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*CHANGES IN THE COMPOSITION OF THE XYLEM EXUDATE OF TEA PLANTS (*CAMELLIA SINENSIS* L.) DURING RECOVERY FROM PRUNING

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A method of collecting the xylem sap from tea plants in quantities sufficient for analysis is described. Organic nitrogenous compounds accounted for most of the nitrogen present in the sap. Glutamine, theanine, glutamic acid, aspartic acid, and lysine were quantitatively the most important nitrogenous compounds. Also present in lesser amounts were leucine/isoleucine, phenyl alanine, valine, and asparagine. Inorganic phosphate, sulphate, glucose, fructose, and sucrose were also present. Phosphoryl choline was not detected.

During recovery from pruning there was a definite change in proportions of some of the constituents in the sap. The total amino-acid content decreased to a very low level 9 days after pruning followed by an increase with bud break. Glutamine and theanine were the most important constituents quantitatively. These tended to decrease relatively more than the other amino acids during the first few days after pruning. The increase in glutamine and, to a lesser extent, theanine was more marked with bud break. Phosphoryl choline was not detected in the sap at any stage during the recovery period. The changes in the level of phosphate, sulphate, and sugars were somewhat parallel to the changes in the level of amino acids. All these constituents decreased to a low level a few days after pruning and increased with bud break. These findings suggest that new growth was made largely at the expense of translocated nitrogenous and other compounds.

A tea plant if unchecked will grow into a multi-stemmed tree of about 6 m high. In commercial practice, however, the plants are periodically pruned to keep the plucking table at a convenient height. Pruning also stimulates new shoot growth and encourages the plant to remain in the vegetative phase for an extended period by delaying the production of reproductive organs. Maximum crops are harvested when pruning cycles and plucking are correlated. In Ceylon two types of pruning, namely clean-pruning and lung-pruning, are practised. A plant is said to be clean-pruned when all leaf-bearing branches are removed. In a lung-pruned plant a few side branches with leaves are retained. The pruning cycle in Ceylon ranges from 1·5 to 2 years in the low-country and 3 to 5 years in the high-grown areas. In the low-country and drier parts of the mid-country it is usual to 'lung-prune'. The lung-branches ('lungs') reduce the subsequent die-back of the plants. It has been suggested that the function of the 'lungs' is to supplement the root reserves as they are used up in the recovery and to ensure more efficient utilization of root reserves (Priestley 1962).

Studies on the total available carbohydrates of tea roots during recovery from pruning has shown that there is a decline in the reserves which continues steadily in both clean-and lung-pruned plants (Nagarajah & Pethiyagoda 1965). The decrease is very sharp during the first few days after pruning, after which it steadies itself for a few weeks, followed by another sharp decrease. The second decrease coincides more or less with bud break, suggesting the utilization of root reserves for bud growth. One of the methods of investigating the utilization of root reserves for recovery is to study the composition of the xylem exudate which originates in the root system during the recovery period.

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The root system can regulate metabolic activities in the shoot by exporting reduced or unreduced nitrogenous and other compounds to it (Carr 1966). Extensive evidence had accumulated over recent years in favour of a regular movement of substantial quantities of organic substances in the xylem of a variety of woody and herbaceous plants (Bollard 1960; Pate 1968). As far as nitrogen metabolism is concerned, it has been recorded that amino acids are the major constituents of xylem sap or exudate. By contrast, inorganic nitrogen, as nitrate or ammonium, may be absent or present only in trace amounts in the xylem sap. These findings are generally interpreted as suggesting that the root system may act as the normal centre of assimilation of inorganic nitrogen, its metabolic activities resulting in the formation of a series of simple amino compounds by amination reactions which may subsequently be delivered to the top of the plant in the transpiration stream for growth. The α-keto acids required for amination reactions are believed to be derived from sugars translocated from the leaves. During recovery from pruning there is no supply of carbohydrates from the aerial parts of the plant, therefore, most of the constituents required for new growth should arrive from mobilization of reserves in the root system.

In the present study samples of xylem exudate (sap) were obtained by applying gentle suction to the cut end of the stems of pruned plants. These samples were used to follow the composition of the ascending sap throughout the recovery period. This exudate is thought to represent the contents of the xylem vessels not seriously contaminated with materials from living cells. A similar study was made with stem sections eluted with water.

MATERIALS AND METHODS

Plants used in the investigation

Plants of clone TRI 2142 were grown in polythene bags under standard nursery conditions. They were transferred after 6 months to cement pots (31 cm (1 ft)X31 cm X 46 cm (1.5 ft) high) filled with fumigated soil, placed in the field and regularly fertilized with T 200 fertilizer mixture (Tolhurst, 1961), up to 2 months from the commencement of the experiment. The present study was made when the plants were 4 years old.

Method of collection of sap

The sap was collected by applying gentle suction to the cut end of the stem. Plate 1 illustrates the mode of collection of the sap. The stem was cut at a height of 300-400 mm and 20 mm of bark was peeled off below the cut end. The cut surface was then trimmed with a sterilized knife, and the stem and cut surface wiped with alcohol. Using a rubber tube, a capillary tube bent at right angles was then fitted on to the cut end. The other end of the capillary tube was connected to a test tube with a side arm. The test-tube was fitted with a two-holed rubber cork which was used to regulate the pressure in the tube. A trap was incorporated to prevent any water from the filter pump accidentally entering the collecting tube. On reducing the pressure inside the tube, the sap rose in the capillary tube and collected in the test-tube. No sap could be collected without reduction of pressure, and steady flow of sap was maintained by adjusting the pressure. The sap collected was removed from the field as soon as possible and stored in a deep freeze.

The composition of the sap collected in the above manner compared well with that of the solution obtained on eluting tea stems (200 mmX10-15 mm diameter) with distilled water (120ml), using a similar apparatus.

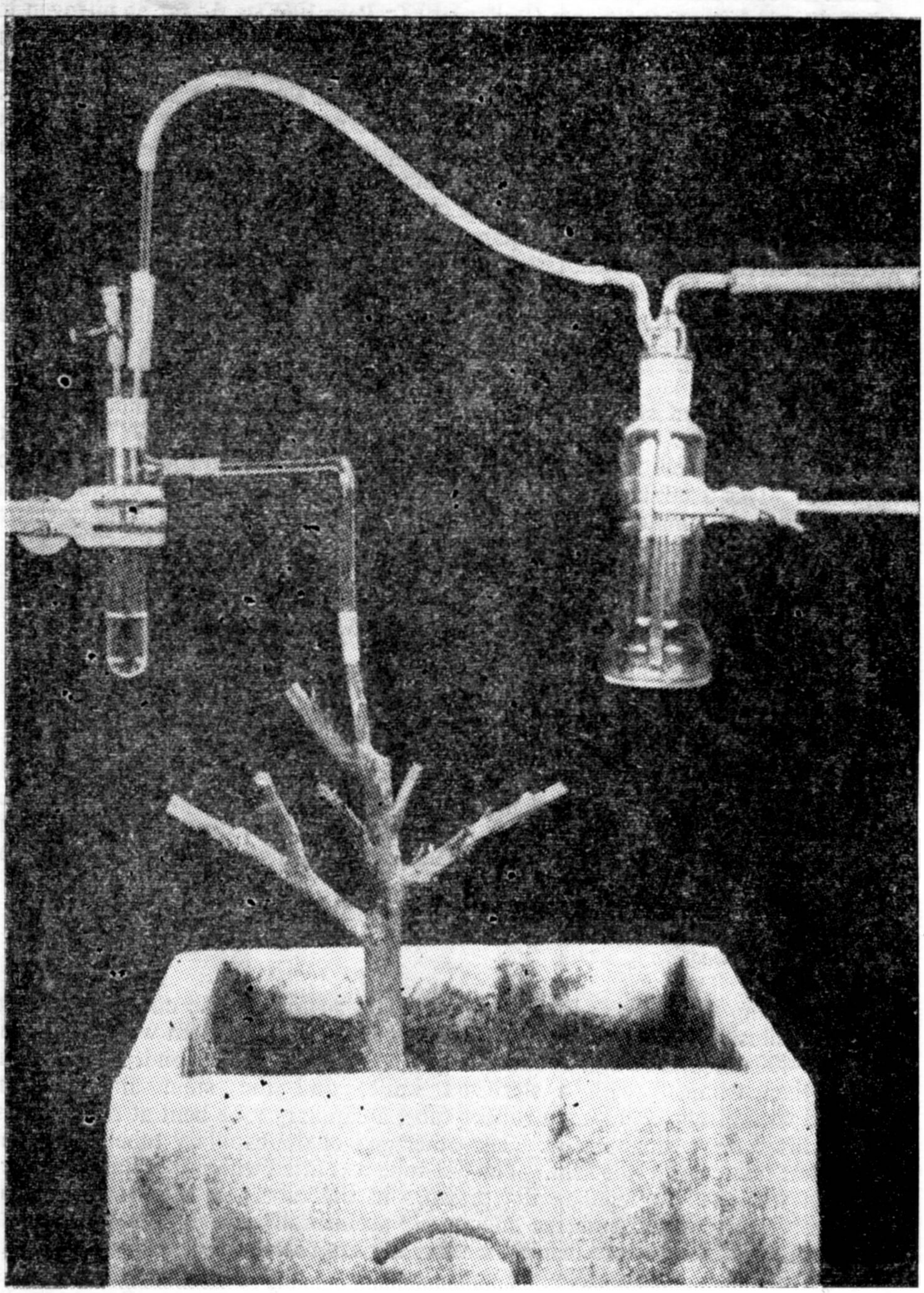

FIG. 1—*Apparatus for collection of xylem exudate from tea plant.*

Collection of sap from plants during the recovery period

The plants were pruned on 18 July 1969 and sap was collected from the clean-and lung-pruned plants at o, 4, 9, 22, 33, and 64-day intervals. In the case of lung-pruned plants, the sap was always collected from stems not carrying 'lungs'. It was found that the sap collection was not possible without removal of 'lungs'. Therefore, the 'lungs' were removed prior to collection of sap. The quantity of sap collected and the general vegetative conditions of the pruned plants, that is presence or absence of buds, leaves, and shoots, at the time of sap collection were recorded (Table 1).

Analysis of sap

The sap was concentrated by evaporation under reduced pressure at 40 °C and the concentrate was passed through ion-exchange columns to separate the charged and uncharged compounds.

Amino acids

The concentrate from 10 ml of sap was passed through a column (20 mmX 10 mm diameter) of Dowex AG-50 (8-10 per cent cross linked ; H^+ form ; 200-400 mesh) and the column washed subsequently with four bed volumes of distilled water. The effluent was used for the isolation of organic acids and sugars. The amino acids were eluted with 30 ml of 2N-aqueous ammonia, evaporated to dryness under reduced pressure, and taken up in 1 ml of distilled water. The individual amino acids after separation on paper chromatograms were estimated by the method of Yemm and Cocking (1955) using alanine as the standard. The total amino acid content of the sap was estimated before purification.

TABLE 1 — *Records of observations made on the pruned plants during the recovery period*

Days from pruning	Condition of pruned plants	Rate of sap collection (ml/h)	
		Clean-pruned	Lung-pruned
0	Position of leaf scars only visible	7·5	7·8
4	Position of leaf scars only visible	4·0	5·2
9	No buds visible yet	3·1	4·1
22	Appearance of buds—0·1 to 0·2 cm long	3·0	4·0
33	Length of developing shoots—1 to 2 cm (1 to 2 small leaves and bud)	3·3	4·2
64	Average length of shoots—10 to 20 cm (4 to 6 expanding leaves and bud)	1·8	5·0

Organic acids

The effluent from the cation column was concentrated and passed through a column (20 mm x 10 mm diameter) of Amberlite CG-4B (OH form, 100–200 mesh) and the column washed subsequently with four bed volumes of distilled water. The organic acids were eluted with 40 ml of 2N-aqueous ammonia evaporated to dryness under reduced pressure and taken up in 1 ml of distilled water.

Sugars

The effluent from the anion column was concentrated and used for sugar analysis.

Chromatography

The amino acids were separated by paper chromatography as described by Roberts and Sanderson (1966). For comparative studies the concentrates were run in one direction in phenol-water.

The organic-acid fraction was chromatographed on silica gel as described by Sanderson and Selvendran (1965). The anions and sugars were identified by paper chromatography using the solvent ethyl acetate/pyridine/water (2 : 1 : 2-top layer) (Jermyn and Isherwood, 1949).

RESULTS

Constituents present in the sap

pH values

The pH values of the sap were found to be between 5.5 and 6.

Amino acids

The main amino acids present in the sap were always glutamine, theanine (γ-N ethyl glutamine), glutamic acid, aspartic acid, and lysine. Judged by the intensity of spots these compounds account for up to 90 per cent of all the amino nitrogen present. Also present in lesser amounts were leucine/isoleucine, phenyl alanine, valine, and asparagine.

Careful analysis showed that no phosphorus-containing compounds were retained on the cation-exchange column. Comparable experiments with phosphoryl choline showed that it was retained on the cation-exchange column and could be eluted with 2N-aqueous ammonia. Phosphoryl choline is also adsorbed by Dowex-AG 50 from weakly acidic solutions (Selvendran, 1968).

Organic acids

Silica-gel chromatography of the eluate from the anion-exchange column showed that it did not contain detectable quantities of organic acids usually present in the tea plant (Sanderson and Selvendran, 1965). However, qualitative analysis and paper chromatography confirmed the presence of phosphate and sulphate.

Sugars

The sugars detected in the sap were glucose, fructose, and sucrose.

Variation in the level and compositions of the sap during recovery from pruning

It was apparent in early work that changes occurred in the level of amino acids, anions, and sugars in the xylem sap during the recovery period.

Amino acids

Chromatography of the sap samples showed that the same amino acids were generally present throughout the recovery period. The results are given in Table 2. The total amino-acid level decreased to a very low level 9 days after pruning followed by an increase before bud break, 3 to 4 weeks from the time of pruning. Glutamine and theanine were the most important constituents quantitatively, and they tended to decrease relatively more than the other amino acids during the first few days. The increase in glutamine and, to a lesser extent, theanine was more marked with bud break. These results were qualitatively verified in three separate experiments.

TABLE 2 — *Amounts (μ moles ml^{-1}) of amino acids in xylem sap during recovery from pruning of clean-pruned plants*

	Days after pruning					
Compound	0	4	9	22	33	64
Glutamine	2·84	0·52	0·06	0·33	1·02	1·40
Theanine	1·26	0·43	0·01	0·05	0·45	0·80
Glutamic acid	0·09	0·06	0·04	0·10	0·18	0·22
Aspartic acid	0·04	0·03	0·04	0·05	0·06	0·11
Lysine	0·07	0·03	0·01	0·04	0·08	0·11
Total amino acids	4·84	1·38	0·20	0·65	1·91	3·43

TABLE 3 — *Amounts (μ moles ml^{-1}) of amino acids in xylem sap during recovery from pruning of lung-pruned plants*

	Days after pruning					
Compound	0	4	9	22	33	64
Glutamine	2·61	0·08	<0·01	0·06	0·97	1·54
Theanine	1·12	0·16	<0·01	0·04	0·43	0·94
Glutamic acid	0·10	0·01	<0·01	0·05	0·15	0·28
Aspartic acid	0·16	0·01	<0·01	0·03	0·05	0·10
Lysine	0·05	0·01	<0·01	0·06	0·06	0·08
Total amino acids	4·52	0·32	0·03	0·18	1·77	3·26

In Table 3 the results of experiment with lung-pruned plants are given. The rate of sap exudation was higher with lung-pruned plants throughout the recovery period. In this experiment too the total amino-acid content decreased to a very low level 9 days after pruning and increased with bud break. The relative decrease in the level of glutamine, theanine, and glutamic acid during the first few days after pruning was more marked compared with the clean-pruned plants.

Phosphoryl choline was not detected in the exudate at any stage during the recovery period.

Studies with xylem exudate collected by eluting stem sections of the pruned plants with distilled water confirmed the above findings. The concentration of glutamine and theanine which was appreciable immediately after pruning decreased to a very low level 9 days after pruning and increased with bud break.

Phosphate, sulphate, and sugars

Qualitative paper chromatography of the anion-exchange eluates and sugar fractions showed that the changes in the level of phosphate, sulphate, and sugars were somewhat parallel to the changes in the level of amino acids. All these constituents decreased to a low level a few days after pruning and increased with bud break.

DISCUSSION

The results from the present study confirmed the findings of earlier workers that the nitrogen in the xylem sap of woody plants is mostly present in organic compounds and that a substantial portion, at least, of the nitrogen reaching the shoots must come through this channel. Glutamine and theanine were the major amino acids in the exudate of tea plants. Ammonia nitrogen was present in a small proportion only (0.12 μ moles/ml) and nitrate nitrogen has not been detected. These results are in accordance with those of other workers (Bollard, 1957, 1960). Nitrates are not normally present in the leaves of tea plants. Nitrate reductase has been found to be present in appreciable quantity in tea feeder roots but has not been detected in tea leaves (Bhavanandan 1969, personal communication).

The presence of appreciable quantities of theanine in the xylem exudate suggests that this major amino acid of the tea leaves is synthesized in the roots and translocated to the aerial parts of the plant. It is clear that, in the tea plant, as in other woody plants, stages in the assimilation of nitrogen are separated morphologically. Normally, incorporation of inorganic nitrogen into organic compounds seems to be largely accomplished in roots. The root system then supplies the aerial parts of the plant with a defined range of amino acids and amides. Further metabolism of nitrogen in leaves can occur using the compounds supplied in the xylem sap as starting materials. This general mode of assimilation is altered when the plant is fertilized with ammonium sulphate. Preliminary experiments indicated that when ammonium sulphate was applied to the roots of tea plants, the ammonia and glutamine content of the sap increased considerably 3 to 4 days after application. However, there was no parallel increase in the level of theanine and glutamic acid. Because the quantity of carbohydrates made available to the root system by the aerial parts of the plant is not altered, the above observation implies that reserve polysaccharides, like starch are being mobilized for nitrogen assimilation.

Tolbert and Wiebe (1955) and Maizel, Benson, and Tolbert (1956) have detected an appreciable quantity of radioactive phosphoryl choline in the sap, after feeding herbaceous plants with radioactive phosphate. From these studies they have suggested that one of the functions of phosphoryl choline is as a phosphate carrier in plants. In the present study careful analysis of the xylem sap at different stages during the recovery period showed absence of phosphoryl choline. Because Sheldrake and Northcote (1968) have reported the presence of phosphatases in xylem sap of plants, the sap was collected in two experiments over cold trichloroacetic acid (TCA). This was carried out by keeping cold TCA in the collecting tube and occasionally shaking the tube to mix the contents. Even then no phosphoryl choline could be detected in the sap. These findings query the role of phosphoryl choline as a phosphate carrier in tea plants and possibly in other woody plants. Phosphorus appears to be translocated in tea plants as inorganic phosphate. However, on eluting stems of tea plants with 0·1 N hydrochloric acid, phosphoryl choline was detected in the eluate. Phosphoryl choline was also detected in tea roots and leaves (Selvendran, unpublished results.)

The presence of sulphate and phosphate in the sap clearly shows that these anions are readily translocated to the aerial parts of the plant in the xylem. The presence of glucose, fructose, and sucrose in the sap is in keeping with the findings of other workers (Sheldrake and Northcote 1968). It is difficult to comment on the mode of origin of these sugars with the experimental technique used in the present study. However, it is possible that some sugars diffuse into the xylem from the bark and are transported upwards in the transpiration stream, During recovery from pruning the major changes in the constituents present in the sap were in amino acids. No definite conclusions could be drawn from the changes in the content of anions and sugars. It is likely that considerable changes in the concentration of hormones also take place; however, this aspect was not investigated.

The second marked decrease in the total available carbohydrates of tea roots during the recovery period which coincides more or less with bud break (Nagarajah and Pethiyagoda 1965) indicates that reserve materials stored in the roots are mobilized and used to support growth of young shoots. It has been found that the time lapse between pruning, bud-break, and subsequent growth of shoots is dependent amongst other factors on the age of the plant; buds tend to develop earlier on younger plants. With bud break cambial activity is initiated over the whole plant, and this in turn is apparently the signal for hydrolysis of reserves in the plant and the resulting sugars are translocated to the actively growing regions. Cambial initials depend on nutrients and water from the xylem vessels and the survival of these initials depends on the contact they make with the xylem vessels. The developing shoots require large quantities of translocated carbon skeleton before an effective photosynthetic mechanism has developed. In addition large quantities of nutrients are used by the intense respiration of the rapidly growing shoots.

The results of the present investigation suggest that new growth was made largely at the expense of translocated nitrogenous compounds. It is possible that the metabolism of amino acids and carbohydrates is closely linked in the storage organs. For example, the fall in concentration of total available carbohydrates in roots during bud break may be related to the increase in the concentration of amino acids in the sap and in the newly developing shoots during this period. Comparison of the amino acids present in the xylem sap with those of newly developed shoots (33 days from pruning) revealed interesting features. The major amino acids of the shoots were glutamine, theanine, and glutamic acid (Selvendran, unpublished results) which compare well with those present in the sap at the corresponding stage of development (see Tables 2 and 3). It is, therefore, reasonable to suppose that the buds receive a significant portion of their nutrients from the xylem sap. Although it is not clear whether hormones or carbohydrates are more important in influencing bud growth, great emphasis must be placed on the importance of translocated amino acids for shoot growth after buds appear. This conclusion is reinforced by the large and rapid increase in dry weight of developing shoots.

From the present study no definite role could be attributed to the lung branches. The observation that there is a greater rate of sap collection with lung-pruned plants, may be because the root system of lung-pruned plants is more active compared with that of clean-pruned plants. In future work it would be of interest to compare the hormone levels in the sap of clean- and lung-pruned plants.

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LITERATURE CITED

BOLLARD, E. G., 1957. Composition of the nitrogen fraction of apple tracheal sap. *Aust. J. biol. Sci.* **10**, 279-87.

BOLLARD, E. G., 1960. Transport in the xylem. *A. Rev. Pl. Physiol.* **11**, 141-66.

CARR, D. J., 1966. Metabolic and hormonal regulation of growth and development. In *Trends in Plant Morphogenesis*, ed. E. G. Cutter, 253-83. Longmans Green, London.

JERMYN, M. A., and ISHERWOOD, F. A., 1949. Improved separation of sugars on the paper partition chromatogram. *Biochem. J.* **44**, 402-7.

MAIZEL, J. V., BENSON, A. A., and TOLBERT, N. A., 1956. Identification of phosphoryl choline as an important constituent of plant saps. *Plant Physiol. Lancaster* **31**, 407-8.

NAGARAJAH, S., and PETHIYAGODA, U., 1965. The influence of 'lungs' on carbohydrate reserves and growth of shoots. *Tea Q.* **36**, 88-102.

PATE, J. S. 1968. Physiological aspects of inorganic and intermediate nitrogen metabolism (with special reference to the legume *Pisum arvense* L). In *Recent Aspects of Nitrogen Metabolism on Plants'*, ed. E. J. Hewitt and C. V. Cutting, 219-40. Academic Press, London and New York.

PRIESTLEY, C. A., 1962. Carbohydrate resources within the perennial plant. *Tech. Commun. Bur. Hort. East Malling* **37**, 116.

ROBERTS, G. R., and SANDERSON, G. W., 1966. Changes undergone by free amino acids during the manufacture of black tea. *J. Sci. Fd. Agri.* **17**, 182-8.

SANDERSON, G. W., and SELVENDRAN, R. R., 1965. The organic acids in tea plants. A study of the non-volatile organic acids separated on silica gel. *J. Sci. Fd. Agri.* **16**, 251-8.

SELVENDRAN, R. R., 1968. *Metabolism of Phosphate Esters and Nucleotides in Mature Strawberry Leaves.* Ph.D. Thesis, Cambridge University, pp. 200.

SHELDRAKE, A. R., and NORTHCOTE, D. H., 1968. Some constituents of xylem sap and their possible relationship to xylem differentiation. *J. exp. Bot.* **19**, 681-9.

TOLBERT, N. W., and WIEBE, H., 1955. Phosphorus and sulphur compounds in plant xylem sap. *Plant Physiol. Lancaster* **30**, 499-504.

TOLHURST, J. A. H., 1961. Manuring young tea. *Tea Q.* **32**, 152-4.

YEMM, W. E., and COCKING, E. C., 1955. The determination of amino acids with ninhydrin. *Analyst* **80**. 209-12.

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