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†THE DISTRIBUTION OF SOME NITROGENOUS CONSTITUENTS IN THE TEA PLANT

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A survey was made of the various nitrogenous constituents of the tea plant using paper chromatography. The following parts of the plant were examined: leaves, bark and wood of stems and roots, feeder roots and fruits (pericarp and cotyledons).

Theanine was present in every organ except the fruit and tended to be the major amino acid of the plant. Glutamic acid, aspartic acid and glutamine also occurred universally and in relatively high concentration, and serine, alanine and γ-aminobutyric acid, though generally present, occurred in smaller quantities. The wood of stems and roots contained appreciable quantities of a ninhydrin-reactive compound which streaked like histidine. Pipecolic acid was present in appreciable quantities only in the fruits.

The amino acids of the hot-water-soluble proteins of the various tissues have been determined and certain differences between them were noted.

1. Introduction

Extensive cultivation of the tea plant under conditions of specialised agricultural practice in Sri Lanka has produced an increasing number of nutritional, developmental, pathological and entomological problems. A comprehensive knowledge of the physiology and biochemistry of this plant should help to elucidate some of these problems. Most of the biochemical investigations concerning the tea plant have been almost entirely restricted to the shoot tips of the plant comprising the buds, the first two leaves and the included stems (collectively called the "flush"), and the changes in the constituents of the "flush" during black tea manufacture.[1–5] Hence, there is a notable lack of information on parts of the plant body other than the leaves and on aspects of metabolism other than those occurring during black tea manufacture. For example, very little information is available on the occurrence, distribution and metabolism of nitrogenous constituents of the plant.

This paper presents a quantitative survey of the nitrogenous constituents of the various tissues of the plant, particularly those present in the alcohol-soluble fraction and in hydrolysates of the alcohol-insoluble material.

2. Experimental

2.1 Plant material

All material (leaves, bark and wood of stems and roots and feeder roots) used in this investigation was collected from 2-year-old plants (Clone Ken 16/3) grown in a field near the laboratory (1500 m elevation). Mature fruits were picked from

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seed-bearing plants. The sap was collected from a decapitated plant by the method described before.[6] The quantity of amino acids present in the sap gives some measure of the nitrogenous compounds translocated from the roots to the aerial parts of the plant.

2.2 Extraction of amino acids

Fresh material (5 g). irrespective of its nature, was plunged into 25ml of boiling 80% (v/v) ethanol and boiled gently for 2 min. It was then cooled and macerated in an Atomix homogeniser for 5 min. The macerate was centrifuged and the supernatant decanted. The residue was re-extracted twice with 30 ml of hot 80% (v/v) ethanol to remove all alcohol-soluble constituents. The ethanol-insoluble material was dried in a dehumidified room and retained for analysis of the hot-water-soluble proteins. Comparative studies showed that incorporation of 0.05 to 0.1 N-HCl in the extraction media had negligible effect on the quantity of amino acids extracted. The combined alcohol extracts were concentrated under vacuum and taken up in a small quantity of water. The total ninhydrin-reactive substances present in the extracts were estimated before purification. The amino acids were isolated from the concentrates by the methods described earlier.[3,6]

2.3 Hydrolysis of the hot-water soluble proteins

The ethanol-insoluble material was extracted with hot water for 6 h to solubilise some of the proteins. The hot-water soluble proteins were hydrolysed with 6 N-HCl for 24 h. The hydrolysates were centrifuged and the acid extract was evaporated to dryness under vacuum. After repeated addition of water and redrying to remove the excess acid, the liberated amino acids were isolated by adsorption on a cation exchange column and analysed by paper chromatography.

2.4 Chromatographic procedures

The amino acids were separated by two-directional paper chromatography using Whatman No. 4 chromatographic grade papers. The solvent combinations were butanol/acetic acid/water (6:1:2 by vol.) in the first direction and phenol/water (8:2 by vol.) in the second. After the chromatography the papers were treated with ninhydrin containing cobalt chloride in the usual way. Notes were taken of the relative intensities of the spots on the paper—these were useful guides for the quantitative study.

Amino acids that could not be readily recognised on the chromatograms were recorded by number, their positions determined by measurements relative to alanine and other characteristics such as ninhydrin colour etc. were noted. The occurrence of pipecolic acid in the extracts of fruits was suspected by its position on the chromatograms and colour reaction with ninhydrin. Supplementary evidence was obtained from its characteristic fluorescence in u.v. light. The individual amino acids (except proline) after separation on paper chromatograms were estimated by the method of Yemm and Cocking[7] using alanine as the standard.

2.5 Nitrogen determination

The total nitrogen of the various tissues and extracts was determined by the micro-Kjeldahl procedure.

3. Results

3.1 Total nitrogen

The total nitrogen contents of the various tissues are recorded in Table 1, together with the results of the fractionation into 80% ethanol-soluble non-protein nitrogen and insoluble protein nitrogen components. In all the tissues, the protein nitrogen accounted for about 40 to 80% of the total nitrogen content of the tissue.

TABLE 1 — *The results of the fractionation of the nitrogenous compounds of the various tissues of the tea plant into 80% (v/v) ethanol-soluble and insoluble components (date expressed as g of N/100 g of dry wt of tissue)*

Tissue	Total N	Soluble N	Insoluble N	Soluble N as a % of total N
Immature (1st leaf)	5.04	1.40[a]	3.64	27.7
Mature (6th leaf)	3.73	0.88[a]	2.85	23.6
Stem-bark	1.80	0.45	1.35	25.0
Stem-wood	0.61	0.21	0.40	34.5
Root-bark	2.02	1.13	0.89	56.0
Root-wood	1.11	0.65	0.46	58.5
Feeder roots	2.52	0.95	1.57	37.7
Pericarp	1.64	0.56	1.08	34.2
Cotyledons	2.62	0.46	2.16	17.6

[a] Caffeine is a major soluble nitrogenous constituent of tea leaves. It accounts for 0.50 and 0.54 g of N/100 g of dry weight of immature and mature leaves, respectively.

However, direct analytical results, such as those in Table 1, disguise the differences in weight between tissues in the whole plant and comparisons between the results from different tissues can give misleading impressions about the distribution of nitrogenous compounds within the plant as a whole. The analytical results, therefore, have to be multiplied by the total dry weight of the tissue as found at sampling to give the total weight of nitrogen present in its various forms in the tissues. These results would then give the distribution of nitrogen within the whole plant.

3.2 Soluble amino acids

The various parts of the tea plant contain a large number of free amino acids and amides which vary considerably as to their relative proportions (Table 2). Furthermore, the total amount of free amino acids and amides may vary from only 450 μg in the stem-wood to about 5000 μg/g of fresh material in the immature leaf. Application of ammonium sulphate to the plant resulted in an increase in the free

TABLE 2 — *The levels of the free amino acids present in the various tissues and sap of the tea plant [data expressed as μg of amino acid (as alanine)/g of fresh wt of tissue]*

Amino acid	1st leaf	%	6th leaf	%	Stem-bark	Stem-wood	Root-bark	Root-wood	Feeder roots	Pericarp	Cotyledons	Sap[a]
Aspartic acid	251	8	97	15	25	27	32	34	16	150	224	2
Glutamic acid	495	15	178	27	30	69	72	58	18	210	176	6
Serine	156	5	43	7	32	2	13	14	6	142	58	Trace
Asparagine	34		—		—	—	—	—	—	177	—	—
Threonine	46		—		—	—	—	—	—	23	Trace	—
Alanine	46		43	7	42	11	14	17	11	72	152	—
Glutamine	69		78	12	51	45	115	24	20	124	366	48
Peptide?	—		—		—	50	—	71	—	—	—	—
γ-AB[b]	—		8		61	33	29	15	6	50	74	—
Valine	18		—		—	Trace	—	Trace	—	27	58	—
Leucine(s)	25		Trace		—	Trace	—	Trace	—	—	—	—
Unknown-1[c]	—		—		—	Trace	19	10	12	—	—	4
Pipecolic acid	—		—		—	—	—	—	—	210	298	—
Theanine	2140	65	213	32	89	69	490	138	383	Trace	—	35
Lysine	—		—		—	—	—	—	—	—	60	—
Cysteic acid?	—		Trace		Trace	Trace	Trace	Trace	—	Trace	Trace	—
Total by addition	3280	100	661		330	304	784	381	472	1260	1518	95
Total ninhydrin-reactive substances	4990		1175		550	430	994	660	750	1800	2300	120

[a] μg amino acid/ml sap.

[b] γ-AB = γ-amino butyric acid.

[c] The R_{Ala} values of unknown-1 in butanol/acetic acid/water and phenol/water were 0.82 and 1.19 respectively.

amino-acid content of the various tissues. The tissues most affected were the roots and leaves (Selvendran, unpublished results). Of the identified amino acids, theanine was the most prominent one in the various tissues except the fruits. Glutamic acid, aspartic acid, glutamine, serine, alanine and γ-amino butyric acid predominate in one or more tissues of the plant. Stem-wood and root-wood are conspicuous by the presence of appreciable quantities of a ninhydrin-reactive compound which streaked on the chromatograms like histidine. It could be a peptide. Pericarp and cotyledons of mature fruits were the only tissues in which pipecolic acid was the predominant amino acid. It is recognised, however, that the failure to report the presence of a given amino acid may be due, not to its absence, but its being present in amounts too low to detect by the methods used.

3.3 Unidentified ninhydrin reactive substances

In addition to the readily identifiable amino acids which are described in Tables 2 and 3, the chromatograms of roots, sap and hydrolysates of the hot-water-soluble

TABLE 3 — *Percentage amino-acid composition of the hot-water-soluble proteins from the various tissues of the tea plant*

Amino acid	1st leaf	Stem-bark	Stem-wood	Root-bark	Root-wood	Pericarp	Cotyledons
Aspartic acid	·14.7	16.0	13.2	13.1	11.8	12.6	6.5
Glutamic acid	·18.9	24.0	26.6	36.6	18.9	16.6	28.3
Serine	5.8	9.2	5.2	8.2	11.3	11.6	4.5
Glycine	7.4	12.8	8.4	9.0	8.5	12.9	7.5
Threonine	4.0	1.6	1.4	1.5	2.4	2.2	0.5
Alanine	5.2	3.2	3.8	4.7	4.7	9.5	5.0
Histidine[a]	4.0	5.2	15.0	4.0	8.5	6.2	23.3
Lysine	·10.5	14.4	9.5	7.8	16.5	10.2	9.0
Arginine	8.9	2.4	1.9	3.1	2.8	—	—
Hydroxyproline	4.2	1.2	—	1.5	—	—	0.5
Unknown-2[b]	2.1	2.4	3.2	2.8	Trace	1.0	—
Valine	4.7	1.6	4.1	3.0	2.8	6.0	3.2
Leucine(s)	7.4	6.0	7.7	4.7	11.8	10.2	9.8
Phenylalanine	2.2	Trace	Trace	Trace	Trace	—	—
Tyrosine	Trace	—	—	—	—	1.0	0.5
Cysteic acid?	—	—	—	—	—	—	2.0

[a] The histidine streak may contain arginine as well.

[b] The R_{Ala} values of unknown 2 in butanol/acetic acid/water and phenol/water were 1.47 and 1,29 respectively.

Proline was detected in most of the hydrolysates but was not estimated.

proteins from the different tissues revealed the presence of substances which gave colour reaction with ninhydrin on paper. The R_{Ala} values of these substances are given in the footnotes to Tables 2 and 3.

4. Discussion

The chemistry of the alcohol-soluble amino acids of the tea plant is to a large extent characterised and dominated by the unique presence of theanine (γ-N-ethyl glutamine). The roots are the chief sites of synthesis of theanine,[9] from which it is translocated to the aerial parts of the plant in the xylem sap.[6] The universal distribution of theanine throughout the plant suggests an underlying function for it. However, at present nothing definite is known about its role.

The occurrence of γ-amino butyric acid in almost all the tissues analysed ranging from virtual absence in the immature leaves to its occurrence as a major constituent of stems is reported for the first time. However, this acid is ubiquitous in the plant kingdom.[10] Pipecolic acid (piperidine-2-carboxylic acid) was detected and identified in the fruits. It arises by elimination of ammonia and by ring formation from lysine and has been reported to be present in the reproductive organs of many angiosperms.[10] The role of this amino acid is, however, not known.

The large differences in the nitrogen content of the ethanol-insoluble material of leaves, cotyledons, stem-bark and feeder roots, compared with stem and root-wood, may be entirely due to the presence of more cytoplasmic protein in the living cells of the former. In view of the fact that the nitrogen-containing compounds of the various tissues supply the nitrogen needed by insects and micro-organisms living on the plant, a knowledge of the nature of these compounds should help to elucidate their feeding habits. The survey of the nitrogenous compounds present in the tea plant provides the background against which quantitative changes under various physiological conditions may be studied.

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