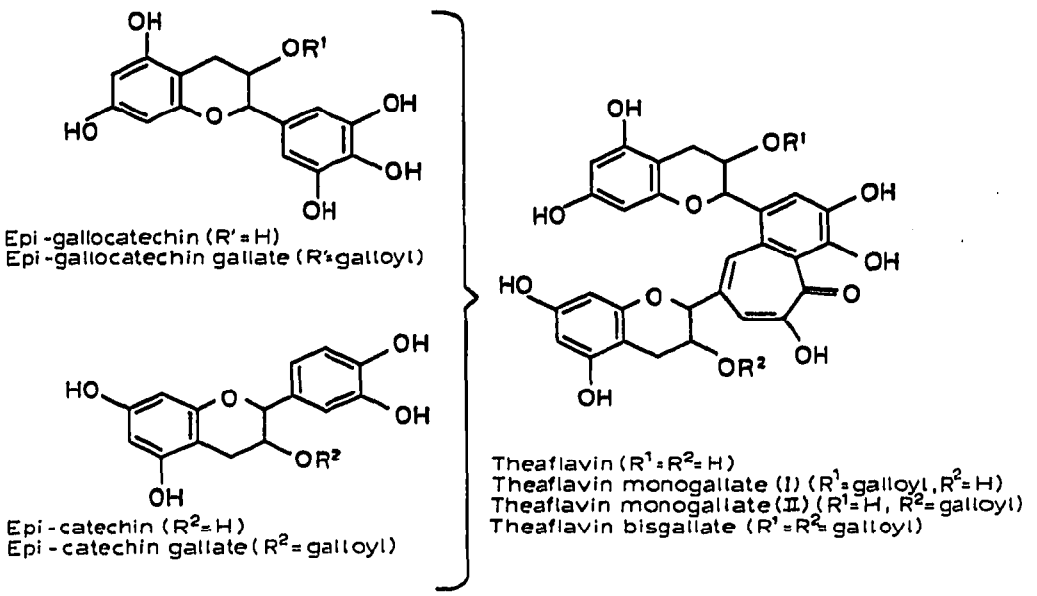
CHROM. 5071

The separation of theaflavins on Sephadex LH-20

Theaflavins, orange-red pigments with an important contribution to the appearance and "mouthfeel" of black tea liquors[1], are formed during tea manufacture by the oxidative condensation of epi-catechin, epi-gallocatechin and their galloyl esters (Fig. 1)[2,3]. They may be detected in tea extracts by column chromatography on



Sephadex LH-20 in 60% aqueous acetone[4], although this method gives no separation of the individual theaflavin species. It has now been found that separation of theaflavin, theaflavin monogallates and theaflavin bisgallate may be achieved by adsorption chromatography on Sephadex LH-20 in 35% aqueous acetone. Apart from a brief recent mention[5] this is the first published demonstration of the existence of the bisgalloyl ester of theaflavin in black tea.

Preparative method

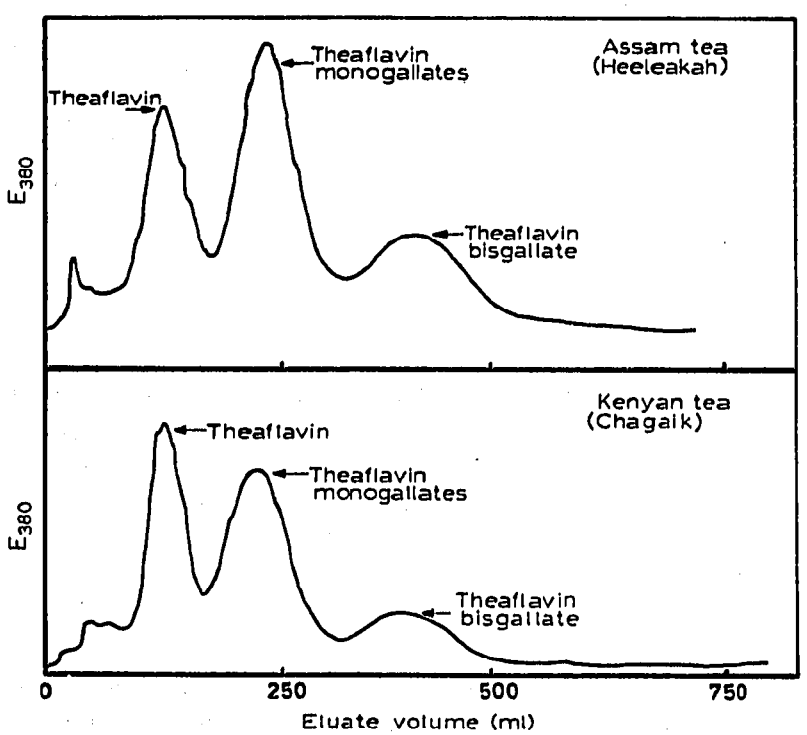
A slurry of Sephadex LH-20 (Pharmacia Ltd.) in 35% aqueous acetone was packed in a Whatman glass chromatography column of 2.54 cm × 30 cm (Reeve Angel Ltd.) and equilibrated with the same solvent. A crude theaflavin sample was prepared by extracting a chloroform-washed aqueous tea brew with ethyl acetate, washing briefly with 2.5% aqueous sodium bicarbonate solution followed by distilled water, and removing the ethyl acetate by rotary evaporation. The crude extract was dissolved in a water-*tert.*-butanol mixture, from which it was freeze dried, and 500 mg of solid was dissolved in a small volume of 35% acetone and applied to the Sephadex column.

Elution at the natural flow rate (0.5 ml/min) was continued for approx. 24 h. Initially, three purple bands were eluted (probably corresponding to decomposition

products of theaflavins) followed by two yellow bands (which paper chromatography revealed to occupy similar positions to the spots Q and Z of ROBERTS *et al.*[6] or the spots F and G of VUATAZ AND BRANDENBERGER[7]). The theaflavins remained on the column and moved only slowly, separating into three orange bands. By the end of the 24 h period the first orange band had been eluted, and the remaining two orange bands were collected by extruding the column, sectioning it, and extracting the Sephadex with 60% acetone. All three samples, after concentrating and freeze-drying, migrated as single spots on two-dimensional paper chromatography with 2-butanol–acetic acid–water (14:1:5) and acetic acid (2%) as solvents.

Analytical method

A smaller Whatman column (1.0 cm × 10 cm) was packed with Sephadex LH-20 in 35% acetone and equilibrated. A flow rate of 1 ml/min was maintained by a micropump (F. A. Hughes & Co.) and 20 mg of crude extract was applied to the column. The eluate was monitored at 380 nm by a Vitatron flow-through photometer unit (Fisons Scientific Instruments Ltd.) and displayed on a logarithmic-scale recorder. Typical traces from standard extracts of different teas are shown in Fig. 2.



Results and discussion

The identity of the theaflavin fractions was established by comparison with authentic synthetic samples of theaflavin, the isomeric monogallates and theaflavin bisgallate prepared by ferricyanide oxidation of the relevant flavanols[8]. Paper chromatography was in agreement with these conclusions.

The choice of 35% acetone as eluting solvent seemed to be optimum between satisfactory resolution and reasonable speed of operation. The resolution was noticeably improved, however, when the column was operated at 4° rather than at room

temperature. Further attempts to separate the monogallates by using more dilute acetone solutions as eluents have failed. This would seem to indicate that adsorption by hydrogen bonding to the phenolic and carbonyl oxygens is probably a major factor responsible for the separation[9], with a contribution also due to London forces which are proportional to molecular volume [10].

The analytical method is of use in comparing different tea samples. Thus Fig. 2 depicts a comparison between single estate teas from Assam and Kenya and indicates a marked difference between the degree of esterification of the theaflavin fraction in these two samples.

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