The Metabolism of Chlorotic Leaves

1. AMINO ACIDS

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Considerable attention has been paid to the enhanced amounts of free amino acids in plant leaves affected by iron-deficiency chlorosis. Bennett (1945) considered this to be the most typical symptom of iron-deficient leaves, and Iljin (1951) noted a marked increase of amino acids in the leaves of plants affected by 'lime-induced' chlorosis, a form of iron deficiency.

Using the technique of paper chromatography, Demetriades (1956) demonstrated the increase of amino acids which occurred in iron-deficient leaves of *Hibiscus esculentus*, an increase in arginine being especially prominent. Iron-deficient blueberry leaves were found to contain up to ten times more arginine (determined with Sakaguchi's reagent) than the healthy leaves (Holley & Cain, 1955). This was true also of leaves in which an iron deficiency was induced by toxic concentrations of nickel or cobalt (Cain & Holley, 1955).

Increases in arginine have similarly been recorded for the chlorophyll-deficient areas of variegated *Pelargonium* leaves by Euler & Burstrom (1933), but this was not true for the variegated leaves of *Euonymus* or *Tradescantia*. Bennett (1945) recorded increased free amino acids in variegated leaves and also in virus-infected leaves. Drever & Larv (1954) found a general increase of free amino acids in the leaves of peach affected by Western X virus; proline and pipecolinic acid were present only in the virus-affected leaves, a fact noted by others (Diener & Dekker, 1954). Similar increases in free amino acids have been recorded by Vavrich (1952) for virus-infected sugar-beet leaves and for leaves of *Acalypha indica* affected by mosaic virus (Laloraya, Govindjee & Raja Rao, 1955).

Young tissues have been found to contain more amino acids than mature tissues. Allsopp (1948) found an abundance of free amino acids in the growing points of ferns and much smaller amounts in the leaves. Steward, Wetmore, Thompson & Nitsch (1954) found that the free amino acids in *Lupinus* leaves decreased as the leaves expanded, especially lysine, arginine, valine, leucine, phenylalanine and tryptophan. Similar results were obtained by Mansford & Raper (1954) for *Equisetum*, by McKee, Nestel & Robertson (1955) for the developing pea, by Shibamoto, Shoji & Tagawa (1955) for bamboo shoots and by Petronici (1956) for *Vicia faba* leaves. This effect of age on the free amino acids in plant leaves is also mentioned by Iljin (1951) and by Bennett (1945), both investigators noting that differences in amino acid patterns of healthy and chlorotic leaves were most striking in the spring.

Since leaves suffering from iron deficiency, genetical chlorosis or certain virus infections, and also young leaves, contain more phosphorus relative to iron and more potassium relative to calcium (DeKock & Hall, 1955), it was of interest to determine whether these large amounts of free amino acids were always found in leaves with high phosphorus:iron and potassium:calcium ratios, irrespective of the cause.

METHODS

Extraction. Samples of about 25 g. of leaf tissue from various sources were macerated for 3 min. in a Waring Blendor with 100 ml. of 70% acetone and allowed to stand overnight. After filtration, the residue was extracted with hot 0·01 N-HCl, filtered and combined with the first extraction after removal of the acetone by distillation. The pH was then adjusted to 7 and the volume of extract reduced to about 150 ml. *in vacuo* at 40°. The extract was then passed through a Zeo-Karb 225 column (28 cm. × 1·5 cm., H^+ form) at less than 10 drops/min., the amino acids being subsequently eluted from the column with aq. 2 N-NH_3 soln. after a water wash. The eluate was freed from excess of NH_3 by evaporation under reduced pressure, and the residue dissolved in water and made up to a suitable volume (10–50 ml.): propan-2-ol was incorporated to give a final concentration of 10%.

Paper chromatography. The amino acids were separated by two-dimensional paper chromatography on sheets of Whatman no. 1 filter paper (28·5 cm. × 23·5 cm.). The amino acid solutions were applied to the paper by means of an automatically adjusting micropipette (Meinhard & Hall, 1950). Solvents used were phenol–ethanol–water (3:1:1, by vol., containing 0·05% of 8-hydroxyquinoline) in an atmosphere containing NH_3, and butan-1-ol–acetic acid–water (30:6:14, by vol.), both mixtures being single-phase systems at 18°. The ascending-boundary technique (Williams & Kirby, 1948) was employed for the phenol run along the smaller dimension of the paper. After the run,

which required about 16 hr., the papers were dried, first in a current of air at room temp. and then in an oven at 80°. For the second dimension it was necessary, in order to obtain a sufficiently long run, to use the descending technique (Consden, Gordon & Martin, 1944), and to carry the solvent from the trough to the chromatogram a strip of filter paper was tacked temporarily with cotton thread to the leading edge of the sheet; the trailing edge was serrated (Hird, 1949). A run of about 20 hr. duration at 18° was required. The papers were dried in a current of air at room temp. for 18–20 hr. and then dipped in a solution of ninhydrin (0·2%) in acetone and heated at 65° for 20 min. Two standard papers with known amounts of the amino acids to be determined, at levels of about 0·02 and 0·08 μmole, were run simultaneously and under the same conditions as the samples.

Determination of amino acids. Paper strips, 2·5 cm. wide, containing the amino acid spots were cut from the chromatograms after being lightly demarcated in pencil with the aid of a Perspex template. The strips, which might contain one or two spots, were scanned in a semi-automatic recording reflectance densitometer (model 2 SR, Joyce, Loebl and Co., Newcastle upon Tyne) and an absorption curve was obtained for each spot. The area under the curves, obtained by taking the product of the height and the width at half the height, was plotted against the weight of amino acid for the standard spots, giving a reference curve for each amino acid from which the amount in the samples could be obtained. Since the relationship between the area under the absorption curve and the weight of amino acid was not usually strictly linear, although nearly so, particularly for small amounts, it was necessary for maximum accuracy to adjust the samples so that the weight of each amino acid to be determined was well within the reference curve for the standards. It was usually necessary to prepare two chromatograms for each sample, one for those amino acids present in relatively high concentrations and another for the remainder.

Some amino acids were best determined by special methods. Tryptophan (when present) was determined on one-dimensional chromatograms after a short ascending run in the butanol–acetic acid solvent. The dried papers were dipped in a 1% solution of *p*-dimethylaminobenzaldehyde in acetone–10 N-HCl (9:1, v/v) and suspended for 80 min. at room temp. (18°). The violet tryptophan spots were then scanned in the densitometer as described above. Standards were run along with the samples. Histidine was determined on similar chromatograms, after spraying with a solution of diazotized sulphanilic acid [0·3 % in 8% (w/v) HCl] followed by 20 % Na_2CO_3 (Bray, Thorpe & White, 1950), by means of the reflectance densitometer. Comparative standards were used.

Proline was determined by the densitometer on one-dimensional chromatograms run in the butanol–acetic acid solvent, dipped in solution of isatin (0·2% in acetone), and heated at 75° for 4 min.

Replication experiments showed that the accuracy of determination of individual amino acids by this method was better than ±20%. Methionine, cystine, β-alanine and citrulline were sometimes detected but only in minor quantities and were not determined. Glycine was not well separated from serine and glutamine by the methods used and may sometimes have escaped detection, but it was never present in large quantities.

RESULTS

Comparison of extraction procedures. In order to test the accuracy of the extraction method, the leaves of mustard plants (*Sinapis alba*) grown under identical conditions in a greenhouse were divided into three portions, two of which were extracted as described above and the third was boiled in 200 ml. of water for 3 min., the leaves then being blended and filtered; after removal of acetone, subsequent procedures for the three samples were identical. Table 1 gives the results of the trial, showing that both the extraction procedure and reproducibility are satisfactory, although the γ-aminobutyric acid figure is somewhat higher in the water extract.

Iron-deficiency chlorosis. Bracken (*Pteris aquilina*) was gathered from calcareous and acid soils at Ullapool, Wester Ross, the former sample showing severe lime-induced chlorosis. The results are shown in Table 2, together with normal and iron-deficient spinach beet (*Beta vulgaris*) grown in sand culture in the greenhouse and healthy and nickel-toxic pea leaves (*Pisum sativum* var. Onward) grown in soil. Striking increases occurred in aspartic and glutamic acids, serine and most other amino acids in chlorotic bracken. In both the iron-deficient spinach beet and peas the increases in various amino acids were not so marked. In

Table 1. *Some free amino acids of mustard leaves*

Results are expressed as mg./100 g. of fresh leaf. Samples nos. 1 and 2 were extracted with acetone and no. 3 was extracted with boiling water. The upper and lower figures refer to chromatograms with slightly different loadings. Amino acids also present in mustard leaves but not determined were: arginine, asparagine, leucine, isoleucine, lysine, phenylalanine, tyrosine. Histidine and tryptophan were absent.

	Sample no.		
	1	2	3
Aspartic acid	29·1 34·6	27·2 22·2	34·5 27·7
Glutamic acid	58·4 63·6	64·1 58·2	58·2 57·4
Glutamine	7·2	7·7	10·9
Serine	27·2	30·9	28·4
Threonine	9·6 9·3	8·5 7·8	11·4 9·4
Alanine	13·9	10·1	11·0
γ-Aminobutyric acid	6·4 7·8	8·0 6·7	16·4 12·5
Valine	6·0	8·7	9·0
Proline	111·0*	114·0*	140·0*
Carboxyl nitrogen	37·6	33·4	36·0
Kjeldahl nitrogen	65·0	65·0	66·0

* Determined with isatin, after chromatography.

Table 2. *Free amino acids of healthy and iron-deficient leaves*

—, Not detected; P, present in fair quantity but not determined. Results are expressed as mg./100 g. of fresh leaf for both normal (N) and iron-deficient (D) leaves.

	Bracken (23)*		Spinach beet (50)*		Peas (43)*	
	(N)	(D)	(N)	(D)	(N)	(D)
Aspartic acid	0·7	10·8	6·0	13·2	9·0	7·0
Glutamic acid	1·0	80·4	17·3	26·5	14·1	26·9
Serine	1·3	59·0	3·9	15·3	2·5	4·5
Threonine	10·5	70·4	1·6	5·5	16·0	45·7
Alanine	5·7	118·1	4·9	6·6	3·0	4·5
Tyrosine	2·8	23·8	1·0	4·1	0·6	0·6
γ-Aminobutyric acid	22·9	54·0	2·1	8·6	1·9	3·7
Valine	38·0	208·3	2·3	3·1	1·3	3·9
Leucine–isoleucine	70·0	258·0	4·1	6·2	1·4	2·5
Phenylalanine	178·0	418·2	0·7	1·4	3·1	6·6
Histidine	6·5	P	2·8	3·9	—	—
Lysine	0·8	3·3	1·4	1·0	1·2	4·2
Arginine	2·1	5·6	3·8	3·3	18·7	24·9
Asparagine	0·4	66·5	6·5	19·6	4·4	12·5
Glutamine	7·8	7·4	0·7	2·3	2·2	0·4
Proline	6·0	40·3	44·1	48·1	4·4	11·1
Tryptophan	38·5	30·5	1·3	1·9	0·7	1·1
Total	393·0	1454·6	104·5	170·6	84·5	160·1

* Sample reference number (Table 8).

Table 3. *Free amino acid of mustard leaves* (78)* *grown in nutrient solutions A, B, C and D containing* 0·1, 0·5, 2·5 *and* 12·5 *p.p.m. of iron respectively*

—, Not detected; T, detected but too weak for determination.

	Concn. (mg./100 g. of fresh leaf)			
	A	B	C	D
Aspartic acid	43·6	31·1	36·2	32·7
Glutamic acid	136·3	79·8	54·1	51·1
Serine	24·7	24·1	12·3	15·3
Threonine	10·5	8·6	8·0	7·4
Alanine	20·9	8·9	6·5	8·3
Tyrosine	3·0	2·7	2·2	1·0
γ-Aminobutyric acid	9·1	4·7	3·6	4·5
Valine	12·1	6·0	6·7	5·4
Leucine–isoleucine	9·4	5·2	2·6	4·4
Phenylalanine	2·0	T	T	T
Lysine	2·5	0·7	T	—
Arginine	70·1	18·2	5·2	T
Asparagine	15·8	5·1	4·5	1·7
Glutamine	8·0	8·8	3·8	3·0
Histidine	5·0	—	—	—
Proline	39·5	37·7	31·8	28·2
Total	412·5	241·6	177·5	163·0

* Sample reference number (Table 8).

Table 3 the free amino acids of mustard (*Sinapis alba*) plants grown in nutrient solutions containing 0·1, 0·5, 2·5 and 12·5 p.p.m. of iron as the *NN'*-ethylenebis-(2-*o*-hydroxyphenyl)glycine chelate are given. The differences in free amino acids were most marked at the lower levels of iron; at the higher levels there were only small differences. The decrease in arginine was especially marked.

Genetical chlorosis. Normal green leaves and completely albino leaves were obtained from a variegated plant of *Bougainvillea glabra* in the greenhouse of the Macaulay Institute. Two sets of samples were collected on successive years. The results are presented in Table 4, and show the remarkable accumulation of arginine which occurs in the chlorotic leaves. Histidine was also much higher in the first albino sample, but actually lower in the second sample.

Effects of age. The decrease of free amino acids which takes place when cabbage leaves age is shown in Table 5. Analyses of the unexpanded 'heart' leaves and the expanded green leaves of four cabbage varieties, Greyhound, Winningstadt, January King and Myate are given. The difference between varieties is shown, as also is the greatly enhanced amount of arginine present in the young leaves.

Grass samples (mainly *Cynosurus cristatus*) taken in May 1956 and July 1956, and samples of beech leaves (*Fagus sylvatica*) taken in June 1956 and August 1956 showed abundant amino acids in the young leaves and marked decrease in the older leaves, to such an extent that only proline was detected in older grass by the method employed (Table 6).

Virus infection. Samples of healthy and diseased sugar beet (*Beta saccharifera*) affected by virus yellows, collected on 2 successive years, are shown in Table 7. The yellow leaves were also found to be boron-deficient. It is apparent that such virus-infected leaves contain much the same amounts of free amino acids as do the healthy leaves. A similar

picture was found when chlorotic leaves of mustard plants grown in high concentrations of ferric ethylenediaminetetra-acetate were compared with healthy green leaves.

Table 8 represents the inorganic analyses of the various samples quoted, together with the calculated phosphorus:iron and potassium:calcium ratios. The sodium and magnesium values have been included so that ratios of univalent to bivalent cation can be calculated if desired. Sample numbers are given in parentheses. No analyses were obtained for the grass samples (39, 42).

Table 4. *Free amino acids of leaves of variegated* Bougainvillea

T, Detected but too weak for determination.

	Concn. (mg./100 g. of fresh leaf)			
	(38)*		(52)*	
	Green	Albino	Green	Albino
Aspartic acid	26·3	29·3	14·2	22·1
Glutamic acid	45·5	28·4	35·5	21·6
Serine	10·6	5·0	4·4	3·2
Threonine	5·7	7·2	3·5	7·5
Alanine	6·0	4·1	6·3	4·0
Tyrosine	1·5	1·2	1·6	1·4
γ-Aminobutyric acid	7·4	5·9	3·8	4·8
Valine	1·7	3·4	2·2	4·6
Leucine–isoleucine	4·4	6·9	1·7	6·6
Phenylalanine	3·0	3·0	2·3	8·9
Histidine	12·6	73·5	10·4	4·2
Lysine	3·0	5·7	4·4	12·0
Arginine	37·1	157·0	24·5	213·0
Asparagine	7·6	2·1	4·8	6·0
Glutamine	3·2	0·7	1·8	2·6
Tryptophan	T	T	4·2	8·0
Proline	27·0	23·3	38·0	40·5
Total	202·6	356·7	163·6	371·0

* Sample reference number (Table 8).

DISCUSSION

Since methods of estimation of individual amino acids may be inaccurate to the extent of 20 %, and in view of the results of replicated extractions given in Table 1, it is considered unlikely that differences between samples of less than 50 % are significant. However, differences between the free amino acids of normal and chlorotic leaves are frequently very large and certainly greater than 100 %, which leaves little doubt of their significance.

That deficiency of iron in leaves does not cause merely one or a few amino acids to increase, but rather an overall increase is shown in Table 2. It is immaterial whether the iron deficiency was caused by absence of iron (spinach), by its unavailability in calcareous soils (bracken) or by heavy-metal toxicity (pea). Moreover, it would appear impossible to single out one particular amino acid which shows consistent trends in the three plants studied. Thus although Holley & Cain (1955) found large increases of arginine in iron-deficient and 'cobalt-toxic' blueberry leaves, this is not shown by any of the above plants, nor is it shown by iron-deficient tomato plants (Possingham, 1956).

Table 5. *Free amino acids of young (W) and mature (G) leaves of four varieties of cabbage*

—, Not detected; T, detected but too weak for determination.

	Concn. (mg./100 g. of fresh leaf)							
	Greyhound (45)*		Winningstadt (53)*		January King (61)*		Myate (71)*	
	(W)	(G)	(W)	(G)	(W)	(G)	(W)	(G)
Aspartic acid	—	T	61·5	18·1	53·0	33·1	41·2	18·0
Glutamic acid	—	T	72·1	30·8	131·4	51·8	135·4	23·1
Serine	—	—	22·0	5·7	23·5	7·6	30·6	6·9
Threonine	—	—	7·1	4·3	18·5	7·5	12·8	4·9
Alanine	—	—	24·4	6·4	35·6	25·6	32·6	8·0
Tyrosine	—	—	3·4	0·6	1·0	—	3·4	—
γ-Aminobutyric acid	0·1	—	13·0	6·3	58·3	30·7	4·3	4·4
Valine	—	—	24·4	8·4	11·2	7·0	11·9	2·5
Leucine–isoleucine	—	—	18·7	3·7	6·9	5·0	11·7	2·1
Phenylalanine	—	—	7·9	2·4	5·1	1·8	2·6	T
Histidine	—	—	15·1	1·3	—	—	13·1	—
Lysine	1·8	0·1	5·2	0·4	—	—	7·2	1·2
Arginine	13·2	1·5	119·0	5·9	264·0	53·0	32·2	2·2
Asparagine	—	—	28·8	4·0	58·3	9·9	40·8	T
Glutamine	—	—	18·5	1·0	53·1	—	15·2	—
Pipecolinic acid	—	—	1·0	4·5	T	T	T	T
Tryptophan	T	—	3·6	1·1	—	—	1·6	T
Proline	2·2	2·2	80·7	24·6	315·0	231·0	11·0	6·7
Total	17·3	3·8	526·4	129·5	1034·9	464·0	407·6	80·0

* Sample reference number (Table 8).

Mustard plants (Table 3) do, however, show a progressive decrease in arginine with improved iron status. This has been recorded by Steinberg (1955) for tobacco plants.

Table 6. *Free amino acids of old and young grass and beech leaves*

—, Not detected; T, detected but too weak for determination.

	Concn. (mg./100 g. of fresh leaf)			
	Grass (39)* (young)	Grass (42)* (old)	Beech leaves (40)* (young)	Beech leaves (44)* (old)
Aspartic acid	9·8	—	32·0	5·9
Glutamic acid	33·6	—	21·3	23·1
Serine	5·6	—	3·2	1·2
Threonine	1·5	—	0·4	0·5
Alanine	14·6	—	4·7	2·5
Tyrosine	1·1	—	5·7	0·7
γ-Aminobutyric acid	5·3	—	7·4	2·7
Valine	4·3	—	1·3	1·0
Leucine–isoleucine	2·6	—	1·6	0·6
Phenylalanine	1·7	—	1·4	1·7
Histidine	—	—	1·9	—
Lysine	0·3	—	0·8	T
Arginine	0·6	—	0·4	—
Asparagine	1·3	—	23·6	1·4
Glutamine	—	—	0·8	0·4
Tryptophan	—	—	0·8	—
Proline	24·2	3·6	6·6	6·6
Total	106·5	3·6	113·9	48·3

* Sample reference number (Table 8).

The increase in arginine in the albino leaves of variegated *Bougainvillea* is, however, quite striking (Table 4). Here a general increase of all amino acids is not apparent; in fact amino acids such as glutamic acid and alanine were higher in the green leaves. Arginine does not, however, show an increase in all variegated leaves (Euler & Burstrom, 1933).

The immature leaves of the various varieties of cabbage (Table 5) also showed large accumulations of arginine. It is, however, apparent that immature or young tissues contain larger quantities of most amino acids, as can be seen in young and old grass and also in beech leaves (Table 6). The process of ageing of leaves thus appears to be accompanied by a progressive disappearance of free amino acids. This is in apparent contradiction to the results of Yemm (1956) who reported an increase of amino acids in senescent leaves. It must be remembered, however, that final death of a tissue such as a leaf is preceded by a 'climacteric bump' in which both respiration rate and free amino acids increase.

The results obtained with chlorotic leaves of virus-infected sugar beet together with chlorotic leaves of iron-toxic mustard (Table 7) are in apparent disagreement with the results discussed above and with those of other workers. Reference to Table 8, however, shows that whereas the previous results are for leaves of which the chlorotic ones show higher ratios of phosphorus to iron and

Table 7. *Free amino acids of healthy and virus-infected leaves of sugar beet and healthy and 'iron-toxic' mustard leaves*

Gr, Green; Y, yellow; —, not detected; T, detected but too weak for determination.

	Concn. (mg./100 g. of fresh leaf)					
	Sugar beet (27)*		Sugar beet (48)*		Mustard (28)*	
	(Gr)	(Y)	(Gr)	(Y)	(Gr)	(Y)
Aspartic acid	0·7	T	3·9	5·8	0·8	1·2
Glutamic acid	2·8	T	10·5	19·3	6·0	6·6
Serine	0·9	—	2·8	4·6	1·6	3·3
Threonine	0·9	T	0·9	2·6	2·0	2·7
Alanine	0·8	T	3·4	1·9	2·4	4·2
Tyrosine	0·9	0·5	11·7	10·1	0·6	0·3
γ-Aminobutyric acid	0·9	0·2	6·7	7·4	1·0	1·2
Valine	2·4	—	12·2	15·6	2·7	3·0
Leucine–isoleucine	3·3	—	15·8	13·8	2·8	2·2
Phenylalanine	3·4	0·5	17·7	7·1	0·7	0·6
Histidine	2·2	2·0	2·8	3·2	0·3	0·1
Lysine	0·6	0·1	3·7	1·4	0·3	0·3
Arginine	0·6	0·3	6·1	3·1	1·6	1·1
Asparagine	1·4	T	3·2	4·0	0·1	0·2
Glutamine	2·7	T	2·7	6·0	—	—
Pipecolinic acid	—	—	—	—	0·2	0·1
Tryptophan	2·6	1·9	9·9	10·7	0·6	T
Proline	1·0	—	6·8	7·3	7·2	7·4
Total	28·1	5·5	120·8	123·9	30·9	34·5

* Sample reference number (Table 8).

Table 8. *Analyses* (%) *of the ash of various plant leaves*

Y, Young; O, old; W, white; G, green; Chl, chlorotic. Sample numbers are given in parentheses.

		P	Fe	K	Ca	Na	Mg	P/Fe	K/Ca	Total amino acid	Ash (%)
Beech (40)	Y	5·72	0·153	21·1	7·3	0·7	3·6	37·4	2·89	113·9	4·4
Beech (44)	O	3·48	0·180	19·8	11·8	1·2	4·0	19·3	1·68	48·3	5·0
Bougainvillea (38)	W	2·68	0·283	33·1	8·6	0·2	1·4	9·5	3·85	356·7	20·0
	G	0·72	0·146	13·6	26·7	0·4	1·8	4·9	0·51	202·6	24·0
Bougainvillea (52)	W	2·81	0·111	29·2	7·7	0·2	2·2	25·3	3·79	371·0	19·2
	G	2·5	0·125	14·2	25·2	0·3	2·4	20·0	0·56	163·6	20·6
Bracken (23)	Chl	4·28	0·087	12·1	20·0	2·4	2·1	49·2	0·61	386·5	5·3
	G	3·76	0·048	13·9	21·6	4·8	2·0	78·3	0·64	1454·6	9·3
Cabbage (45)	W	6·49	0·070	34·7	4·6	0·9	2·0	92·7	7·54	17·3	9·7
(Greyhound)	G	2·82	0·076	16·1	18·6	0·7	1·8	37·1	0·87	3·8	18·5
Cabbage (53)	W	6·57	0·067	37·3	2·1	0·6	2·4	98·1	17·76	526·4	8·2
(Winningstadt)	G	3·65	0·058	18·7	16·7	0·8	1·7	62·9	1·12	129·5	12·0
Cabbage (61)	W	6·67	0·065	39·0	4·0	0·19	2·3	102·6	9·75	1034·9	8·0
(January King)	G	2·67	0·033	24·4	16·5	0·69	1·1	80·9	1·48	464·0	13·1
Cabbage (71) (Myate)	W	8·13	0·099	40·9	1·9	0·38	2·2	82·1	21·5	407·6	8·1
	G	1·78	0·033	14·9	15·9	2·2	1·5	53·9	0·9	80·0	12·0
Mustard (28)	G	3·11	0·050	10·6	24·1	0·2	4·6	62·2	0·44	30·9	—
	Chl	0·81	0·080	2·0	32·1	0·5	3·7	10·1	0·06	34·5	—
Spinach beet (50)	Chl	7·96	0·207	19·1	6·4	3·6	3·6	38·5	2·97	104·5	12·1
	G	3·20	0·058	29·6	2·9	5·7	3·5	55·2	10·21	170·6	12·9
Sugar beet (27)	G	1·11	0·046	20·5	5·1	17·1	5·6	24·1	4·02	28·1	10·1
	Chl	0·97	0·089	15·4	7·6	13·3	2·3	10·9	2·03	5·5	18·1
Sugar beet (48)	G	2·43	0·083	24·0	4·5	13·2	2·3	29·3	5·33	120·8	11·4
	Chl	1·73	0·110	23·3	6·0	12·2	2·4	15·7	3·88	123·9	12·5
Pea (43)	Chl	1·9	0·076	17·2	13·7	2·4	3·7	25·0	1·26	84·5	10·5
	G	2·3	0·023	24·0	13·5	2·7	3·9	100·0	1·77	160·1	11·1
Mustard (78)	A	7·98	0·081	20·1	11·7	2·4	3·2	98·5	1·72	—	11·3
	B	4·29	0·088	20·2	14·2	2·8	3·1	48·8	1·42	—	11·4
	C	3·66	0·098	20·2	15·8	2·5	3·4	37·3	1·27	—	12·2
	D	3·64	0·117	19·5	16·9	2·6	3·2	31·1	1·15	—	12·3
Kale (54)	G	4·52	0·074	26·6	6·8	2·6	1·7	61·08	3·91	—	8·2
	W	6·29	0·086	35·8	2·8	0·9	1·7	73·14	12·79	—	8·9

potassium to calcium, in the chlorotic sugar-beet leaves affected by virus yellows and in iron-toxic mustard the reverse is true, the chlorosis being of an iron-toxic type, and in such chlorotic leaves it would appear that free amino acids are little altered.

The above studies seem to indicate a close relationship between the mineral analysis of the leaf and its content of free amino acids, the latter being high when the phosphorus:iron ratio is high and declining as the phosphorus:iron ratio declines, whether this is brought about by improving the iron status or as an ageing effect. Such a relationship between the content of free amino acids and 'active' iron (i.e. iron extractable from dried leaves by N-HCl) in leaves was pointed out by Bennett (1945) and by Holley & Cain (1955). Though our understanding of the metabolism of amino acids within the plant cell is far from complete, their intimate relationship with the organic acids is known (Steward & Pollard, 1957). It is the purpose of the next paper to compare the changes in the organic acids with the changes in amino acids.

SUMMARY

1. The free amino acid content of healthy and chlorotic leaves has been studied. Iron-deficient chlorotic leaves contained enhanced amounts of free amino acids, even when the iron deficiency was induced by heavy metals or was due to genetical causes.

2. In iron-toxicity chlorosis, however, the content of free amino acids was not very different from that of healthy leaves.

3. It would appear that the free amino acid content of leaves depends upon their iron status, as reflected by the phosphorus:iron ratio.

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The Metabolism of Chlorotic Leaves

2. ORGANIC ACIDS

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DeKock (1956) has pointed out that chlorosis in plant leaves, whether induced by excess of lime, by iron deficiency, by toxicity of heavy metals or by genetical or virological factors, usually appears to be characterized by higher ratios of phosphorus to iron and of potassium to calcium than are found in the normal green leaf. The levels of some of the organic constituents in normal and chlorotic leaves have been examined to determine whether any consistent distortion of the normal pattern occurs in chlorotic leaves, irrespective of the cause of chlorosis.

In a study of the free amino acids of such chlorotic and healthy leaves of various plants, it was found that significantly larger amounts of free amino acids were associated with tissues in which higher phosphorus:iron and potassium:calcium ratios were found (DeKock & Morrison, 1958).

Various plant leaves with high potassium contents have been shown to contain large amounts of citric acid (Cooil, 1948; Iljin, 1951; Kurchatov, 1940), whereas leaves with high calcium contents contain either large amounts of oxalic acid (Olsen, 1939; Chandler, 1937; Scharrer & Jung, 1953, 1954; Pierce & Appleman, 1943) or of malic acid (Cooil, 1948). McGeorge (1949) found a marked correlation between iron extractable with N-HCl ('active iron'), citric acid and oxalic acid in citrus and deciduous fruit-tree leaves, chlorotic leaves containing less active iron, more citric acid and less oxalic acid than green leaves. Determinations of the organic acids of healthy and chlorotic leaves used in the previous study (DeKock & Morrison, 1958) were therefore made to see if the citric acid:(malic plus oxalic acid) ratios would vary as the phosphorus:iron and potassium:calcium ratios.

METHODS

Samples were those used in the investigation of the free amino acids of chlorotic leaves (DeKock & Morrison, 1958). The effluent from the Zeo-Karb 225 column was run through an Amberlite-IR4B column, which absorbed the organic acids. The effluent was discarded. The organic acids were then eluted from the column with 200 ml. of aq. N-NH_3 soln., and the eluate was concentrated to a small volume by evaporation on a steam bath and made up to 10 ml. A portion of this was then used for analysis by partition chromatography.

Partition chromatography of plant organic acids. The non-volatile organic acids were separated by partition chro-