological evolution within the genus and in the tribe as a whole.

Summary

The amino acid pools of three *Lathyrus* species, *L. niger*, *L. maritimus* and *L. sylvestris*, have been studied at four developmental stages. Different plant organs have been analysed separately and their amino acid composition compared. The reasons for the apparent dissimilarities have been clarified with the aid of feeding experiments and the metabolism of the most characteristic compounds simultaneously investigated.

Some protein amino acids were found to accumulate in certain parts of a given species but no general trends could be discerned regarding their occurrence in these species. The prominent protein amino acids are alanine, valine, leucine-isoleucine, serine, aspartic acid and asparagine. γ-Aminobutyric acid is also found in high concentrations. The amount of proline is greater in L. *maritimus* than in the other two species studied.

The occurrence of rare non-protein amino acids is the most characteristic feature of each species. They all contain pipecolic acid but in *L. maritimus* it is present in high

concentrations and this species also synthesizes 5-hydroxypipecolic acid, which has not previously been found in any member of the genus. *L. maritimus* also contains abundant γ-methylglutamic acid, which was not found in the other two species. Homoserine and α,γ-diaminobutyric acid are characteristic of *L. sylvestris*. *L. maritimus* does not contain these two compounds but *L. niger* has homoserine in the stems and leaves.

Homoarginine occurs only in traces in the shoot of *L. maritimus*, despite the fact that it is the precursor of lathyrine, a compound which is abundant in all the organs of this species. There is thus a shift towards lathyrine synthesis in this species, just as there is towards pipecolic acid in the synthesis of this acid via lysine. Lathyrine accumulates in the wintering organs of *L. niger*.

Using radioactive homoarginine as tracer, it could be demonstrated that this amino acid was effectively catabolized in the leaves of *L. niger* but served as a precursor of lathyrine in the fruits of this plant. The natural occurrence of an amino acid is mostly determined by the ability of plant organs to synthesize this compound rather than its transport from other parts. The seedlings of *L. sylvestris*, which do not contain homoarginine, catabolized it only slightly. This species has lost the ability to form lathyrine and homoarginine and was unable to catabolize lathyrine. Lathyrine was catabolized in young seedlings of *L. niger* except in the white underground part of the stem, which under natural conditions contains larger amounts of this amino acid than the other parts. Its concentration in this part may be due to both accumulation and weak catabolism. No clear diurnal variation could be detected in the leaves of *L. niger*.

Some evolutionary relationships deduced from the metabolism of homoarginine, lathyrine, homoserine, α,γ-diaminobutyric acid, pipecolic acid and γ-methylglutamic acid are discussed.

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Appendix: Tables

TABLE 1. Lathyrus niger, *some details of the material collected*

Seedlings

Length of the aerial shoots ca. 7—10 cm (A in fig. 3)

22. 5.—66: rootstocks, 5.0, 28.8; white underground shoots, length 1—3 cm, 1.3; white underground shoots, length 3—7 cm, 2.5; white underground parts of the vertical stems, 2.5, 23.4; aerial shoots, 6.0, 13.1.

Length of the aerial shoots ca. 10—12 cm (B in fig. 3)

20. 5.—66: white underground parts of the vertical stems, 5.5; aerial stems, 6.0; leaves, 4.5.

Length of the aerial shoots ca. 18—20 cm (C in fig. 3)

20. 5.—66: white underground parts of the vertical stems, 5.0; aerial stems, 2.5; leaves over 0.1 g, 2.0; leaves under 0.1 g, 2.0.

Length of the aerial shoots ca. 30 cm (D in fig. 3)

20. 5.—66: aerial stems, 5.0; leaves over 0.1 g, 2.5; leaves under 0.1 g, 2.0.

Budding plants

Length of the aerial shoots ca. 50 cm

13. 6.—64: rootstocks, 5.0, 24.3; white underground parts of the vertical stems, 5.0, 21.2; aerial stems 0—10 cm over the soil surface, 5.0, 20.1; aerial stems 10—20 cm over the soil surface, 5.0, 21.2; aerial stems 20—35 cm over the soil surface, 5.0, 18.8; aerial stems 35—50 cm over the soil surface, 5.0, 20.1; leaves over 0.5 g, 5.0, 22.1; leaves under 0.5 g, 5.0, 25.7; peduncles, 0.3; buds, 1.0.

5. 7.—65: petals, 0.5; stamens 0.015; pistils 0.02.

Flowering plants

Length of the aerial shoots ca. 60 cm

5. 7.—65: rootstocks, 10.0, 35.7; white underground parts of the vertical stems, 10.0, 21.6; aerial stems 0—30 cm over the soil surface, 10.0, 23.0; aerial stems 30—60 cm over the soil surface, 10.0, 27.8; leaves over 0.5 g, 10.0, 27.2; leaves under 0.5 g, 5.0, 27.7; peduncles, 1.0, 38.6; petals (flowers just opened), 0.5, 18.4; petals (flowers fully opened), 1.0, 19.0; stamens (flowers not opened), 0.1, 26.4; stamens (flowers just opened), 0.3, 15.2; anthers (flowers not opened), 0.03; pistils (flowers not opened), 0.1, 21.1; pistils (flowers just opened), 0.2, 20.0.

3. 7.—64: petals (flowers fully opened), 1.2; stamens, 0.21; pistils, 0.19.

Fruiting plants

Length of the aerial shoots ca. 65 cm

26. 7.—67: rootstocks, 10.0, 40.5; white underground vertical parts of the stems, 3.0, 27.0; aerial stems 0—25 cm over the soil surface 10.0, 25.0; aerial stems 25—45 cm over the soil surface, 3.9, 34.2; aerial stems 45—65 cm over the soil surface, 2.0, 38.0; leaves over 0.2 g, 3.0, 26.9; leaves under 0.2 g, 4.0, 33.1.

5. 7.—66: peduncles, 0.3; pods, length 1.5—2 cm, 0.5; pods, length 2.0—2.5 cm, 0.8; peduncles, 0.3; pods, length 2.5—3 cm. 0.9

15. 7.—64: peduncles, 1.0; pods, length 4—5 cm, 4.0.

22. 7.—64: peduncles, 0.5; pericarps, 3.0; seeds 0.24 g/100, 0.144; seeds 0.41 g/100, 0.325.

28. 7.—63: peduncles, 1.0; pericarps, 4.5; seeds 3.8 g/100, 2.0.

5. 8.—63: peduncles, 1.0; pericarps, 10.0; seeds 4.5 g/100, 4.5.

15. 8.—63: peduncles, 2.0; pericarps, 10.0; seeds 5.1 g/100 3.5, 44.7; seeds 4.8 g/100, (half mature), 2.0, 46.6; pericarps, (dry), 5.0.

(The fresh weight of each plant organ has been marked in grams and after it, if determined, the dry weight in per cents.)

TABLE 2. L. maritimus, *some details of the material collected*

Seedlings

Length of the aerial shoots ca. 15 cm

2. 7.—64: rootstocks, 2.0, 19.0; white underground shoots, length 2.5—5 cm, 0.75; stems, 2.0; leaves under 0.5 g, 4.0, 16.6; tops of shoots, 3.0, 16.1.

Budding plants

Length of the aerial shoots ca. 20 cm

2. 7.—64: rootstocks, 2.0, 26.2; stems, 5.0, 15.7; leaves over 0.5 g, 5.0; leaves under 0.5 g, 5.0; tops of shoots, 4.0; peduncles, 0.3; buds, 2.0.

Flowering plants

Length of the aerial shoots ca. 20—25 cm

2. 7.—64: rootstocks, 1.5; stems, 3.0; leaves over 0.5 g, 5.0, 16.5; leaves under 0.5 g, 1.5; tops of shoots, 3.0; peduncles, 2.3; petals, 1.2; stamens, 0.012; pistils, 0.032.

Fruiting plants

Length of the aerial shoots ca. 25—30 cm

20. 7.—65: rootstocks, 2.5; white underground shoots, 1.0, 17.9; stems, 5.0, 27.0; leaves over 0.5 g, 5.0; leaves under 0.5 g, 5.0; tops of shoots, 1.6.

2. 7.—63: peduncles, 2.0; pericarps of small pods, 4.0; seeds 1.25 g/100, 1.0.

20. 7.—65: pericarps, 10.0; seeds 8.3 g/100, 5.0, 28.6;

7. 8.—63: pericarps, 10.0, 20.4; seeds 9.3 g/100, 5.0, 30.5; pericarps, 5.0; seeds 7.6 g/100 (half mature), 2.5, 39.5; seeds 3.8 g/100 (dry), 2.5, 81.5.

TABLE 3. L. sylvestris, *some details of the material collected*

Seedlings

Length of the aerial shoots ca. 25 cm

8. 6.—67: rootstocks, 5.0, 19.8; white underground parts of the vertical stems, 3.0, 15.5; aerial stems 0—10 cm over the soil surface, 5.0, 15.1; aerial stems 10—25 cm over the soil surface, 2.0, 12.8; leaves over 0.5 g, 5.0, 19.4; leaves under 0.5 g, 2.0, 18.8.

Budding plants

Length of the aerial shoots ca. 80 cm

12. 7.—65: rootstocks, 10.0, 23.5; white underground parts of vertical stems, 3.0, 25.1; aerial stems 0—40 cm over the soil surface, 10.0, 21.0; aerial stems 40—80 cm over the soil surface, 10.0, 13.8; leaves over 0.5 g, 10.0, 23.4; leaves under 0.5 g, 10.0, 18.6; peduncles, 4.0, 13.0; buds (small), 2.0, 17.5.

19. 7.—66: buds (intermediate), 0.25; buds (big), 0.5; petals 0.4; stamens (small), 0.012; stamens (big), 0.040; anthers, 0.028; pistils (small), 0.017; pistils (intermediate), 0.047.

Flowering plants

Length of the aerial shoots ca. 120 cm

10. 9.—65: rootstocks, 5.0, 21.5; white underground parts of the vertical stems, 5.0, 22.8; aerial stems 0—60 cm over the soil surface, 5.0, 27.1; aerial stems 60—120 cm over the soil surface, 1.5, 17.6; leaves over 0.5 g, 5.0, 26.2; leaves under 0.5 g, 2.0, 19.1.

19. 7.—66: peduncles, 0.5; petals, 2.76; stamens, 0.364; anthers, 0.045; pistils, 0.413.

Fruiting plants

Length of the aerial shoots ca. 120 cm

10. 9.—65: rootstocks, 2.5, 26.7; white underground parts of the vertical stems, 4.0, 28.2; aerial stems 0—60 cm over the soil surface, 1.5; aerial stems 60—120 cm over the soil surface, 1.5; leaves over 0.5 g (in the lower part of the stem), 1.4; leaves over 0.5 g (in the middle part of the stem), 2.0, 27.0; leaves under 0.5 g, 2.5, 29.2.

6. 9.—63: pericarps of small pods, 2.9; seeds 2.9 g/100, 1.45.

25. 9.—63: peduncles, 4.0; pericarps, 5.0; seeds 8.9 g/100, 5.0; peduncles, 0.65; pericarps, 10.0; seeds 10 g/100, 10.0, 33.2; pericarps, 5.0; seeds 11 g/100, 5.0, 35.8.

20. 9.—63: peduncles, 0.5; pericarps, 2.5; seeds (dry) 3.6 g/100, 2.0, 62.8.

TABLE 4. *Some climatic records for date and period of collection of material*[1]

Date	Day temperature			Mean temperature of the month	Mean temperature of the month for a 30-year period	Total solar radiation grkal/cm²	Sunshine hours per month
	mean	max.	min.				
L. niger							
28.7.—63	18.4	23.2	14.0	16.7	17.8	652	269
5.8.—63	18.1	21.3	15.2	17.1	16.3	531	198
15.8.—63	14.9	18.6	9.7	17.1	16.3	341	198
20.5.—64	11.4	15.2	5.5	9.5	9.3	635	299
13.6.—64	15.9	21.6	10.6	14.6	14.5	616	317
15.7.—64	21.7	26.6	15.6	17.2	17.8	560	304
22.7.—64	16.9	20.1	14.6	17.2	17.8	577	304
5.7.—65	13.6	17.5	7.0	14.8	17.8	541	254
22.5.—66	8.7	12.0	6.6	9.1	9.3	620	292.5
5.7.—66	17.8	21.8	13.0	18.0	17.8	610	302.7
26.7.—67	17.0	19.1	15.5	16.8	17.8	260	347.6
L. maritimus							
2.7.—63	16.0	19.6	10.4	16.7	17.8	652	269
20.7.—63	17.3	22.5	13.7	16.7	17.8	415	269
7.8.—63	17.1	19.5	15.2	17.1	16.3	293	198
2.7.—64	16.4	21.1	11.1	17.2	17.8	667	304
20.7.—65	20.0	25.0	14.1	14.8	17.8	579	254
L. sylvestris							
6.9.—63	16.1	17.8	12.6	13.6	11.5	119	161
25.9.—63	14.4	17.3	12.1	13.6	11.5	202	161
12.7.—65	13.0	17.2	10.0	14.8	17.8	415	254
10.9.—65	15.7	18.4	13.2	13.4	11.7	301	91
19.7.—66	16.3	19.7	14.7	18.0	17.8	580	302.7
8.6.—67	10.5	12.4	9.2	13.7	14.5	500	280.3
19.7.—67	17.7	23.1	11.0	16.8	17.8	545	347.6

[1] The data have been collected from the monthly reports of Finland's Meteorological Institute, which has an observation point in the Botanical Garden of the University of Helsinki.

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