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DISTRIBUTION OF L-DOPA AND RELATED AMINO ACIDS IN *VICIA*

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Abstract—European legumes with the highest L-DOPA content are *Vicia narbonensis* and *V faba minor*, a forage plant For *V faba minor*, the fruit-bearing period is the best time for the production of this amino acid The valves of the pods contain the greatest amount Edible fava beans (seeds of *V faba*) are practially free of L-DOPA, this amino acid is, therefore unlikely to be the cause of favism A diet rich in fava beans, on the other hand, cannot affect favorably the course of Parkinson's disease L-DOPA, present in the free form in all vegetative organs of *V faba minor* and of broad beans, is found as a glucoside only in the tegument of the seeds, while its precursor, L-tyrosine, is present in the whole plant, mostly as a glucoside The cotyledons of green fava beans contain appreciable quantities of methionine only in the form of glucoside The drying of green *V faba minor* (fodder) always involves loss of L-DOPA The smallest loss (11%) occurs when drying is effected in shaded ventilated environment

INTRODUCTION

THE IMPORTANCE of L-dihydroxyphenylalanine (L-DOPA) as an anti-Parkinson drug prompted us to examine the possibility of finding it in indigenous plants Acetic acid extracts of the plants were analyzed by the accelerated system for automated aminoacid analysis (A A A)[1] and by a colorimetric technique

RESULTS AND DISCUSSION

Quantitative determination of free L-DOPA in European legumes

Table 1 shows the quantitative data obtained with the various plants examined On account of the sizable L-DOPA content in the green plant *Vicia faba minor*,* a legume widely cultivated and commonly used as fodder, this plant was examined in more detail The study was also extended to edible fava beans (*V faba* L) because of their importance in nutrition

L-DOPA production in Vicia faba minor

Table 2 shows the quantitative data on the presence of free L-DOPA in cultivated *V faba minor* at various vegetative stages The best time for L-DOPA production is the fruit-bearing period, the valves of the pods are particularly rich in this amino acid (Table 1)

* *Faba vulgaris* Moench *minor* Beck = *Vicia faba minor* Systematics of *Vicia faba* L in (a) TASSINARI, G (1960) *Manuale dell'Agronomo*, p 460, R E D A, Roma, (b) PANTANELLI, E (1955) *Coltivazioni erbacee*, Edizioni Agricole p 149, Bologna, (c) *Enciclopedia Agraria Italiana* (1960) Vol 4, p 406, R E D A Roma

[1] SPACKMAN, D H (1963) *Federation Proc* **22**, 244, (1964) *Federation Proc* **23**, 371
[2] ARNOW L E (1937) *J Biol Chem* **118**, 531

Though the L-DOPA content as a percentage shows maximum and minimum values production of the compound in the plant steadily increases during development However, owing to the different distribution of L-DOPA in the various organs of the plant its content does not increase proportionally with increase in weight (Table 3)

TABLE 1 QUANTITATIVE DETERMINATION OF FREE L-DOPA IN INDIGENOUS LEGUMINOUS PLANTS

Plant	Plant organ	Method of analysis	% of L-DOPA in sample 1	2	3	4
Cicer arietinum L	Dry seeds (chick pea)	Colorimetric	Nil			
Phaseolus vulgaris L	Dry seeds (white Spanish beans)					
Galega officinalis L	Green plant					
Lens culinaris Medik	Dry seeds (lentils)					
Lupinus albus L	Dry seeds (lupines)					
Medicago sativa L	Dry seeds					
Vicia faba L (see Table 5)	Green valve of the pod	Amino acid Analyzer	0·2	0·4	0·54	0·75
	Flowering green plant		0·09			
	Green seeds (green fava)		0·01	0·006	0·007	
	Dry seeds (dry fava)	Both	Nil	Tr		
	Dry cotyledons (peeled dry fava)		Tr	Tr		
Vicia faba minor (forage)	Dry seed		0·07			
	Green pods (whole unripe fruit)	Amino acid Analyzer	0·63			
	Green plant with pods		0·56			
	Green flowering plant		0·16		0·40	
	Green vegetative plant		0·33	0·24	0·49	0·57
Vicia narbonensis L	Green seeds	Colorimetric	Nil			
	Green pods (valves only)	Amino acid Analyzer	0·5			
	Green plant with pods		0·6			
Vicia sativa L (vetch)	Dry seeds	Colorimetric	Nil			
	Green plant		Nil			

During flowering, the increase of L-DOPA in the plant is arrested (Table 2) The dark melanin[3] of the flower (dark spots on the lateral petals) is likely to be produced at the cost of the amino acid, following the known reaction pattern whereby L-DOPA is converted into melanin All types of petal of the flower (flag, lateral petals carina) contain free L-DOPA

TABLE 2 L-DOPA AND L-TYROSINE CONTENT OF *Vicia faba minor* SEEDLINGS OF VARIOUS AGES

Age of seedling (days) (sample 1 of Table 1)	Mean total weight of seedling (g)	Free L-DOPA and L-tyrosine content (AAA) mg seedling L-DOPA	L-Tyrosine	% in the plant L-DOPA	L-Tyrosine
0 (April 4th)	0·3 (seed)	0·22	0·03	0·075	0·010
21	0·5 (6)*	1·6	0·15	0·32	0·030
33	4·2 (10)	15·2	0·36	0·36	0·009
40	8·9 (6)	28·5	0·63	0·32	0·007
54	27 (7)	61·0	1·62	0·22	0·006
61 (flowering)	34 (7)	57·0	2·72	0·16	0·008
89 (fruiting)	67·2 (4)	380	9·48	0·56	0·044

* Number of replicates

Distribution of L-*DOPA in the various organs of* Vicia faba minor

Table 3 gives an indication of the distribution of the amino acid in the various organs of *V faba minor* Pods and stalk are the richest and poorest sources respectively L-DOPA does not appear in the plant (leaves stalk, roots) in the glucosidic form whilst L-tyrosine is present exclusively in this form The high concentration of free L-DOPA in the valves

[3] THOMAS M (1955) in *Modern Methods of Plant Analysis* Vol IV p 663 Springer Berlin

of the pods, and its scarcity in the seeds, both in *V faba minor* and in edible fava beans (Table 1), prompted us to effect a selective analysis of the various organs constituting the pod of *V faba* (Table 4) This may contribute to the clarification of the role of L-DOPA in favism

TABLE 3 L-DOPA AND L-TYROSINE DISTRIBUTION IN THE VARIOUS ORGANS OF *Vicia faba minor*

Plant organ	% in the organ L-DOPA Direct analysis	L-DOPA Analysis after β-glucosidase treatment	L-Tyrosine Direct analysis	L-Tyrosine Analysis after β-glucosidase treatment
Whole plant before flowering	0 24	—	Tr	—
Leaves of the plant before flowering	0 360	0 360	Tr	0 158
Stalk before flowering	0 170	0 162	Tr	0 100
Root before flowering	0 305	0 325	Tr	0 145
Whole green pods	0 69	—	0 156	—

Table 4 shows that in the edible part of the fava beans (the seeds), L-DOPA is virtually absent in the cotyledons, and is present in the teguments in such minimal quantities as to make a possible causal relationship between L-DOPA and favism most unlikely The assumption of some authors, (see review of Liener[4]), that L-DOPA plays a causal role in the disease, may be due to incomplete interpretation of the data reported by the first research workers who extracted L-DOPA from "Fava" [5] In reality this term designated only the green valves of the pods and not the edible seeds Conversely, recent observations on the importance of L-DOPA in the treatment of Parkinson's disease and the high dosage necessary to counterbalance peripheral decarboxylation, proves that the consumption of broad beans (*V faba*), even in large amounts, cannot exert any useful therapeutic effect on parkinsonism [6]

TABLE 4 DISTRIBUTION OF L-DOPA, L-TYROSINE AND THEIR GLUCOSIDES IN THE GREEN PODS OF *Vicia faba*

Plant organ	% in the green plant organ (amino acid analyzer) L-DOPA Sample 1 A	B	Sample 2 A	B	Sample 3 A	B	L-Tyrosine Sample 1 A	B	Sample 2 A	B	Sample 3 A	B
Green valve of pod	0 54	—	0 755	0 755	—	—	0 18	—	0 13	0 240	—	—
Tegument of seed	0 01	—	0 03	0 11	Tr	0 058	0 034	—	0 05	0 21	0 006	0 108
Naked cotyledons of seed	Tr	Tr	Tr	Tr	—	—	0 009	0 68	0 006	0 076	—	—

A—Samples analysed directly B—samples analysed after incubation with β-glucosidase

[4] LIENER, I E (1969) in *Toxic Constituents of Plant Foodstuffs*, p 421, Academic Press, New York
[5] TORQUATI T (1913) *Archivio Farmacol Sperim* **15**, 213, GUGGENHEIM M (1913) *Z Physiol Chem* **88**, 276
[6] In Sicily, people eat the whole pods, including the valves while they are still tender Since L-DOPA resists cooking, it must be assumed that people ingest not merely traces but quantities from 200–800 mg of L-DOPA for 100 g of pods At any rate the most severe cases of favism are observed after the ingestion of *Vicia faba* seeds without the pods (LUISADA, A (1941) *Medicine* **20**, 229)

Glycosides of L-*DOPA and of* L-*tyrosine*

The action of β-glucosidase on extracts of various organs of *V faba* reveals the presence, in some of them, of a glucoside of L-DOPA already identified by another procedure [7]

TABLE 5 PERCENTAGE CONTENT IN FREE AMINO ACIDS IN A *Vicia faba minor* SEEDLING AT VARIOUS PHASES OF DEVELOPMENT*

Free amino acid	% composition at day 0 (seed)	21	33	40	54	61 (flowers)	90 (fruits)
Aspartic acid	0 025	0 040	0 014	0 021	0 016	0 012	0 020
Threonine	Nil	Tr	0 009	0 007	0 008	0 004	0 019
Serine	0 015	0 460	0 038	0 049	0 049	0 025	0 100
Glutamic acid	0 035	0 090	0 032	0 042	0 028	0 016	0 032
Proline	Tr	0 030	Tr	Tr	0 006	0 004	0 025
Glycine	0 010	0 013	Tr	Tr	Tr	Tr	Tr
Alanine	0 010	0 030	0 006	0 007	0 008	0 014	0 025
Cysteine	Nil	Nil	Nil	Nil	Nil	Nil	Tr
Valine	Tr	0 050	0 009	0 009	0 008	0 006	0 019
Methionine	Tr	Tr	Nil	Tr	Tr	Tr	Tr
Isoleucine	Tr	0 025	0 006	0 007	0 006	0 004	0 010
Leucine	0 005	0 025	0 006	0 009	0 008	0 004	0 011
L-DOPA	0 075	0 320	0 360	0 320	0 220	0 165	0 560
Tyrosine	0 010	0 030	0 009	0 007	0 006	0 008	0 044
Phenylalanine	0 010	0 040	0 006	0 007	0 006	0 004	0 011

* Sample 1 of Table analysed by A A A

Data in Table 3 and 4 indicate that this glucoside is absent in the leaves, stalk and roots of the vegetative plant, the cotyledons of the seeds and the green valves of the pods, and is found only in the teguments of the seeds However the glucoside of L-tyrosine is found in all the above-mentioned organs It is of interest to note that the whole plant before

TABLE 6 DETECTION OF CYSTEINE AND METHIONINE IN VARIOUS ORGANS OF *Vicia faba minor* (FORAGE) AND *V faba* (FAVA) AFTER TREATMENT WITH β-GLUCOSIDASE

Plant organ	% of aminoacid in the green organ: Cysteine, Direct analysis	Cysteine, After β-glucosidase treatment	Methionine, Direct analysis	Methionine, After β-glucosidase treatment
V faba minor vegetative stalk	Nil	Tr	Nil	0 027
V faba minor, vegetative root	Nil	Tr	Nil	0 050
V faba minor vegetative, leaves	Nil	Tr	Nil	0 058
Green fava, tegument of seed	Nil	0 006	Tr	0 039
		0 023	Tr	0 038
Green fava, cotyledons	Tr	0 008	Tr	0 023
			Tr	0 290

flowering contains both free L-DOPA and the glucoside of L-tyrosine, whilst free tyrosine is absent (Table 3) The glucoside seems to constitute a reserve for L-tyrosine which once released, is oxidized to L-DOPA The direct oxidation of the glucoside of tyrosine to glucoside of L-DOPA by phenoloxidase (tyrosinase) could not be demonstrated In fact, on

[7] ANDREWS R S and PRIDHAM J B (1965) *Nature* **205**, 1213

treating extracts of cotyledons of *V faba* (containing glucosides of L-tyrosine and lacking free L-tyrosine and L-DOPA) with fungal tyrosinase, the glucoside of L-tyrosine remains unaltered [8]

Amino acid content of Vicia faba minor

The free amino acid content of *V faba minor*, at various stages of its development, is shown in Table 5 Sulphur-containing amino acids are almost entirely absent in the free form, but occur in the form of glucosides (Table 6)

Loss of L-*DOPA incurred during drying of* Vicia faba minor

Green *V faba minor* contains about 90% water All methods of drying, except that effected in a shaded, ventilated environment, resulted in a substantial loss of L-DOPA (Table 7)

TABLE 7 LOSS IN L-DOPA IN *Vicia faba minor* WITH DIFFERENT DRYING METHODS

Drying method	Content after drying	% loss during drying	Drying method	Content after drying	% loss during drying
Natural on field	0 41	41	Industrial desiccator with hot air stream		
Shaded, ventilated environment	0 62	11	Drying at 90–100°	0 09	88
			120°	0 17	77
			150°	0 16	79

Initial L-DOPA content in the fresh plant 0 75%

EXPERIMENTAL

Extraction ca 10 g of fresh plant tissue were homogenized 3 × with 50 ml (100 ml for dry plants) of 1% AcOH containing 0 5% satd SO_2 soln The solns were filtered and the combined filtrates were diluted to standard vol

Colorimetric determination of L-*DOPA* The method of Arnow[2] was used In the visible region of the spectrum, this method gives absorption curves that overlap the A A A values when the determination is made on pure L-DOPA When acetic acid extracts of leguminous plants of the genus *Vicia* are used, this method gives values higher than those obtained with A A A very likely on account of the presence of other *o*-dihydroxy-compounds The colorimetric method is useful to ascertain the absence of L-DOPA when the chromatic reaction is negative

The method of Maggi *et al* [9] published after all the experiments reported in this paper had been completed is based on the same principle as the method of Arnow, the detection of *o*-dihydroxy phenolic compounds This method also yielded higher values on acetic acid extracts of *Vicia* than those obtained with A A A

Detection of the glucosides of L-*DOPA*[7] *and of tyrosine in various plant organs* Extracts were prepared as described above with the exception that acetate buffer (pH 5) replaced 1% AcOH Each extract was divided into two equal portions, one of which was analyzed directly β-Glucosidase (10 mg/ml) was added to the other and kept at 37° for 48 hr prior to analysis Finally a third soln for analysis was obtained by extracting the plant with acetate buffer at pH 5 (with 0 5% SO_2) in a boiling H_2O bath for 20 min in order to destroy any glucosidase that may be present in the plant

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[8] This finding confirms the statements of DUCKWORTH J and COLEMAN (1970) *J Biol Chem* **245**, 1613 of a *para*-oxidation of catecholes In L-tyrosine-glucoside, the *para*-hydroxy group is protected by glucose Oxidation of L-tyrosine and L-tyrosine glucoside was performed with Worthington polyphenol oxidase in 0 5 M phosphate buffer, pH 6 5 according to (1972) *Worthington Enzyme Manual*, p 39, Freehold New Jersey, L-tyrosine was oxidized to melanin

[9] MAGGI N, CASAVECCHIA, G and CAVATORTA, L (1970) *Il Farmaco Ed Pr* **25**, 297, MAGGI, N and COMETTI, A (1972) *J Pharm Sci* **61**, 924