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A new chromatographic spray for the detection of catechins

The colour tests hitherto described in the literature for the detection of catechins on paper chromatograms are rather non-specific and apply to phenolic compounds in general[1] (*e.g.* vanillin–sulphuric acid, sulphanilic acid, *p*-nitroaniline and ferric chloride–potassium ferricyanide reagents). A colour reaction is now described which appears to be specific for the catechins on paper chromatograms. It possesses a high degree of sensitivity and very stable red spots are formed.

The dry chromatogram was sprayed with a 1 % solution of 2,4,6-trinitrophenol in 95 % ethanol, and when the papers were visibly dry, the chromatograms were sprayed again with a 5 % solution of potassium hydroxide in 80 % ethanol (Jaffé-reaction). After 30 sec, stable red spots were given by the catechins. (This colour could be matched with Chinese Red of the Permoglaze colour chart or Geranium Lake of the Derwent colour chart).

It has been recorded rather ambiguously in the literature that the formation of orange or red colours with the above reagents was a characteristic of the guanidines[1] (for a detailed discussion of this reaction see *e.g.* ref. 2). Tests were carried out in our laboratories in order to verify the validity of this statement. The common guanidines such as creatinine, glycocyamine and guanidine gave orange colours which were quite distinct from the characteristic red given by the catechins. Tests were also carried out to determine whether other polyphenols would give a similar colour reaction with these reagents. The polyphenols tested included quercitin, myricetin, caffeic acid, naringenin, coumarin, catechol, phloroglucinol and leucocyanidin (polymer). None of these compounds responded to the test.

The four main catechins found in tea leaves, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate, all gave positive results. The attachment of the galloyl residue in position 2 did not seem to affect the colour reaction (*e.g.* epigallocatechin), but the formation of galloyl esters in position 3 (*e.g.* epigallocatechin gallate) retarded the colour formation to some extent. Similarly the theaflavins formed during tea fermentation answered this test as the basic catechin residue remained unaltered. It was also observed that the closely related leucoanthocyanins (flavane-3,4-diols) present in tea leaves did not give this test. A similar observation was made with the flavones such as naringenin and quercitin which possess double bonds between positions 2 and 3. It was also interesting to note that the use of *o*-nitrophenol, *p*-nitrophenol or even 2,4-dinitrophenol in place of the trinitrophenol, did not give the characteristic red colour with the catechins.

On paper, this colour was very stable and the chromatogram could be preserved for a week or even more unlike the pink colour given by the vanillin–sulphuric acid reagent which faded after 20 min. This method could detect concentrations of catechins of the order of 10 p.p.m. by weight (0.01 mg) and was more sensitive than most of the other reagents, except the vanillin–sulphuric acid reagent which could detect even smaller concentrations (1 p.p.m.).

Tea Research Institute of Ceylon,
Talawakelle (Ceylon)

A. S. L. TIRIMANNA*
K. P. W. C. PERERA

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* Present address: Ceylon Institute of Scientific and Industrial Research, Colombo 7, Ceylon.