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THE ORGANIC ACIDS IN BANANA LEAVES

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Abstract—The concentration of total non-volatile organic acids in eleven banana leaf samples was found to be 93·2 (± 10·2) m-equiv./100 g dry weight; with a twelfth sample containing only 57·5 m-equiv. The leaf tissue represented three cultivars of banana and various stages of plant maturation, grown either in the tropics or the greenhouse. Oxalic acid constituted 83 per cent of the total acidity, malic acid about 2 per cent and "citric peak" acidity (citric acid plus certain phosphates) about 9 per cent. The remaining acidity consisted of a series of acids, each present in trace quantities. These included glutamic, aspartic, glutaric, glyceric, glycolic, glyoxylic, shikimic, succinic, pyruvic, malonic, and α-ketoglutaric acids. At least nine trace acids, including five keto acids, remain unidentified. Approximately 50 per cent of the oxalate in field-grown leaves is in water-soluble form; the greenhouse leaves contained 28 per cent soluble oxalates. The results are compared with those obtained with the leaves of temperate-zone plants.

INTRODUCTION

THE non-volatile organic acids are known to play a key metabolic role in the leaves of temperate-zone plants. Relatively little is known about the occurrence or role of these acids in tropical leaves.

The banana leaf exhibits the rapid growth so typical of tropical plants. It is additionally interesting because of its exceptional size. The leaves are typically 2–4 m long, with a 2–3 m^2 surface area for the largest leaves.[1] A banana plant normally produces 40–45 leaves in the 9-month growing period prior to flowering. It would be interesting to know more about the metabolic systems involved in such luxuriant growth. The present study was undertaken to identify and determine the organic acids of the banana leaf.

RESULTS

Identification of the Acids

Figure 1 shows the separation by ion-exchange chromatography of the organic acids from each fraction. The individual acids were then isolated from appropriate fractions by paper, silica gel or ion-exchange chromatography. Confirmation of their identity was obtained in most cases by specific tests (Table 1) and by comparison with authentic acids in silica gel chromatography. Glutaric and malonic acids were identified only by comparison with authentic samples in the three chromatographic systems.

The keto acids were isolated as their 2,4-dinitrophenylhydrazones from a separate sample of the dried leaf tissue.[7] Chromatography of the hydrazones[7] and of the amino acids[2] resulting from their hydrogenation revealed the presence of α-ketoglutaric, pyruvic and glyoxylic acids plus five unknown keto acids.

[1] A. F. SKUTCH, *Botan. Gaz.* **84**, 337 (1927).

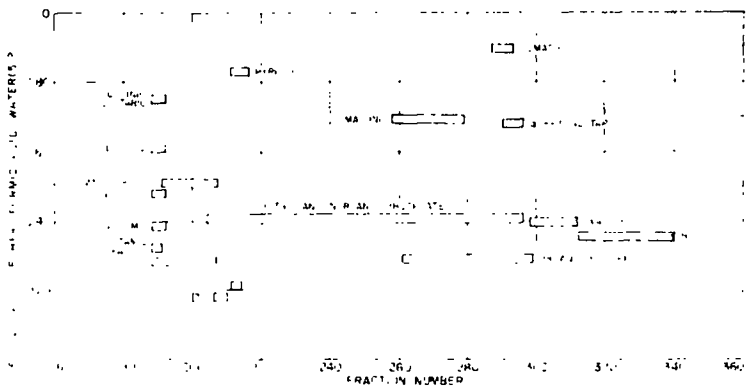

FIG. 1. DISPLACEMENT SEPARATION OF THE NON-VOLATILE ORGANIC ACIDS OF BANANA LEAF

Organic acids from 280 g dried tissue displaced with 0·1 N HCl from Dowex 1, × 10, 100–200 mesh, acetate form coupled columns of decreasing volume (1·9 × 8 cm, 1·2 × 6 cm, 0·9 × 5 cm, 0·6 × 3 cm). Fraction volume, 1·0 ml, flow rate, 0·2 ml/min. Aliquots of each fraction were assayed by paper chromatography. The bars are plotted to represent the acids found in each fraction versus their R_f values in the solvent employed.

TABLE 1. IDENTIFYING TESTS APPLIED TO THE ORGANIC ACIDS IN BANANA LEAF TISSUE*

Acid	Criteria of identity
Glutamic and aspartic	Two-dimensional chromatography and color test with ninhydrin-dicyclohexylamine.[2]
Shikimic	M.p. 191 °; m.p. authentic acid 190–191 ° with some sublimation; mixed m.p. 190–192 °; red color with aniline.[3]
Glyceric	Blue color with naphthoresorcinol-H_2SO_4;[4] calcium glycerate m.p. 137 ° with no depression of mixed m.p.
Glycolic	Red color with 2,7-dihydroxynaphthalene-H_2SO_4;[4] brown color with naphthoresorcinol-H_2SO_4.[4]
Succinic	Sublimation starting at 135 °, complete sublimation at 160 ° for both authentic and isolated acids.
Malic	M.p. 102 °, m.p. authentic acid 101 °; mixed m.p. 101 °, yellow color with 2,7-dihydroxynaphthalene-H_2SO_4;[4] gas chromatography of methyl ester derivative on diethylene glycol succinate, column temp. 125 °, flow rate 50 ml/min. Identical retention volume (510 ml) for both isolated and authentic acid.
Citric	Pentabromoacetone derivative;[5] m.p. 73–74 °, m.p. authentic sample 72–73.5 °, mixed m.p. 73–74 °.
Pyruvic	2,4-Dinitrophenylhydrazone derivative,[6] m.p. 218–220 °, m.p. of authentic sample 219–220 °; mixed m.p. 217–219 °.
α-Ketoglutaric	2,4-Dinitrophenylhydrazone derivative,[6] hydrogenation and chromatography in amino acid system.[2]
Fumaric	Sublimation of authentic sample started at 145 °, completely sublimed at 202 °; isolated sample started at 146 °, completely sublimed at 199 °.
Oxalic	Conversion to glycolic acid;[4] sublimation of isolated and authentic acid started at 95 °, completely sublimed at about 140 °.

* All melting and sublimation points were determined on a Fisher–Johns apparatus and are uncorrected.

[2] T. L. HARDY, D. O. HOLLAND and J. H. C. NAYLER, *Analyt. Chem.* **27**, 971 (1955).
[3] S. YOSHIDA and M. HASEGAWA, *Arch. Biochem. Biophys.* **70**, 377 (1957).
[4] F. FEIGL, *Spot Tests in Organic Analysis* (6th Ed.), pp. 377–380, Elsevier, N.Y. (1960).
[5] R. ROPER and T. S. MA, *Microchem J.* **1**, 245 (1957).
[6] P. J. ELVING and R. E. VAN ATTA, *Analyt. Chem.* **26**, 295 (1954).
[7] A. MEISTER and P. A. ABENDSCHEIN, *Analyt. Chem.* **28**, 171 (1956).

Quantitative Determination of the Organic Acids

The primary purpose of this phase of the study was to estimate the mean organic acid content of the banana leaf. This was accomplished by analysis of a series of twelve leaf samples representing three varieties, green house vs. field-grown plants, and several stages in

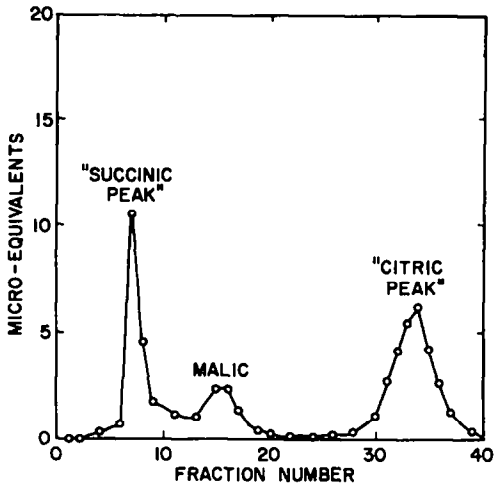

FIG. 2. ION-EXCHANGE SEPARATION OF THE NON-VOLATILE ORGANIC ACIDS OF BANANA LEAF

Dowex 1, × 10, 100–200 mesh, acetate form, 0·6 × 4·0 cm. Gradient elution, 3·5 N formic acid feeding into mixing chamber initially containing 200 ml of water, flow rate, 2·0 ml/min; fraction volume 2·0 ml. Sample—water extract of 0·5 g dried leaf. See under Results for identity of acids included in "succinic peak" and "citric peak".

the growth of the plant. Since we were seeking only gross differences in the organic acid content of the samples, no attempt was made to separate all of the acids present. Figure 2 shows a typical separation of the organic acids and the results for all the samples are summarized in Table 2.

TABLE 2. ORGANIC ACID CONTENT OF BANANA LEAF

Variety and where grown:	Cv. Gros Michel, greenhouse grown						Cv. Gros Michel, field grown		Cv. Lacatan, field grown			Cv. Lidi, field grown
Age of plants (months):	4	7	9	12	15	20	4	7	7	12	20	~ 9
Organic acids												
Malic	1·2*	1·2	1·3	1·9	1·2	1·3	2·4	5·4	1·9	2·2	2·4	1·5
"Citric peak"	7·0	6·0	8·0	10·6	6·1	6·6	6·5	16·7	10·5	7·7	13·4	5·3
"Succinic peak"	3·7	3·0	2·9	4·4	3·6	3·4	9·9	3·7	4·7	5·9	4·5	5·0
Total oxalate	89·5	72·0	68·7	40·6	64·7	80·5	89·3	66·8	79·9	89·8	75·4	82·5
H_2O soluble oxalate	22·5	26·5	18·5	2·0	15·6	20·7	52·6	30·0	36·8	40·7	28·4	46·0
Total	101·4	82·2	80·9	57·5	75·6	91·8	108·1	92·6	97·0	105·6	95·7	94·3

* Results as m-equiv./100 g dry weight.

Paper chromatography showed the "succinic peak" to include succinic, glycolic, glyceric, shikimic, glutamic and aspartic acids. The malic acid peak was predominantly malic acid, but sometimes contained traces of two other unidentified acids. The "citric peak" included traces of malonic acid and always included significant amounts of phosphates. Oxalic acid was determined separately in aliquots of water extracts and in acidified extracts. No attempt was made to obtain quantitative data on the keto acids.

DISCUSSION

Our results indicate that there are twenty-four organic acids which occur in the banana leaf at concentrations of 0·004 m-equiv./100 g dry weight or greater. Although each of the fifteen identified acids has been found in the leaves of at least one other higher plant,[8] our study appears to be unique in identifying the entire series in a particular leaf. Notably absent in the banana leaf were quinic, cis-aconitic and isocitric acids; these acids occur quite generally in temperate-zone leaves.[8-10]

With one or two exceptions, the individual banana leaf samples contained remarkably similar concentrations of total organic acids, malic acid and total oxalic acid. The "succinic peak" and "citric peak" results showed considerably more variation. However, the results seem adequate to estimate the mean organic acid content of the banana leaf for comparison with other leaves.

The mean total concentration of acids in the banana leaves (exclusive of the 12-month Gros Michel) was 93 ($\pm$ 10) m-equiv./100 g dry weight. This is at the lower end of the 70–400 m-equiv. range reported for temperate-zone leaves.[11-14] The organic acidity of the banana leaf is low because this leaf contains no significant pools of Krebs cycle acids. Temperate-zone leaves, even those with low total acidity, tend to accumulate relatively high concentrations (10 to more than 200 m-equiv./100 g dry weight) of one or more of the Krebs cycle acids, most often malic, citric or isocitric acids.[11-14] Possibly vigorously growing tropical leaves turn over their acids at a higher rate than temperate-zone leaves, since the tropical Bougainvillea leaf is also reported to contain low concentrations of Krebs cycle acids.[12]

Oxalic acid made up 83 ($\pm$ 6) per cent of the total acidity of the banana leaf. This is comparable to beet, spinach and buckwheat leaves; about 77 per cent of their total acidity is oxalic acid.[12, 14] However, these three leaves normally contain more oxalic acid (150–323 m-equiv./100 g dry weight) than the banana leaf. The tropical Bougainvillea leaf contains about the same concentration and proportion of oxalic acid as the banana leaf.[12]

On the average, 28 per cent of the oxalic acid occurred in water-soluble form in the greenhouse-grown banana leaves while 48 per cent was soluble in the field-grown leaves. The reason for this significant difference is not clear. If oxalic acid is involved in ion balance in plant tissues, as postulated by Pierce and Appleman[14] and by Osmond,[15] the differing contents of water-soluble oxalate may reflect differences in the soil nutrient availability or utiliza-

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[9] A. C. HULME and A. RICHARDSON, *J. Sci. Food Agric.* **5**, 221 (1954).
[10] J. K. PALMER, *Science* **126**, 504 (1957).
[11] J. BONNER, *Plant Biochemistry*, p. 142, Academic Press, New York (1950).
[12] P. C. DEKOCK and R. I. MORRISON, *Biochem. J.* **70**, 272 (1958).
[13] J. K. PALMER, *Conn. Agric. Expt. Station (New Haven) Bull.* 589 (1955).
[14] E. C. PIERCE and C. O. APPLEMAN, *Plant Physiol.* **18**, 224 (1943).
[15] B. OSMOND, *Nature* **198**, 503 (1963).

tion for the two groups of plants. It is of some interest that the oxalic acid in unripe banana fruits is entirely water-soluble and represents about 50 per cent of the total organic acidity.[16]

EXPERIMENTAL

The banana leaves used in this study were from three cultivars: *Musa acuminata* cv. Hort. Gros Michel; cv. Hort. Lacatan; cv. Hort. Lidi. Leaf sampling was restricted to healthy, green leaves, fully expanded and at the third or fourth position below the newly emerging leaf. The leaves were wiped with damp cheese-cloth to remove any pesticide residues, cut into small strips, oven-dried for 3 hr at 80°, and finely ground. The samples were stored in well-stoppered bottles and their moisture contents were determined just prior to extraction.

For identification purposes, the organic acids were extracted with hot water from 280 g of dried (Gros Michel) leaf, utilizing the extraction procedure described earlier.[13] The aqueous extract was added directly to an anion exchange column (Dowex 1, × 10, acetate, 2·2 × 13 cm) and the total organic acid fraction was obtained by displacement with 0·1 N HCl. The organic acids were further separated by displacement from multiple columns, as described by Anet and Reynolds[17] (see Fig. 1 for details). The organic acid front was located by testing the fractions for acetate and the organic acids were located in the subsequent fractions by paper chromatography in ether: 88% formic acid: water (5:2:1).[13,19] Fractions were appropriately pooled for isolation of individual acids. Glutamic, shikimic and succinic acids were obtained by gradient elution from Dowex 1 resin in the acetate form.[13] Glyceric, glycolic and aspartic acids were isolated by preparative paper chromatography in the ether: formic acid: water solvent.[13,19] Glutaric, malic, malonic, fumaric and α-ketoglutaric acids were purified by silica gel chromatography.[18] The citric, pyruvic and oxalic acid fractions from the displacement column were essentially free of contaminating organic acids. These were crystallized as the free acid, hydrazone, and calcium salt respectively.

The keto acids were extracted as the 2,4-dinitrophenylhydrazones which were separated on paper chromatograms.[7] The amino acid derivatives of the keto acids were prepared by the procedure of Towers *et al.*,[20] and were separated on paper chromatograms[2] and on an amino acid analyzer (Beckman/Spinco Model 120).

For quantitative determinations, the organic acids in 0·5–1·0 g of oven-dried tissue were extracted with hot water and analyzed by ion-exchange chromatography, as described earlier.[13] See Fig. 2 for details of chromatographic procedure.

Preliminary studies in which the organic acids were extracted from comparable samples of fresh, lyophilized and oven-dried leaf with both hot water and 70% alcohol demonstrated the simpler oven-drying, hot water extraction procedure to be adequate.

Oxalic acid was not eluted from the ion-exchange column. It was determined on aliquots of the water extracts (soluble oxalate) or in separate 0·1 N HCl extracts (total oxalate) by precipitation as calcium oxalate and titration with perchlorato-cerate.[13,16]

The phosphates were located on the paper chromatograms and characterized as inorganic or organic with the Bandurski–Axelrod reagents.[21]

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[17] E. F. L. ANET and T. M. REYNOLDS, *Australian J. Chem.* **8**, 267 (1955).
[18] H. G. WAGER and F. A. ISHERWOOD, *Analyst* **86**, 260 (1961).
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