REFERENCES

Allsopp, A. (1948). *Nature, Lond.*, **161**, 833.
Bennett, J. P. (1945). *Soil Sci.* **60**, 91.
Bray, G. H., Thorpe, W. V. & White, K. (1950). *Biochem. J.* **46**, 271.
Cain, J. C. & Holley, R. W. (1955). *Proc. Amer. Soc. hort. Sci.* **65**, 49.
Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
DeKock, P. C. & Hall, A. (1955). *Plant Physiol.* **30**, 293.
Demetriades, S. D. (1956). *Nature, Lond.*, **177**, 95.
Diener, T. O. & Dekker, C. A. (1954). *Phytopathology*, **44**, 643.
Drever, T. O. & Larv, N. O. (1954). *Phytopathology*, **44**, 486.
Euler, H. von & Burstrom, D. (1933). *Hoppe-Seyl. Z.* **215**, 47.
Hird, F. J. R. (1949). *Aust. J. Sci.* **11**, 170.
Holley, R. W. & Cain, J. C. (1955). *Science*, **121**, 172.
Iljin, W. S. (1951). *Plant & Soil*, **3**, 239.
Laloraya, M. Govindjee, M. & Raja Rao, T. (1955). *Curr. Sci.* **24**, 203.
McKee, H. S., Nestel, L. & Robertson, R. N. (1955). *Aust. J. biol. Sci.* **8**, 467.
Mansford, K. & Raper, R. (1954). *Nature, Lond.*, **174**, 314.
Meinhard, J. E. & Hall, N. F. (1950). *Analyt. Chem.* **22**, 344.
Petronici, C. (1956). *Ann. Sper. agr.* **x**, 841.
Possingham, J. V. (1956). *Aust. J. biol. Sci.* **9**, 539.
Shibamoto, T., Shoji, R. & Tagawa, K. (1955). *Bull. Tokyo Univ. For.* **48**, 209.
Steinberg, R. A. (1955). *Inorganic Nitrogen Metabolism.* Ed. by McElroy, W. D. & Glass, B. Baltimore: The Johns Hopkins Press.
Steward, F. C. & Pollard, J. K. (1957). *Annu. Rev. Pl. Physiol.* **8**, 65.
Steward, F. C., Wetmore, R. H., Thompson, J. F. & Nitsch, J. P. (1954). *Amer. J. Bot.* **41**, 123.
Vavrich, I. (1952). *Chem. Listy*, **46**, 453.
Williams, R. J. & Kirby, H. (1948). *Science*, **107**, 481.
Yemm, E. W. (1956). *Ciba Foundation Colloq., Ageing*, **2**, 202.

The Metabolism of Chlorotic Leaves

2. ORGANIC ACIDS

BY P. C. DEKOCK AND R. I. MORRISON
The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen

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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-

matography on a column of silica gel, a gradient-elution technique being used, and determined by titration with standard alkali.

Silica gel. This was prepared from sodium metasilicate essentially as described by Neish (1949), but in order to obtain sufficiently high flow rates on the column it was necessary before drying the gel to remove the finer particles by suspending the material repeatedly in water and decanting the supernatant liquid after the coarser material had settled.

Solvents. Solvents used were mixtures of $CHCl_3$ and technical grade *tert.*-amyl alcohol (2-methylbutan-2-ol) saturated with 0·1 N-H_2SO_4. Before use, the $CHCl_3$ was washed with water to remove ethanol, and the *tert.*-amyl alcohol was shaken with solid NaOH (5 g./l.) and redistilled to remove traces of acidic material. The mixtures generally employed were 2% and 50% (v/v) $CHCl_3$ in *tert.*-amyl alcohol (afterwards referred to as CA2 and CA50 respectively); these were equilibrated with 0·1 N-H_2SO_4 and passed through filter paper to remove suspended water droplets.

Preparation of column. The silica-gel column was contained in a glass tube 25 cm. long and 1·2 cm. internal diameter, constricted to 0·4 cm. at the lower end and having a B19 standard ground-glass joint at the upper end. Dry silica gel (4 g.) was mixed with 3·5 ml. of 0·1 N-H_2SO_4 and slurried with 20 ml. of $CHCl_3$. A small plug of cotton wool was packed into the bottom of the tube and the slurry was poured in. Excess of $CHCl_3$ was allowed to drain and any gel adhering to the tube above the liquid level was washed down with a little $CHCl_3$. The gel was then packed down by inserting a circle of filter paper with a diameter slightly greater than that of the tube and pressing down with a stainless-steel plunger which was a sliding fit for the tube.

Application of sample. A suitable volume (usually 0·5–2·0 ml.) of the sample solution was made just alkaline to thymol blue and evaporated to dryness on a steam bath. The residue was cooled and dissolved in 0·1 N-H_2SO_4 (0·5 ml.) and mixed with 0·7 g. of dry silica gel to give a free-flowing powder, which was transferred to the top of the column. It was washed down and slurried with a small volume (5 ml.) of $CHCl_3$ and packed under a filter-paper disk. The tube was then filled with solvent mixture CA2.

Gradient elution. The simple arrangement used for gradient elution, although evolved independently, is similar to that described by Kellie & Wade (1957), when a solvent gradient is applied to the column from two reservoirs, a recipient and a donor of suitable cross-sectional areas, connected by a siphon, the column being fed from the recipient reservoir which is stirred magnetically. Such a system delivers solvent of composition

$$C = C_2 - (C_2 - C_1)(1 - V)^{A_2\rho_1/A_1\rho_2},$$

where A_1 and A_2 are the respective areas of cross-section of recipient and donor reservoirs, C_1 and C_2 are the initial composition of the solvents, ρ_1 and ρ_2 are the densities of the solvents and V is the fraction of the total volume delivered (cf. Bock & Ling, 1954).

In practice the reservoirs were two glass bottles, of capacities 500 and 250 ml. such that the ratio A_2/A_1 was 0·637. The two solvents CA2 (C_1) and CA50 (C_2) had densities at 18° of 1·460 (ρ_1) and 1·145 (ρ_2) respectively, and the total volume of solvent was 500 ml., comprising 278 ml. of CA2 and 222 ml. of CA50. The bottles were connected by a siphon tube, and another siphon conducted the mixed solvent to the top of the column. By mounting the reservoirs about 80 cm. above the column sufficient pressure was obtained to give a suitable rate of flow (approx. 25 ml./hr.). The rate was controlled when necessary by a valve consisting of a stainless-steel rod sliding in a closely fitting glass capillary (Partridge & Brimley, 1951) fitted to the lower end of the tube. The effluent was collected in 4·5 ml. fractions by means of a siphon measuring device and an automatic fraction collector.

Titration of fractions. Fractions were titrated with 0·02 N-NaOH with thymol blue as indicator, by means of a microburette, the tubes being shaken vigorously during the titration.

Results obtained for the separation of a synthetic mixture of acid are shown in Fig. 1*A*, where the titre for each fraction is plotted against the fraction number. It can be seen that the technique gave a good separation with well-defined peaks of fumaric, succinic, malic and citric acid. The pairs, malonic acid–*trans*-aconitic acid and oxalic acid–*cis*-aconitic acid, coincided under these conditions, although it was later found that they could be separated from each other and from the acids previously mentioned by the use of 0·1 N-H_2SO_4 in 50% glycerol–water as the stationary phase (Fig. 1*B*). All results given were obtained with aq. 0·1 N-H_2SO_4 as the stationary phase. Recovery of individual acids was always better than 95%.

Oxalic acid. Because of its volatility, oxalic acid was determined by Baker's method as outlined by Palmer (1955) on separate samples. Although determinations by the silica-gel column frequently agreed very well with the chemical method, it could not always be relied upon and separate determinations were usually made.

Identification of acids. After elution from the silica-gel column, fractions were identified by paper chromatography (Buch, Montgomery & Porter, 1952). It was not always possible to determine the identity of acid in the peak with maximum about fraction 39, although both malonic and *trans*-aconitic acid could be present and have in a few instances been identified, as is indicated in the tables of results.

Total acidity. In a number of samples, the total organic acidity was determined by Palmer's (1955) method. It was found that Deacidite FF (50–100 mesh) could be substituted for Dowex 1, with equally good results, the procedure remaining unaltered. Values obtained by this method agreed very well with those obtained by the gradient-elution technique.

RESULTS

Comparison of extraction procedures. The reproducibility of the extraction was tested on mustard leaves grown in a standard nutrient medium with 2 p.p.m. of iron as ferric ethylenediaminetetraacetate. The procedure is described in the previous paper (DeKock & Morrison, 1958), the eluted acid from the IR4b columns being separated by partition chromatography on silica gel. Results are given in Table 1.

As reproducibility was deemed satisfactory, the acetone-extraction procedure was adhered to.

Iron-deficiency chlorosis. Mustard plants grown in nutrient culture with 0·1, 0·5, 2·5 and 12·5 p.p.m. of Fe as the *NN'*-ethylenebis-(2-*o*-hydroxyphenyl)-glycine chelate were harvested at the flower-bud stage. The mature leaves were separated and organic acids estimated as before. Results are shown in Table 2.

Genetical chlorosis. Colourless (albino) and green leaves were harvested from a plant of *Bougainvillea glabra variegata*. The analysis of the organic acids are given in Table 3. The leaves of a variegated variety of kale grown in a garden soil were harvested

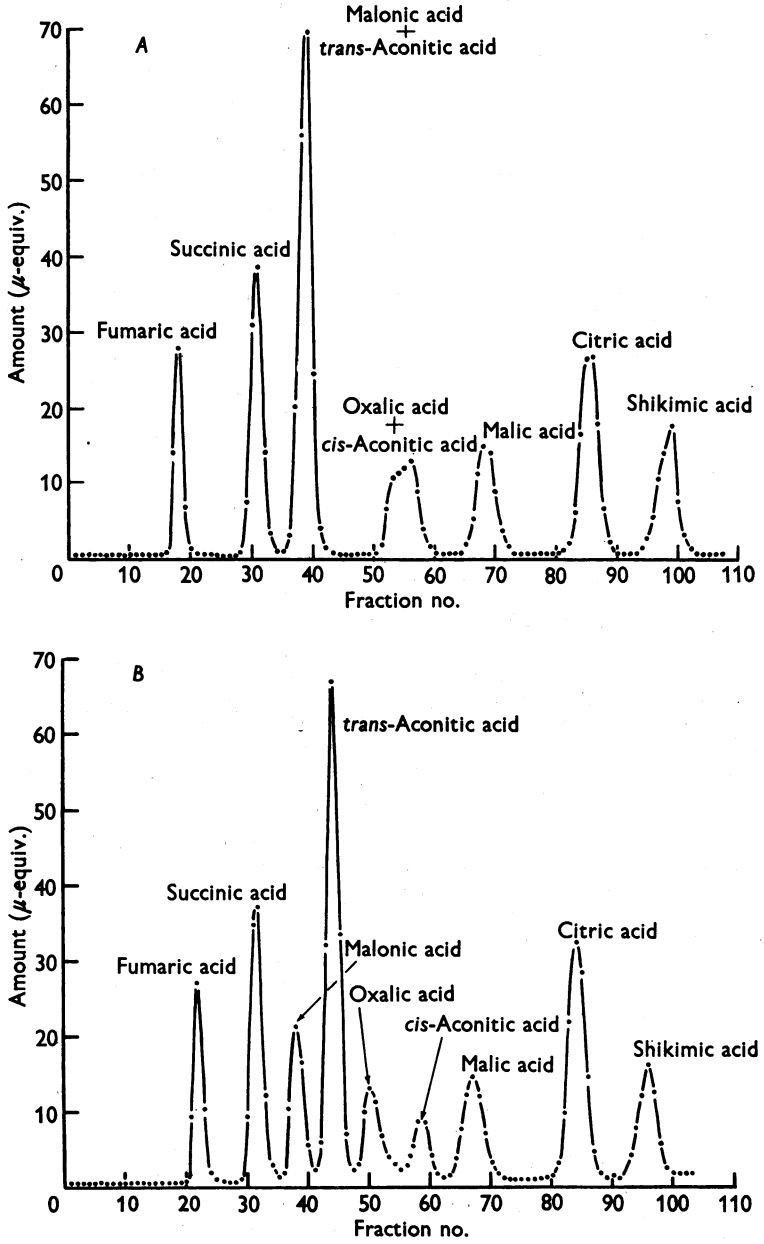

Fig. 1. Separation of synthetic mixtures of organic acids by partition chromatography on silica gel. Acid (μ-equiv.) in each fraction (4·5 ml.) is plotted against the fraction number. *A*, Separation obtained with 0·1 N-H_2SO_4 as stationary phase; *B*, separation with 0·1 N-H_2SO_4 in glycerol–water (1:1, v/v) as stationary phase.

and the green areas cut away from the chlorophyll-free areas.

Metal toxicity. Peas grown in a soil to which a considerable quantity of nickel had been added were severely chlorotic. The organic acid pattern of these leaves compared with that of healthy pea leaves is given in Table 4.

Ageing. Organic acids of the inner white leaves and the outer green leaves of four varieties of cabbage are shown in Table 5, and the analyses of young and old leaves of bracken are shown in Table 6.

Virological chlorosis. Leaves of sugar beet infected with virus yellows, and healthy leaves, were obtained from Cambridgeshire. The organic acid analysis of these leaves is shown in Table 7.

Mineral constituents. The analyses of the ash of the various leaves utilized in these organic acid studies are given in Table 8 of the preceding paper (DeKock & Morrison, 1958).

Table 1. *Organic acids of mustard* (70)* *leaves*

Samples A and B were extracted with acidified 70% acetone and sample C with boiling water. Oxalic acid was determined chemically, the remainder by silica-gel chromatography. For further details see text.

Acid	Content (m-equiv./kg. of leaf) A	B	C
Fumaric	0·6	0·5	0·7
Succinic	1·8	1·7	2·1
Unknown†	12·9	10·5	9·7
Oxalic	9·0	9·0	—
Malic	112·7	118·3	118·7
Citric	33·9	28·9	33·4
Citric: (malic + oxalic) ratio	0·30	0·24	0·28

* Sample reference number.
† Mainly malonic acid found.

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

Acid	Content (m-equiv./kg. of leaf) A	B	C	D
Fumaric	1·7	2·0	1·7	0·9
Succinic	2·6	1·8	1·9	2·1
Unknown†	11·6	11·7	11·5	11·0
Malic	38·9	58·4	68·1	78·5
Citric	19·5	23·5	23·5	26·8
Total acidity	74·3	97·4	106·7	119·3
Citric: malic ratio	0·50	0·40	0·35	0·34

* Sample reference number.
† Mainly malonic; identification by infrared spectrum.

Table 3. *Organic acids of albino* (*W*) *and green* (*G*) *leaves in genetical chlorosis*

Acid	Content (m-equiv./kg. of leaf) *Bougainvillea* (52)* (G)	(W)	Kale (54)* (G)	(W)
Fumaric	0·6	0·1	3·75	2·65
Succinic	1·0	0·4	5·5	3·05
Unknown†	1·9	0·2	1·85	1·65
Oxalic	93·8	25·0	0·9	0·7
Malic	1·9	1·0	57·65	24·4
Citric	1·5	4·1	14·9	34·1
Total acidity	100·7	29·8	84·55	66·55
Citric: (malic + oxalic) ratio	0·02	0·16	0·25	1·36

* Sample reference number (Table 8, DeKock & Morrison, 1958).
† Probably contains *trans*-aconitic acid or malonic acid or both.

Table 4. *Organic acids of leaves from normal* (*G*) *and 'nickel-toxic' (Chl) peas* (43)*

Acid	Content (m-equiv./kg. of leaf) (G)	(Chl)
Fumaric	8·8	6·5
Succinic	13·5	17·0
Unknown†	1·1	1·0
Oxalic	0·3	0·7
Malic	59·0	42·7
Citric	30·7	64·5
Total acidity	113·4	132·4
Citric: (malic + oxalic) ratio	0·51	1·49

* Sample reference number (Table 8, DeKock & Morrison, 1958).
† Probably contains *trans*-aconitic acid or malonic acid or both.

DISCUSSION

Increasing the iron supply to mustard plants in nutrient culture causes the phosphorus in the leaf ash to decrease, whereas the iron and calcium contents increase (Table 8, DeKock & Morrison, 1958). The total acidity of the leaf also increases owing to a considerable increase in malic acid and to a slight increase in citric acid. It will thus be seen that the citric acid:malic acid ratio is highest in the severely iron-deficient leaves and progressively declines as the iron status improves (Table 2). Both the phosphorus:iron and the potassium:calcium ratios show similar trends (Table 8, DeKock & Morrison, 1958).

The albino areas of both *Bougainvillea* and kale tissues, which are chlorophyll-free for genetical reasons, are found to contain more phosphorus and potassium and very much less calcium than the

Table 5. *Organic acids of the outer green (G) and inner white (W) leaves of four varieties of cabbage*

Acid	Content (m-equiv./kg. of leaf)							
	Greyhound (45)*		Winningstadt (53)*		January King (61)*		Myate (71)*	
	(G)	(W)	(G)	(W)	(G)	(W)	(G)	(W)
Fumaric	0·7	0·5	5·0	—	4·0	3·9	1·1	0·5
Succinic	1·5	0·4	22·7	1·0	1·0	2·0	0·9	1·1
Unknown†	0·6	1·35	4·5	5·9	1·3	8·0	0·8	7·6
Oxalic	0·2	0·4	2·8	0·9	—	0·4	—	—
Unknown	—	—	Trace	Trace	—	1·6	Trace	0·6
Malic	55·7	17·2	100·7	22·6	104·2	33·8	124·8	20·8
Citric	15·5	17·8	21·3	24·1	12·1	28·5	24·2	20·4
Total acidity	74·2	37·65	157·0	54·5	122·6	78·2	151·8	51·0
Citric: (malic + oxalic) ratio	0·28	1·01	0·21	1·03	0·12	0·83	0·19	0·98

* Sample reference number (Table 8, DeKock & Morrison, 1958).
† Probably contains *trans*-aconitic or malonic acid or both.

Table 6. *Organic acids of young (Y) and mature (O) bracken fronds*

Acid	Content (m-equiv./kg. of leaf)	
	Y (72)*	O (77)*
Fumaric	0·4	0·8
Succinic	1·5	0·8
Unknown†	2·4	1·8
Oxalic	0·2	0·2
Malic	4·8	7·0
Citric	4·5	4·4
Shikimic	6·3	4·2
Total acidity	20·1	19·2
Citric: (malic + oxalic) ratio	0·90	0·61

* Sample reference number (Table 8, DeKock & Morrison, 1958).
† Probably contains *trans*-aconitic and malonic acid.

Table 7. *Organic acids of healthy (G) and chlorotic (Chl) leaves of sugar beet* (48)* *infected with virus yellows*

Acid	Content (m-equiv./kg. of leaf)	
	G	Chl
Fumaric	2·2	1·4
Succinic	3·7	2·6
Unknown†	4·7	3·7
Oxalic	150·0	270·3
Malic	3·4	3·4
Citric	15·0	19·8
Total acidity	179·0	301·2
Citric: (malic + oxalic) ratio	0·10	0·07

* Sample reference number (Table 8, DeKock & Morrison, 1958).
† Malonic acid and *trans*-aconitic acid were identified in these fractions by paper chromatography.

corresponding green areas (Table 8, DeKock & Morrison, 1958). In both (Table 3), the citric acid content of the albino-leaf tissue is greater than that of the green-leaf tissue, whereas the reverse is true for the malic acid content. The oxalic acid content of both green and white tissue of kale leaves is quite low, but in *Bougainvillea* it is considerable and nearly four times as high in the green as in the albino. The total acids are also higher in the green tissues of *Bougainvillea*, but this difference is not so apparent in the variegated kale.

The iron-deficiency induced in pea plants (Table 8, DeKock & Morrison, 1958) by excess of nickel is accompanied by higher amounts of citric acid in the chlorotic leaves, whereas the malic acid is lower in these leaves compared with normal green leaves (Table 4).

The increase of malic acid which occurs when leaves age is seen in Table 5. The immature leaves of the cabbage 'heart' contain less total acid and much less malic acid than the green leaves. Citric acid is seen to be greater or only slightly less in the immature leaves than in the green leaves, so that the citric acid: malic acid ratio is once again high in leaves having high phosphorus: iron and potassium: calcium ratios. A similar situation can be seen in young and old bracken fronds (Table 6). In this instance, however, it is evident that shikimic acid makes a major contribution to the total acidity, and appears to be present in greatest amount in the younger leaves, thus behaving much like citric acid.

Sugar-beet leaves infected with virus yellows contain more oxalic acid than do the healthy green leaves (Table 7). Citric acid is slightly increased and the remaining acids are somewhat reduced. This result appears to contradict the previous findings for chlorotic leaves, but in this instance it will be seen that the chlorotic leaves contain more calcium and less potassium and phosphorus than the green leaves (Table 8, DeKock & Morrison, 1958). Thus the citric acid: (malic + oxalic acid) ratio is lower in the chlorotic leaves, as are also the phosphorus: iron and potassium: calcium ratios. Similarly, mustard leaves (sample 28, Table 8, DeKock & Morrison, 1958), which were chlorotic

owing to iron toxicity, were found to contain four times the malic acid content of the healthy green leaves, whereas the citric acid in these leaves was too small to be accurately determined.

Whereas Pierce & Appleman (1943), who estimated oxalic, malic and citric acid by chemical means, frequently found that 70–80% of the total acidity of the leaf could not be accounted for by these acids, no such discrepancies were found in the present studies, apart from skikimic acid in bracken and broad bean. Oxalic, malic or citric acid appears to be the major constituent. Fractions for fumaric, succinic, malonic or *trans*-aconitic acid were never present to any great extent. The *trans*-aconitic acid in these extracts probably arose from the conversion of *cis*-aconitic acid (Krebs & Eggleston, 1944).

From the results presented it would appear that a relation exists in leaves between the phosphorus:iron ratio, the potassium:calcium ratio and the citric acid:(malic+oxalic acid) ratio. The total organic acidity also appears to vary inversely with the total phosphorus in the leaf, as has been noted by others (Cooil, 1948; Vavrich, 1954; Ward & Petrie, 1940). Although the amount of carbonate has not been estimated in these leaves, its contribution to the cation–anion balance of the leaf is not very great (Scharrer & Jung, 1957).

In the previous part of this study it was shown that the free amino acids of leaf tissue were higher when the phosphorus content of the leaf was high (DeKock & Morrison, 1958). They therefore vary inversely as the total organic acids.

SUMMARY

1. Organic acids in healthy leaves and leaves chlorotic due to iron deficiency, heavy-metal toxicity, ageing, virus-infection and genetical constitution were quantitatively studied.

2. All types of chlorotic leaves contained more citric acid relative to malic or oxalic acid, except in virus yellows in sugar beet where the reverse is true.

3. The citric acid:(malic+oxalic acid) ratio of leaves appears to vary as the phosphorus:iron and potassium:calcium ratios. The total amount of organic acid also varied inversely as the phosphorus content of the leaf.

4. Green leaves apparently contain large quantities of either malic or oxalic acid.

REFERENCES

Bock, R. M. & Ling, N. (1954). *Analyt. Chem.* **26**, 1543.
Buch, M. L., Montgomery, R. & Porter, W. L. (1952). *Analyt. Chem.* **24**, 489.
Chandler, R. F. (1937). *J. agric. Res.* **55**, 393.
Cooil, B. J. (1948). *Plant Physiol.* **23**, 403.
DeKock, P. C. (1956). *Ann. Bot., Lond.*, N.S., **20**, 133.
DeKock, P. C. & Morrison, R. I. (1958). *Biochem. J.* **70**, 266.
Iljin, W. S. (1951). *Plant & Soil*, **3**, 339.
Kellie, A. E. & Wade, A. P. (1957). *Biochem. J.* **66**, 196.
Krebs, H. A. & Eggleston, L. V. (1944). *Biochem. J.* **38**, 21.
Kurchatov, P. A. (1940). *Khim. ref. Zh.* **2**, 54.
McGeorge, W. T. (1949). *Soil Sci.* **68**, 381.
Neish, A. C. (1949). *Canad. J. Res.* **B27**, 6.
Olsen, C. (1939). *C.R. Lab. Carlsberg*, **23**, 101.
Palmer, J. K. (1955). *Bull. Conn. agric. Exp. Sta.*, no. 589.
Partridge, S. M. & Brimley, R. C. (1951). *Biochem. J.* **48**, 313.
Pierce, E. C. & Appleman, E. O. (1943). *Plant Physiol.* **18**, 224.
Scharrer, K. & Jung, J. (1953). *Z. PflErnähr. Dung.* **62**, 63.
Scharrer, K. & Jung, J. (1954). *Z. PflErnähr. Dung.* **66**, 1.
Scharrer, K. & Jung, J. (1957). *Z. PflErnähr. Dung.* **76**, 27.
Vavrich, L. (1954). *Chem. Listy*, **48**, 442.
Ward, E. D. & Petrie, A. H. K. (1940). *Aust. J. exp. Biol. med. Sci.* **18**, 21.

The Uptake and Metabolism of Amino Acids by Slices of Carrot

By L. M. BIRT*
Department of Biochemistry, University of Melbourne, Australia
and F. J. R. HIRD
Departments of Agriculture and Biochemistry, University of Melbourne, Australia

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It has been shown that several amino acids disappear from a solution which is in contact with slices of carrot tissue (Birt & Hird, 1956). Further, Webster (1954), Rheinhold & Powell (1956) and Kursanov (1956) have studied the uptake of certain amino acids by various plant tissues. However, actual evidence for the transport of amino acids into plant tissues against a concentration gradient is limited to that provided for the accumulation of histidine by carrot slices (Birt & Hird, 1956).

In an investigation of the mechanism responsible for the uptake of amino acids by carrot slices, it is essential to assess the contribution made by the metabolic destruction of these compounds to the

* Present address: Department of Biochemistry, University of Oxford.