The Distribution of Polyphenols in the Tea Plant (*Camellia sinensis* L.)

By G. I. FORREST* AND D. S. BENDALL
Department of Biochemistry, University of Cambridge

(Received 31 December 1968)

1. Methods for the separation and determination of the polyphenolic components of the tea plant by thin-layer chromatography and colorimetric reactions have been devised. 2. High concentrations of catechins, flavonols and depsides were found to be restricted to the young vegetative and floral shoots, whereas leucoanthocyanins or flavylagens were characteristic of the more bulky axial tissues of the plant. 3. In the young shoots cell growth was correlated with an increasing degree of flavonoid B-ring hydroxylation. 4. Maximal flavylagen concentrations occurred in the outer protective layers of stem and of seed coat. 5. Mature leaves were shown to contain derivatives of the flavones apigenin and luteolin. 6. Developing seedlings showed a steady increase in polyphenol complexity; flavylagens were concentrated at shoot and root apices and accumulated at the stem base. 7. It is postulated that the flavanols (leucoanthocyanins and catechins), because they can co-polymerize, are of use to the plant for protection of wood and bark against infection and decay.

The young vegetative shoots of the tea plant are known to contain a wide range of polyphenols, a number of which, especially the catechins, are present in high concentration. A study of the metabolism of the species may therefore be expected to throw light on fundamental problems of polyphenol function and metabolism in plants generally. In addition, it is the endogenous flavonoids of the young shoots that make the manufacture of black tea from them possible; and since the most important characteristics of a black tea liquor are believed to be primarily dependent on the quantities and relative proportions of the different flavonoids in the fresh shoots, it is very important from the point of view of the quality potential of made tea that the factors controlling the polyphenol constitution of the vegetative shoots should be elucidated.

A prerequisite of any such study is an investigation of the distribution of the polyphenols over the entire plant, so that the relative importance of each class of compounds in the general economy of the plant may be estimated, and to provide a guide not only to the planning of further experiments but also to the interpretation of their results.

There have been several recent attempts to measure the concentrations of individual catechins in different parts of the shoot, e.g. Vuataz, Brandenberger & Egli (1959), Zaprometov (1963), Nakagawa & Torii (1964a,b) and Bhatia & Ullah

(1968); but these observations have been somewhat restricted in the classes of phenol surveyed and the range of tissues examined. The aim of the present work was to provide a reasonably comprehensive account of the polyphenol distribution throughout the plant, so far as our present knowledge of these compounds permits.

MATERIALS AND METHODS

Plant material. Most of the work has been carried out on plants grown in a heated greenhouse in Cambridge Botanic Gardens. Most were about 7 years old and were predominantly of the Assam variety, *Camellia sinensis* var. *assamica* (Masters) Kitamura. They originated from Malawi (then Nyasaland), and were grown in soil in large pots and were watered regularly with a nutrient solution. Several batches of seeds were sent from Malawi by air in June 1965, together with fresh samples of young shoots taken from the mother trees. The seeds were all open-pollinated; the best material proved to be of the Lujeri Estate seedbearers MT7 and MT9. After being soaked overnight, the seeds were planted 1 in. deep in unsupplemented peat in the greenhouse; germination generally took 2–4 weeks.

Preparation of extracts. Tissues were extracted either fresh or after freeze-drying. Freeze-dried samples were stored in small stoppered bottles over silica gel at -15° . All tissues were extracted with aq. 50% (v/v) methanol. Soft tissues were extracted by grinding with Ballotini no. 12 beads in a chilled mortar in cold methanol, followed by centrifuging off the bulk of the residue in a bench centrifuge, and then precipitating chlorophyll and other material by centrifuging in a Servall high-speed angle-head centrifuge

* Present address: The Nature Conservancy, Moor House Field Station, Garrigill, Alston, Cumberland.

or in an MSE 18 centrifuge at 10000 rev./min. for 15 min. at 4°. The pellets were re-extracted several times, and the combined supernatants concentrated in a rotary evaporator. Woody tissues were extracted by grinding them to dust with a heavy file, and the seedcoat was extracted by hammering it to a fine powder and then treating the powders as above. The cotyledon extract was shaken with chloroform to remove the fat; the emulsified milky fat layer together with the bottom chloroform layer were re-extracted several times with methanol, and the clear upper methanol layers were combined and concentrated to give the final extract. A number of embryos (2–3 mm. long) were combined to give an extract sufficiently concentrated for analysis.

Separation techniques. T.l.c. was used for polyphenol separation, having the advantages over paper chromatography of high resolution, sensitivity, speed of development and ease and efficiency of elution from the layers. The equipment was supplied by Desaga Co., Heidelberg, Germany. Cellulose layers were found to be preferable to polyamide or silica-gel ones, and the procedure developed was as follows. Two-dimensional plates were spread with cellulose powder MN-300 made by Macherey, Nagel and Co., Düren, Germany, to a thickness of 0.25 mm. After the layers had dried, the solutions were applied with either micropipettes or a microsyringe (Hamilton Microliter; Hamilton Co., Whittier, Calif., U.S.A.). The plates were run in water or in aq. 5% (v/v) methanol in the first direction (the latter solvent giving somewhat better separation of the flavonol glycosides), and in butan-1-ol-acetic acid-water (4:1:5, by vol., upper phase, aged) in the second direction. The spots were located by their absorption or fluorescence in u.v. light in the absence and presence of NH₃ vapour.

Identification of polyphenols. Roberts and his co-workers (e.g. Roberts, Wight & Wood, 1958) established the identity of a number of spots on paper chromatograms of extracts of tea shoots, using a rather similar solvent system to that presently adopted. The general pattern of spots obtained on cellulose layers in our work was very similar, and it was reasonable to expect the relative positions of most of the major components to tally in the two cases. However, independent evidence of their identification was obtained in the following ways.

(a) Authentic markers. The locations of the six main catechins, rutin and gallic acid were checked by running marker solutions of each. From the position of rutin the locations of the other flavonol glycosides were confirmed.

(b) *R_F* values. Published *R_F* values are available for a large number of polyphenols on paper chromatograms. Most of the values for tea polyphenols have been found to be applicable also to cellulose thin layers, although the *R_F* values on thin layers are somewhat variable.

(c) Colour reaction. Ultraviolet fluorescence and chromogenic sprays confirmed the identities of the main components. The characteristics of the u.v. fluorescence of the main groups of compounds were described by Harborne (1959). The spray reagents used were bis-diazotized benzidine, toluene-*p*-sulphonic acid, vanillin-toluene-*p*-sulphonic acid, aluminium chloride and the ferric reagent; details were given by Roux & Maihs (1960) and Gage & Wender (1950). The benzidine spray reagent was found to be the most generally useful since it is one of the most sensitive and selective.

(d) Spectra of eluates. Spectra of catechins eluted from

chromatograms were found to have maxima within 1–2 nm. of the values given by Vuataz *et al.* (1959) when read against cellulose blanks. Spectral characteristics were also useful confirmatory evidence for the identification of the flavones and flavonols.

Determination of polyphenols

Catechins. After a number of preliminary experiments the following method of determination was worked out. The catechins were located on chromatograms by their purple fluorescence under u.v. light in the presence of NH₃, and the spots were scraped off the plate into 15 ml. glass centrifuge tubes with a microspatula, and homogenized in 3 ml. of aq. 50% (v/v) ethanol in a small glass homogenizing tube (Wesley Coe Ltd., Cambridge), internal volume 5.5 ml., by using an 8 mm. mechanical stirrer [Baird and Tatlock (London) Ltd., Chadwell Heath, Essex] with a strong torque, fitted with a small hard-plastic homogenizer with a metal stem. The cellulose was spun off in a bench centrifuge, and a suitable volume of the supernatant was used for the determination. It was made up to 2 ml. (if necessary) with 50% (v/v) ethanol and cooled to 0° on ice. Recovery of catechins from chromatograms was shown to be quantitative. The acid benzidine and NaNO₂ solutions were cooled to 5° in a stirred cold-water bath, and 6 ml. of the acid benzidine solution (made by stirring 5 g. of benzidine with 14 ml. of conc. HCl and dissolving the suspension in 980 ml. of water) was added to 4 ml. of 1.45 M-NaNO₂ in a small conical flask, and the mixture shaken. After 10 min., when the solution was a clear yellow, 6 ml. was pipetted into 114 ml. of water at 0° on ice, and this solution was shaken. This was the reaction mixture, a clear pale yellow; it was kept at 0°, and was stable for at least 2 hr. To each tube of catechin solution 2 ml. of the reaction mixture was added at a known time, and the tube shaken and kept at 0°. After 24 min. the extinction at 450 nm. was read on the Unicam SP.500 spectrophotometer against a distilled-water blank. Blank values, obtained by adding the reaction mixture to 2 ml. of aq. 50% (v/v) ethanol, were found to rise slightly as the reaction mixture aged, although the net extinction due to catechin in the experimental solutions, when corrected for this blank value, remained constant. When reading a series of solutions it was found sufficient to include one blank tube for every ½ hr. or so during which the reagent was used. Blank values in fact were small, with extinctions in the range 0.015–0.020. This method gave highly reproducible results, and the sensitivity was sufficient to determine accurately less than 1 µg. of catechin; cellulose blanks were negligible. The curves were linear up to extinctions >1.0; the simple catechins on a weight-for-weight basis gave identical extinction coefficients, whereas the catechin gallates gave somewhat lower extinction coefficients. The factors for the conversion of extinction at 450 nm. to weight of catechin in µg. in the 2 ml. sample estimated were: simple catechins, $E_{450} \times 37.8$; catechin gallates, $E_{450} \times 43.3$.

In some analyses an alternative method of catechin determination was used. This method, developed in collaboration with Dr R. T. Ellis, was based on the vanillin reaction as described by Swain & Hillis (1959). To each of the catechin spots scraped off into tubes was added 1 ml. of water, followed after shaking by 5 ml. of the vanillin reagent [1 g. of vanillin dissolved in 100 ml. of 70% (v/v)

H₂SO₄, made up fresh every 3 days and stored at 4°; the solution was shaken to mix it; the acid soon dissolved the cellulose, and after 15 min. the extinction at 500 nm. was read. A blank was prepared by adding the reagent to 1 ml. of water. Conversion factors for the weight of catechin in μ g. from the extinction at 500 nm. were: epigallocatechin gallate, $E_{500} \times 76.9$; epicatechin gallate, $E_{500} \times 72.7$; epigallocatechin, $E_{500} \times 62.9$; galocatechin, $E_{500} \times 62.9$; epicatechin, $E_{500} \times 51.8$; catechin, $E_{500} \times 44.6$.

This method was less sensitive than the benzidine method, and the reagent blanks were higher (extinctions 0.05–0.07), but it was simpler to carry out, the results were reproducible and the cellulose blanks were negligible.

Flavonol glycosides. These are commonly determined by the use of bathochromic shifts induced by aluminium salts (e.g. Dowd, 1959), but difficulty was experienced in obtaining efficient elution from cellulose. Conc. H₂SO₄ also caused large bathochromic shifts in the visible extinction peaks of the flavonols and of their glycosides, and since the cellulose was simultaneously dissolved this was made the basis of a determination procedure. The shifts induced were of the same degree of magnitude as those caused by AlCl₃; for example, the visible extinction peak of rutin was shifted 65 nm. There was a linear relation between the peak extinction and the amount of flavonol present. To render the method sufficiently sensitive, it was found convenient to add 1.0 ml. of 50% (v/v) H₂SO₄ to each tube containing a cellulose spot. After 1 hr., when the cellulose had been dissolved with occasional shaking, the tubes were centrifuged and the extinctions of the resulting yellow solutions read at the peak wavelengths in micro-cuvettes in the Unicam SP.500 spectrophotometer. A cellulose blank extinction, directly proportional to the weight of cellulose, was corrected for by preparing standard graphs of the extinctions at the extinction maxima of the various glycoside-acid complexes, against weight of cellulose. It was necessary then to weigh the cellulose of each spot before determination. Each of the glycosides had its peak extinction at a characteristic wavelength in the presence of H₂SO₄; the bathochromic shift induced was slightly extended in the presence of cellulose by about 10 nm., and it was therefore this wavelength that was used for determinations after dissolution of the cellulose.

Standard curves were prepared by using samples of quercetin and of its two glycosides quercitrin and rutin; they gave similar but distinct graphs that were linear up to extinctions >1.0. Since it was not possible to obtain authentic specimens of the other tea flavonol glycosides, conversion factors derived from these graphs were applied also to kaempferol and myricetin derivatives, with the following results; the factors are for the conversion of corrected extinction to μ g. of glycoside: kaempferol glucoside, $E \times 16.5$; kaempferol rhamnoglucoiside, $E \times 24.4$; quercetin glucoside, $E \times 17.1$; quercetin rhamnoglucoiside, $E \times 25.1$; myricetin glucoside, $E \times 17.7$; myricetin rhamnoglucoiside, $E \times 25.8$.

The technique has been extended to differentiate between flavone and flavonol glycosides, since the extinction peak of the former is shifted to about 400 nm. and of the latter to about 420–440 nm.

Other polyphenols. The chief members of this group were chlorogenic acids, *p*-coumarylquinic acids, gallic acid, theogallin and an unidentified component G-36. The Folin-Ciocalteu reagent was found to be suitable for their deter-

mination (Folin & Ciocalteu, 1927), and the following procedure was adopted. Spots located on chromatograms were eluted by homogenizing them in 1.5 ml. of 50% (v/v) ethanol, and the cellulose was centrifuged off. To 1 ml. samples of the supernatant was added 0.5 ml. of Folin-Ciocalteu reagent followed 3 min. later by 0.5 ml. of 1M-Na₂CO₃, and the tubes were shaken. After 1 hr. the extinction was read at 725 nm. in micro-cuvettes against a distilled-water blank. A reagent blank was made up by adding the reagent to 1 ml. of 50% (v/v) ethanol and also reading this against water after 1 hr. A standard curve was prepared with gallic acid recrystallized three times from water and made up in aq. 50% (v/v) ethanol, and results for other compounds were expressed in terms of the equivalent concentration or weight of gallic acid. Goldstein & Swain (1963) showed that the reaction products of gallic acid and of chlorogenic acid with the Folin-Denis reagent (Folin & Denis, 1915) have very similar molar extinction coefficients.

Flavylogens. This term was given by Swain (see Seakins, 1959) to those flavonoids or flavonoid polymers that form anthocyanidins when heated with mineral acids. Such compounds include not only monomeric leucoanthocyanins (flavan-3,4-diols), but also their polymers, and their copolymers with catechins. In fact the reaction is given by condensed tannins and their flavandiol precursors. The yield of anthocyanidin is low (Swain & Hillis, 1959), and Roux & Paulus (1962) showed that it is dependent on the degree of polymerization of the flavylogen. Estimations were carried out by the procedure of Swain & Hillis (1959). Since flavylogens from different sources vary so much in the extent of their conversion into anthocyanidins, results of the determinations have been given simply in terms of the relative extinctions or of 'total extinction' per organ; the present state of knowledge of these compounds does not

Table 1. *Tests of the specificity of the potassium titanium oxalate method for the determination of phenols*

For details of the method, see the text

Compound tested	Quantity taken (μ moles)	E_{445}
<i>o</i> -Cresol	100	0.013
<i>p</i> -Cresol	100	0.013
Guaiacol	100	0.023
Catechol	2	0.436
Resorcinol	100	0.025
Hydroquinone	100	0.064
Protocatechuic acid	2	0.447
Pyrogallol	2	0.480
Phloroglucinol	100	0.037
Gallic acid	2	0.499
<i>o</i> -Phenylenediamine	100	0.008
Glucose	100	0.007
Mannose	100	0.007
Glycine	133	0
L-Glutamic acid	68	0
L-Tyrosine	55	0
L-Ascorbic acid	8	0.191
DL-Malic acid	20	0.006

warrant the use of more definite methods of expression of results.

Total phenols. The method used was based on potassium titanium oxalate, and was developed by D. S. Bendall & R. Hill (unpublished work). The reagent is believed to be nearly specific for *o*-dihydroxyphenols (although it does react with ascorbic acid and hydrogen peroxide) as shown in Table 1, and is thus suitable for the determination of tea polyphenols. The procedure was to place 2 ml. of 0.1M-potassium titanium oxalate and 1 ml. of 0.35M-tris in each tube (final pH 6.7) and to add a suitable volume of the experimental solution, ensuring that the methanol concentration was the same in each tube. The total volume was made up to 10 ml. with water, and 10 min. after the addition of the experimental solution the extinction at 445 nm. was read against distilled water. A blank contained no polyphenol solution. A standard graph prepared with pyrogallol was linear up to extinctions of at least 1.5, and passed through the origin. Results are expressed in terms of the equivalent weight or concentration of pyrogallol.

Chemicals

(+)-Gallocatechin, (−)-epigallocatechin, (−)-epigallocatechin gallate and (−)-epicatechin gallate were prepared from air-dried green shoots of tea by the method of Vuataz *et al.* (1959) (see Gregory & Bendall, 1966). The catechins were recrystallized once from water and dried *in vacuo* over P₂O₅. (−)-Epicatechin, obtained from L. Light and Co. Ltd., Colnbrook, Bucks., and (+)-catechin, from Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A., were recrystallized from water. All catechins were stored over silica gel at −15°. Quercetin and rutin were supplied by Koch-Light Laboratories Ltd., Colnbrook, Bucks., and a sample of quercitrin was given by the British Dyewood Co. Ltd., Glasgow. Folin-Ciocalteu reagent was supplied by British Drug Houses Ltd., Poole, Dorset, and was stored at 4°.

RESULTS

Distribution in young shoots of mature plants

Vegetative shoots. Fig. 1 illustrates the distribution of the catechins in the young shoot of a seed-bearer of the clone MT 7 from Lujeri Estate, Malawi, and in the normal plucked shoot of the clone Chisunga 5/E4 grown at Cambridge. The two outstanding features were the decrease in catechin gallates and the increase in epigallocatechin as the leaves matured, hydroxylation of the catechins gradually predominating over esterification. There was a close correlation between growth and catechin synthesis: in clone MT 7 the catechins never varied more than between 2.5 and 3.5% of the fresh weight at all stages of leaf development.

The six main flavonol glycosides occurring in tea are the glucosyl and rhamnoglucosyl derivatives of kaempferol (one B-ring hydroxyl group), quercetin (two hydroxyl groups), and myricetin (three hydroxyl groups). The flavonol distribution is shown in Fig. 2: as with the catechins, the leaf as it matured acquired a steadily increasing power to

Fig. 1.* Catechin concentrations in the young vegetative shoots of the clones (a) MT 7 grown at Lujeri Estate, Malawi, and (b) Chisunga 5/E4 grown in Cambridge. Leaves are numbered from apex downwards as L1 etc. ▲, EGCG; ●, EGC; ○, EC; ■, ECG; △, GC; □, C.

* Abbreviations used in figures and tables: AB, apical bud; B, blue; C, (+)-catechin; CA, chlorogenic acids; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; G, green; GA, gallic acid; GC, (+)-gallocatechin; K, kaempferol; Q, quercetin; M, myricetin; KG, QG, MG, 3-glucosides of K, Q and M respectively; KRG, QRG, MRG, 3-rhamnoglucosides of K, Q and M respectively; LA or +, leucoanthocyanin; O, origin; P-, pale; P, purple; QA, *p*-coumarylquinic acids; TG, theogallin (galloylquinic acid); Y, yellow.

Fig. 2. Flavonol glycoside concentrations in the young vegetative shoots of clone MT7. Leaves are numbered from apex downwards as L1 etc. Glycosides of: ●, M; △, Q; □, K.

carry out B-ring hydroxylation. The diglycosides showed a more rapid increase in concentration than the corresponding monoglycosides in younger leaves, but fell off more rapidly during further growth and eventually lagged behind, so that in older leaves the glucosides predominated over the rhamnoglucosides. In this respect there appeared to be a further parallel with the catechins, the decreasing proportion of further glycosylation with a rhamnose unit being similar to the decreasing esterification of catechins with gallic acid. In the clone-MT7 shoot the maximum weight of flavonol glycosides attained per leaf, about 4mg., was about one-tenth of that reached by the catechins, about 43mg.; both maxima occurred in the fourth leaf from the apex.

In the stems of young shoots the most obvious feature was the comparative dearth of polyphenols (compare Figs. 3 and 4). The galloatechins were undetectable in the mature stem and in the very young stem, so that the only three catechins typical

Fig. 3. Thin-layer cellulose chromatogram of a methanolic extract of the third leaf of young vegetative shoot of clone MT7. The solvent fronts are shown as solid lines.

of stem tissues were catechin, epicatechin and epicatechin gallate; however, the three gallo catechins were superimposed on these in the region of maximum enlargement of the young leaves. The overall result of these features was that the catechin concentration of the stems of young shoots as plucked for manufacture of black tea was about half that of the total shoots, the greatest decrease being in the most complex catechins: gallo catechin and especially catechin were more concentrated than in the shoot as a whole. The flavonol composition of the stem was distinct from that of the leaves; myricetin glucoside and rhamnoglucoside were the only known components and were accompanied by two other major unidentified flavonols Y2 and Y3 (Fig. 4), which were also present as minor

components in leaves. The stem flavonols paralleled the catechins in that synthesis was decreased compared with leaves, and the temporary appearance of myricetin recalled that of the gallo catechins in the youngest internodes.

Flavylogen synthesis paralleled leaf growth fairly closely (Table 2), but was typical of stems rather than of leaves. In both stems and leaves there was an increase of chromatographically more mobile components during aging; however, in the stem they were partially replaced by a streak staining orange with the benzidine reagent (Fig. 4), evidence of polymerization enlarged upon by Forrest & Bendall (1969). Gallic acid was maximal in the youngest leaves and stem, the concentration rapidly falling off during maturation (Table 2); theogallin showed a very marked maximum in the apical bud, declining rapidly thereafter, whereas the compound G-36, similar in colour reactions to theogallin and gallic acid and yielding gallic acid on mild acid hydrolysis in 2M-hydrochloric acid at 60° for 10min., was maximal in the first leaf but completely absent from the stem throughout its length. The distributions of these three compounds, as well as of the catechin gallates, serve to show the abundance and the localization of gallic acid in both free and combined forms in the apical regions of the shoot. Chlorogenic acids and *p*-coumarylquinic acids were more concentrated in the stem than in leaves; again, they were characteristic of the young regions of the shoot.

Floral shoots. Compared with the leafy shoot, the most striking features of the floral shoots of the clone Chisunga 5/E4 were the abundance of leucoanthocyanins and of unidentified flavonol glycosides, the high gallo catechin/epicatechin ratio, the very low chlorogenic acid concentrations and the absence of *p*-coumarylquinic acid. Equally important, however, was the broad similarity between the catechin compositions of the two types of shoot, and Fig. 5 shows how the distribution throughout the flower, considered as a modified terminal shoot, paralleled that in the leafy shoot: from the apical

Fig. 4. Thin-layer cellulose chromatogram of a methanolic extract of the mature stem (fourth internode) of young vegetative shoot of clone MT7. The solvent fronts are shown as solid lines.

Table 2. Concentrations of components of the young shoot of clone *Lujeri MT 7*

Results are given as % of fresh wt. as equivalent of gallic acid, except for leucoanthocyanins, where they are given as $E_{550}/g.$ fresh wt. Leaves are numbered from the apex downwards as L1 etc.

	AB	L1	L2	L3	L4	L5
Leaf wt. (g.)	0.10	0.23	0.48	1.14	1.27	0.96
GA	0.098	0.098	0.036	0.013	0.010	0.012
TG	0.263	0.127	0.070	0.040	0.032	0.031
CA	0.176	0.160	0.116	0.087	0.069	0.072
QA	0.041	0.016	0.038	0.020	0.028	0.031
Compound G-36	0.072	0.141	0.118	0.057	0.018	0.016
Total leucoanthocyanins	10.0	7.5	6.8	6.9	7.0	9.4

Fig. 5. Histogram of catechin compositions of different parts of the flower. (a) Ovary; (b) stamens; (c) corolla; (d) calyx.

ovary to the calyx, the most distal part, there was a gradual increase in complexity of the predominating catechins. During development of the flower bud (Table 3) catechin synthesis kept very good pace with growth, even during the final stages when there was a large increase in weight during opening of the flower. None of the individual catechins showed any remarkable changes; galocatechin showed the greatest alteration, decreasing to about half its initial concentration owing to the differential growth in later stages of organs in which its concentration was relatively low. As in the leafy shoot, theogallin was maximal at the apex, in the ovary (Table 4), and also during bud development it was maximal in the youngest buds. Other notable features were the confinement of chlorogenic acid to the calyx and corolla, and the marked increase in the concentration of gallic acid during bud growth, followed by a sharp decrease, not only in concentration but also in weight per flower, during the opening of the flower.

Table 3. Concentrations of components of flower buds from clone Chisunga 4/E5 at different stages of development

Results are given as % of dry wt., except results for theogallin and compound G-36, which are given as % of dry wt. as equivalent of gallic acid, leucoanthocyanins, which are given as $E_{550}/g.$ dry wt., and total phenols, which are given as equivalent mg. of pyrogallol/g. dry wt.

Stage no.	1	2	3	4	5	6
Mean fresh wt. of bud (mg.)	28.1	67.8	97.1	133.8	189.0	355.0
EGC	0.38	0.32	0.36	0.42	0.32	0.31
EGCG	4.76	4.96	6.31	6.30	5.51	3.50
ECG	1.68	1.67	2.21	2.09	1.80	1.21
GC	1.24	0.94	0.78	0.82	0.63	0.50
EC	0.16	0.12	0.11	0.09	0.16	0.18
C	0.24	0.19	0.16	0.16	0.13	0.09
Total catechins	8.46	8.19	9.94	9.87	8.55	5.79
TG	0.52	0.44	0.41	0.38	0.34	0.32
GA	0.17	0.20	0.30	0.33	0.33	0.12
Compound G-36	0.21	0.14	0.10	0.11	0.15	0.13
Total leucoanthocyanins	41.0	35.2	48.6	51.5	33.9	33.0
Total phenols	46.0	48.5	56.0	57.5	55.9	50.0

Table 4. Concentrations of components of the floral shoot

Results are given as % of dry wt. as equivalent of gallic acid, except results for leucoanthocyanins, which are given as $E_{550}/g.$ dry wt., and total phenols, which are given as equivalent mg. of pyrogallol/g. dry wt.

	Ovary	Stamens	Corolla	Calyx
Mean dry wt. (mg./flower)	3.2	38.3	27.2	9.2
Ratio dry wt./fresh wt.	0.251	0.239	0.147	0.391
GA	0.057	0.132	0.068	0.057
TG	0.342	0.191	0.166	0.145
CA	—	—	0.117	0.061
Compound G-36	0.133	0.072	0.072	0.110
Total leucoanthocyanins	105.0	25.5	37.2	70.3
Total phenols	56.5	31.5	36.4	51.3

Distribution in mature tissues

Foliar tissues. (a) Mature leaves. Aging leaves became dark green and leathery with a high proportion of woody tissue in the veins, but catechin synthesis was still active. The trends in the composition set up during maturation were continued, with epigallocatechin becoming increasingly predominant over its gallate (Table 5). Epicatechin gallate in particular was much decreased, to below the concentration of epicatechin. The flavonol glycosides were present only in low concentrations, but two flavone glycosides designated F-29 and F-30, whose structures are considered below, were characteristic of mature and old leaves (see Fig. 6). Synthesis of both depsides and leucoanthocyanins was decreased. Chromatograms were characterized by a number of small spots fluorescing pale yellow in u.v. light, the strongest of them being compounds Y-31 and Y-32 (Fig. 6), which were shown by sulphuric acid-induced spectral shifts to be flavonol

glycosides, and by a smaller number of stronger spots fluorescing blue to blue-purple.

(b) Fruit and seed. In the immature fruit the differences in catechin composition from opening flower buds can be accounted for almost entirely by the loss of stamens and petals, only those parts rich in polyphenols remaining (Table 5). This represents a conservation of catechins by the plant, since the high flavanol concentrations present in the flower are either carried over into the succeeding generation or used in production of the seed.

Mature seeds sent from Malawi were divided into the hard brown seed coat, cotyledons and embryo. Epicatechin and catechin were the only catechins detectable in the seed coat, and were present in very low concentrations, but the flavylogen content was high, about 12 discrete leucoanthocyanin spots appearing on chromatograms together with heavy streaking from the origin in the two solvent systems. Leucoanthocyanins were the only polyphenols detectable in cotyledon extracts; the main com-

Fig. 6. Thin-layer cellulose chromatogram of methanolic extract of old-leaf tissue of the clone S.61, grown in Cambridge. The solvent fronts are shown as solid lines.

Table 5. *Concentrations of polyphenols in various tissues*

Results are given as % of fresh wt. for all tissues except those for pith, which are given as % of dry wt., leucoanthocyanins, which are given as $E_{550}/g.$, and total phenols, which are given as equivalent mg. of pyrogallol/g. The relative quantities of substances, estimated by inspection of chromatograms, are shown by numbers of $\times$ signs.

Clone	Apical banjhi leaf	Mature leaf (L9)	Old leaf	Flower bud	Immature fruit	Mature stem		
						First year	Second year	
						(19)	Pith	Phloem
Clone	S.61	R/52	S.61	S.61	S.61	R/52	S.61	IN/27
EGC	0.89	1.66	1.57	0.07	0.08	—	0.25	$\times \times$
EGCG	0.85	1.04	0.77	0.32	0.52	—	0.17	$\times \times$
ECG	0.25	0.16	0.15	0.17	0.25	0.11	0.12	$\times \times \times$
GC	0.15	0.18	0.08	0.17	0.20	—	0.06	$\times$
EC	0.14	0.27	0.16	0.07	0.18	0.14	0.29	$\times \times \times$
C	0.02	0.02	0.01	0.06	0.07	0.03	0.03	$\times$
Total catechins	2.30	3.33	2.75	0.86	1.30	0.28	0.92	
GA	$\times$	($\times$)	—	$\times$	$\times$	—	—	—
TG	$\times$	$\times$	$\times$	$\times \times$	$\times$	—	($\times$)	($\times$)
CA	$\times$	$\times$	—	$\times \times$	$\times$	—	$\times$	—
QA	$\times$	$\times$	—	$\times$	—	—	—	—
Compound G-36	$\times$	—	($\times$)	$\times$	$\times$	—	—	—
Flavonols	MG	MG, QG	MG, MRG, QRG, KRG	MG, MRG, QRG	—	—	—	MG
Flavones	$\times$	$\times \times$	$\times \times$	—	—	—	—	—
Total leucoanthocyanins	6.0	5.7	4.7	14.0	19.3	6.9	35.0	$\times \times$
Total phenols	10.7	14.6	6.8	11.7	12.6	3.8	1.8	

ponent formed an elongated spot that fluoresced red under u.v. light and green with ammonia, and stained brick-red with the benzidine reagent, the colour developing 2 min. after spraying and slowly increasing in intensity. The total flavylogen content was low. Embryos contained epicatechin and a trace of catechin, but higher amounts of kaempferol glucoside and rhamnoglucoside, the latter predominating; flavylogens were low in amount, but more concentrated than in the cotyledons.

Axial tissues. (a) Mature young stem. While the stem was still in its first year of growth, before the vascular cambium became fully active, only the three catechins typical of young stems were present, the gallo catechins being absent; depsides and flavonols were absent, but flavylogens were quite concentrated (Table 5). Stems between 1 and 2 years old, in which there was an intensely green layer of secondary phloem beneath the brown periderm, had a much richer polyphenol composition. The most abundant catechins in the phloem layer were epicatechin and its gallate, gallo catechins being less concentrated; this recalls the situation in the shoot apex, although here epicatechin was relatively much more concentrated than in the shoot. Leucoanthocyanins were abundant in the phloem, but myricetin glucoside

was the only detectable flavonol. In the pith all catechins were very low in amount, epicatechin predominating as in the young stem (Table 5); leucoanthocyanins accounted for a large proportion of the low total phenol content.

(b) Old stem. Bark peeled off the boles of bushes of several clones separated easily into grey periderm and intensely green phloem (including cortex and cambium). The periderm was extremely deficient in polyphenols, the only compounds present being leucoanthocyanins; chromatograms sprayed with the benzidine reagent showed considerable streaking, indicating polymerization, about six orange-brown leucoanthocyanin spots, and four elongated bright-yellow spots probably indicating 5-deoxy-leucoanthocyanins (Roux & Maihs, 1960). The phloem, however, was relatively rich in polyphenols; the three stem catechins were present, with gallo catechins absent. Gallic acid, theogallin and compound G-36 were present, and flavylogens were extremely concentrated, with a high proportion of brown-to-yellow-staining leucoanthocyanin spots.

Samples of wood were obtained from a 6-year-old branch of a bush of the clone Likanga 8/D4. The wood was pale green, and the extracts were dull purplish grey from the outer wood and grey-green from the middle and inner wood. The three fractions gave rather similar chromatograms, most of the

Table 6. *Summary of polyphenol distribution in the tea plant*

The relative amounts of substances, estimated by inspection of chromatograms, are shown by numbers of x signs.

	EC and C	ECG	EGCG	EGC and GC	LA	CA	QA	Compound G-36	TG	GA	Flavonols	Flavones
Apical bud	x	x x	x	x	x x	x	x	x	x x	x	—	—
Leaf	x	x x	x x	x	x	x	x	x	x x	x	—	—
Young	x	x x	x x	x x	x	x	x	x	x x	x	x	x
Mature	x	x x	x x	x x	x	x	x	x	x x	x	x	x
Old	x	x	x	x	x	—	—	—	x	—	x	x
Flower												
Bud	x	x x	x	x	x x	x	—	x	x	x	x	—
Ovary	x	x x	x	x	x x	—	—	x	x	—	?	?
Stamens	—	x	x	—	x	—	—	—	x	x	?	?
Corolla	—	x	x	—	x	x	—	—	x	—	x	?
Calyx	x	x	x x	x	x x	x	—	x	x	x	?	?
Seed												
Coat	x	—	—	—	x	—	—	—	—	—	—	—
Cotyledon	—	—	—	—	x	—	—	—	—	—	—	—
Embryo	x	—	—	—	x	—	—	—	—	—	x	—
Stem												
Young	x	x x	x	x	x x	x	x	—	x	x	x	—
Mature												—
Phloem	x	x x	x	x	x x	—	—	—	—	—	—	—
Pith	x	x	—	—	x	x	x	—	—	—	—	—
Old												
Periderm	—	—	—	—	x x	—	—	—	—	—	—	—
Phloem	x	x	—	—	x x	—	—	—	—	—	—	—
Xylem	x	—	—	—	x x	—	—	—	—	—	—	—
Root												
Young	x	—	—	—	x x	—	—	—	—	—	—	—
Old												—
Bark	x	—	—	—	x x	—	—	—	—	—	—	—
Xylem	x	—	—	—	x x	—	—	—	—	—	—	—

spots being brown-staining leucoanthocyanins, with streaking also present; yellow-staining leucoanthocyanins were very minor components and few in number. The only identifiable component was epicatechin, which was moderately concentrated; all other catechins were absent.

(c) Root. Samples were taken from the same bush of Likanga 8/D4 of root apices (growing as a mass against the sides of the pot, pale yellow to white in colour), young root (diameter approx. 1.5 mm., with brown bark), and mature root bark and wood taken from the oldest parts of the root near the stem base. All parts of the root system were very deficient in the shoot polyphenols, epicatechin and leucoanthocyanins being the only identifiable components; the latter were, however, very concentrated particularly in the bark and young root, and epicatechin concentration was high in all parts of the root. On chromatograms two striking features of the leucoanthocyanins were the extensive and often heavy streaking accompanying the discrete spots, and the high proportion and number of components staining bright canary yellow with the benzidine reagent.

Table 6 summarizes the polyphenol distribution over the plant.

Germination and growth of seedlings

The only catechins detectable in the embryo (see above) were catechin and epicatechin. During germination of seeds from clones MT 7 and MT 9 (see Table 7), even before the plumule had emerged, the catechin gallates were present, with epicatechin gallate predominating, and after emergence of the plumule epigallocatechin gallate showed a large increase, but it did not become dominant until leaf development occurred (Table 8). The total catechins constituted about 2.5% of the fresh weight of the shoot from plumule emergence until the stem had attained a height of 10 cm. and two normal leaves had grown. During this time an enormous increase in size had taken place, so that catechin synthesis kept very good pace with growth (Tables 7 and 8).

The first normal leaves formed, after three or four 'scale' and dwarfed leaves, had a catechin composition similar to that of the leaves of a mature plant, with epigallocatechin gallate predominating.

However, the distribution of the individual catechins throughout the seedling shoot differed from a normal shoot in that there was no obvious increase in epigallocatechin down the shoot, although the gallates became more concentrated in the more apical leaves (Table 8). Indeed, the galocatechins were maximal in the most apical leaves of the youngest seedlings, whereas epicatechin and catechin increased in the older leaves. This distribution was probably a consequence of the earliest leaves not having achieved the full synthetic capacity of a normal leaf, so that they contained a greater proportion of the simpler catechins, whereas later-formed leaves were better able to elaborate on the simple catechin structure. As the shoot emerged from its first banjhi (dormant) stage after growth of the first three normal leaves, it was found that, in two seedlings examined, two of these older leaves of the first flush had very low catechin concentrations (S4 and S5, Tables 8 and 9). The implication of this is obscure, but translocation would seem to be the only reasonable explanation. As soon as the second flush was under way, there was a renewal of differential gallate synthesis (Tables 8 and 9b); and the shoot soon came to have the catechin distribution typical of that of shoots of mature plants, with galocatechins accumulating in the older leaves.

Flavylogens were more concentrated in the stems of young seedlings than in the leaves, and concentrations were maximal at shoot and root apices (Table 10); however, they tended to accumulate at the base of the stem. The most apical leaves also sometimes had a high flavylogen concentration. In the young plumule and epicotyl the main leucoanthocyanin spots were the most mobile; only when normal leaves had formed did the other spots typical of leaves appear. Very little synthesis of gallic acid and depsides occurred until normal leaves had developed. During the second flush of growth, the flavones F-29 and F-30 were detected in the oldest leaves of the first flush; apart from the kaempferol glycosides present in the earliest stages of embryonic development, these were the only representatives of the flavone and flavonol classes of flavonoids detected in seedlings.

The newly emerged radicle contained catechin and epicatechin; but more important were the

Table 7. *Details of seedlings analysed*

Seedling no.	...	...	S1	S2	S3	S4	S5
Clone			MT9	MT9	MT9	MT7	MT9
Fresh wt. (g.)			1.45	1.59	3.80	2.91	6.69
Shoot fresh wt. (g.)			0.01	0.04	0.36	0.94	2.40
Height of shoot (cm.)			—	0.5	9.5	11.5	20.0
Approx. length of root (cm.)			1.0	3.0	7.0	9	9

Table 8. *Concentrations of the main catechins in leaves of developing seedlings*

Results are given as % of fresh wt. Seedlings are numbered, as S1 etc., as in Table 7; leaves of seedlings are numbered from the base upwards as L1 etc. for the first flush and L1' etc. for the second, in order of development.

Seedling no. ...	...	S1	S2	S3	S4	S5
Second flush						
L2' EGC						0.12
EGCG						0.54
ECG						0.20
Total catechins						0.94
L1' EGC						0.25
EGCG						0.66
ECG						0.18
Total catechins						1.17
First flush						
L3 EGC					1.08	0.28
EGCG					1.10	0.36
ECG					0.20	0.15
Total catechins					2.59	0.93
L2 EGC				1.33	—	—
EGCG				3.21	—	0.19
ECG				0.91	—	0.15
Total catechins				5.82	—	0.51
L1 EGC		0.01	0.12	0.32	—	—
EGCG		0.04	0.92	1.34	0.49	—
ECG		0.26	1.10	0.76	0.33	—
Total catechins		0.44	2.52	2.90	0.99	0.05

Table 9. *Catechins in seedling shoots*

Leaves are numbered as in Table 8, and seedlings as in Table 7.

(a) Weights of total catechins throughout shoot (mg.)

Seedling no. ...	...	S1	S2	S3	S4	S5
L2'						3.00
L1'						3.94
L3				3.43	6.38	4.50
L2				2.87	—	1.25
L1				0.67	1.17	0.07
Dwarfed leaf				0.81	0.47	
Stem				1.26	1.40	
Total		0.06	0.32	9.05	9.78	
% of fresh wt. of shoot		0.44	0.75	2.51	1.09	

(b) Weights of individual catechins in shoots (mg.)

	S3, whole shoot		S4, leaves		S5, leaves	
	Wt.	% of total catechins	Wt.	% of total catechins	Wt.	% of total catechins
EGC	1.44	16	2.65	33	2.56	20
EGCG	4.38	48	3.52	44	6.18	48
ECG	2.11	23	1.14	14	2.36	19
GC	0.15	2	0.10	1	Trace	—
EC	0.88	10	0.54	7	1.65	13
C	0.09	1	0.08	1	Trace	—
Total catechins	9.05		8.03		12.75	

Table 10. *Concentrations and amounts of flavylogens in seedlings*

Concentrations are given as $E_{550}/g.$ fresh wt., and amounts as total $E_{550}/organ.$ Leaves are numbered as in Table 8, and seedlings as in Table 7.

Seedling no.	...	...	Concentrations					Total amounts				
			S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Apical bud						17.1	3.7				0.19	0.23
L2'							2.5					0.81
L1'							2.2					0.74
L3					11.7	4.0	3.1			0.69	1.00	1.51
L2					9.1	4.1	3.8			0.90	0.71	0.94
L1					9.6	5.4	3.8			0.33	0.64	0.47
Dwarf leaves					13.2	7.1	12.7			0.25	0.33	0.58
Total leaves										2.16	2.97	5.27
Stem												
Apical					53.4	15.6	4.8			0.47	1.18	1.43
Middle					26.0	15.0	4.3			1.21	1.49	0.99
Basal					38.8	20.3	8.3			3.65	2.29	3.15
Epicotyl				18.5	45.4	24.4	17.3		0.63	3.80	1.02	2.47
Total stem										9.13	5.97	8.03
Total shoot			16.4	33.2				0.21	0.26	11.30	9.13	13.30
Root			7.0	5.5	9.7	6.5	5.1	0.17	0.70	2.55	2.00	1.57
Root apices					29.7					0.81		
Total plant								0.38	1.59	14.66	11.13	14.87

leucoanthocyanins, the predominant members being the most mobile chromatographically; a yellow-staining component was also present in high concentration. All these flavanols persisted throughout the developmental stages studied.

Leaf flavones

The yellow-fluorescing compounds F-29 and F-30 (see Fig. 6) were characteristic of mature and old leaves of several clones growing in Cambridge, and of China-hybrid material sent fresh from Malawi. Extracts were analysed by preliminary separation on Whatman 3MM paper with water as solvent, followed by chromatography in butan-1-ol-acetic acid-water (4:1:5 by vol., upper phase). Elution and spectral analysis of the F-29 and F-30 bands showed both compounds to possess a free 5-hydroxyl group (Jurd, 1962). Further spectral modifications by addition of standard reagents (Jurd, 1956; Jurd & Horowitz, 1957) showed F-29 to be a derivative of luteolin (3',4',5,7-tetrahydroxyflavone) and F-30 to be a derivative of apigenin (3',5,7-trihydroxyflavone). Acid hydrolysis of the glycosides (Harborne, 1965) reached completion within $\frac{1}{2}$ hr.; the aglycones gave typical flavone spectra.

DISCUSSION

The chemistry of the tea plant is to a large extent characterized and dominated by the presence of gallic acid in both free and combined forms. The

young shoots are the chief sites of synthesis both of gallic acid and of a large number of more complex polyphenolic compounds, with some of which it is able to combine, so that the regions of polyphenol concentration in the plant are the aerial apical regions. Throughout the rest of the plant polyphenols are scarce, apart from leucoanthocyanins, except in the other chief meristematic region, the vascular cambium. The characteristics of distribution may be summarized by stating that the bulk of the complex polyphenols seems to be restricted to regions of active cell growth and maturation (apart from root tips) and therefore accumulates in the apical foliar and floral regions, whereas the leucoanthocyanins are typical of the woody axial tissues of the plant.

An important distributional characteristic is the association between flavonoid B-ring hydroxylation and leaf growth; the vicinal-trihydroxylated derivatives become maximal in regions where the predominant growth activity is cell enlargement, a process brought about by expansion of the central vacuole. Since the flavonoids are located in the vacuole, it appears that synthesis of highly hydroxylated flavonoids is closely associated with secretion into the vacuole. This feature, together with the early decline in gallic acid synthesis, leads to an increasing gallocatechin/catechin gallate ratio in developing leaves. The same features were seen during seedling development, the earliest elaboration on the simple catechins being esterification to the gallates, to be followed later by hydroxylation

to gallo catechins. A similar situation was found by Karimdzhanov, Ismailov & Sadykov (1965) during growth of cotton seedlings.

The restriction of gallo catechin synthesis to vacuolating cells is evident in the stem, where the only two regions containing gallo catechins are those in which active cell enlargement is occurring, i.e. the region of the young stem behind the apex and the region of high vascular cambial activity. In the latter region the function of volume increase is taken over by cell division rather than by cell enlargement, and the differentiation of most of these cells into either xylem or phloem elements, whose functions are structural and translocatory rather than biosynthetic, accounts for the very small amounts of gallo catechins in the stem. Between the two regions it is noteworthy that gallo catechins were not detected; this was a genuine disappearance as the young stem aged rather than a dilution effect, for there was no great increase in either length or girth of the internodes between the regions. The overall tendency is towards elaboration of the catechin structures found in apical buds during leaf development, and towards simplification during stem development.

Epicatechin is the only catechin to persist into such tissues as the hard seed coat, the woody stem and the root, but in these tissues leucoanthocyanins attain great profusion. Griffiths (1958), in his survey of the flavonoids of *Theobroma cacao*, also observed that only epicatechin and two leucocyanidins persisted, to varying degrees, in all tissues; Hillis & Swain (1959*a,b*) found that the wood of plum trees contained predominantly catechins and leucoanthocyanins. The work of Hathway, Roux and others has also shown the predominance of leucoanthocyanin monomers and polymers in the bark and wood of a number of tree species. It is significant that the highest flavylgen concentrations were found in the phloem in both stem and root, which layer included the cambium, for several authors, e.g. Roux (1958), Roux & Evelyn (1958) and Saayman & Roux (1965), have suggested that in certain species the phloem and cambial regions are the site of synthesis of monomers that are subsequently translocated laterally to wood and periderm, where they undergo polymerization to condensed tannins. There is a well-substantiated correlation between woodiness and the incidence of leucoanthocyanins (e.g. Bate-Smith, 1956), and it is notable that the maximum flavylgen concentrations occurred in those tissues that give rise to external woody protective layers, namely the outer regions of mature stem and of seed coat. In addition, young seedlings showed an accumulation of flavylgens at the stem base, where wood and bark formation commenced. The basis of this correlation is generally believed to be the polymerization of

leucoanthocyanins to condensed tannins, which function as preservatives of the wood and bark against infection and decay (see Bate-Smith, 1956, 1957). The results of this survey imply that in the foliar organs the flavonoids and other polyphenols undergo molecular elaboration and diversification for no apparent purpose, whereas in the axial regions and the seed coat they undergo polymerization in order that they may thereby fulfil their function as tannin precursors to inhibit infection of the protective coats encasing the secondary body of the current generation and the embryo body of the succeeding generation.

There are several indications that the flavanols, comprising the catechins and leucoanthocyanins, are of greater importance in the plant than the other groups of polyphenols.

(i) Their universal distribution as a class throughout the plant suggests an underlying function.

(ii) High concentrations of flavylgens and of catechins are more or less inversely correlated (see Table 6); there is thus always a fair concentration of one class of flavanols represented.

(iii) There is a close association between growth and flavanol synthesis, e.g. both catechin and flavylgen concentrations remain remarkably constant during leaf development, flower-bud development, young root growth and the initial rapid growth of young seedlings; with young roots it was found that, although the dimensions showed great variation, the flavanol concentrations were nevertheless relatively constant. The sporadic occurrences of the other groups of polyphenols, e.g. the anthocyanins, flavonols and flavones, are in marked contrast.

(iv) The flavanols are believed to be the only flavonoids to undergo polymerization (Bate-Smith & Swain, 1953), although a recent report (Somers, 1966) suggests that anthocyanins may in some cases become incorporated physically into the structures of condensed tannins.

(v) Monomeric flavanols are usually present in highest concentration at the sites of active metabolism, notably the young shoots and the vascular cambial region, but flavylgens accumulate in maximum amounts in the tissues of wood and bark. This distribution implies a meristematically localized synthesis followed by translocation to the regions of deposition and subsequent polymerization. Other workers have postulated such a scheme, e.g. Hillis (1955, 1956) working with eucalypts found maximal leucoanthocyanin concentration in the leaf tips where cell division was most active, and on the basis of further distributional studies he suggested translocation to the stem, where they acted as the immediate precursors of the bark and heartwood extractives.

On the basis of these distributional studies it is

postulated that the flavanols of the tea plant, and in particular the leucoanthocyanins, have a definite function to perform in the plant that is a consequence of their ability to polymerize to condensed tannins. There is an increasing body of evidence to suggest that the catechins may play an important part not only in the actual structure of these polymers, but also in the regulation of the polymerization process: Bate-Smith & Swain (1965) considered that catechin-leucoanthocyanin dimers are the chief form in which flavandiols exist in plants, the presence of the catechin moiety preventing self-polymerization.

We are grateful to Mr J. Ramsden and to the staff of the Tea Research Foundation of Central Africa for gifts of tea seed and cuttings. G.I.F. thanks the Science Research Council for financial support.

REFERENCES

- Bate-Smith, E. C. (1956). *Scient. Proc. R. Dubl. Soc.* **27**, 165.
 Bate-Smith, E. C. (1957). *J. Linn. Soc. Bot.* **55**, 669.
 Bate-Smith, E. C. & Swain, T. (1953). *Chem. & Ind.* p. 377.
 Bate-Smith, E. C. & Swain, T. (1965). *Lloydia*, **28**, 313.
 Bhatia, I. S. & Ullah, M. R. (1968). *J. Sci. Fd Agric.* **19**, 535.
 Dowd, L. E. (1959). *Analyt. Chem.* **31**, 1184.
 Folin, O. & Ciocalteu, V. (1927). *J. biol. Chem.* **73**, 627.
 Folin, O. & Denis, W. (1915). *J. biol. Chem.* **22**, 305.
 Forrest, G. I. & Bendall, D. S. (1969). *Biochem. J.* **113**, 757.
 Gage, T. B. & Wender, S. H. (1950). *Analyt. Chem.* **22**, 708.
 Goldstein, J. L. & Swain, T. (1963). *Phytochemistry*, **2**, 371.
 Gregory, R. P. F. & Bendall, D. S. (1966). *Biochem. J.* **101**, 569.
 Griffiths, L. A. (1958). *Biochem. J.* **70**, 120.
 Harborne, J. B. (1959). *J. Chromat.* **2**, 581.
 Harborne, J. B. (1965). *Phytochemistry*, **4**, 107.
 Hillis, W. E. (1955). *Nature, Lond.*, **175**, 597.
 Hillis, W. E. (1956). *Aust. J. biol. Sci.* **9**, 263.
 Hillis, W. E. & Swain, T. (1959a). *J. Sci. Fd Agric.* **10**, 135.
 Hillis, W. E. & Swain, T. (1959b). *J. Sci. Fd Agric.* **10**, 533.
 Jurd, L. (1956). *Arch. Biochem. Biophys.* **63**, 376.
 Jurd, L. (1962). In *The Chemistry of Flavonoid Compounds*, p. 107. Ed. by Geissman, T. A. Oxford: Pergamon Press Ltd.
 Jurd, L. & Horowitz, R. M. (1957). *J. org. Chem.* **22**, 1618.
 Karimdzhanov, A. K., Ismailov, A. I. & Sadykov, A. S. (1965). *Khim. Prirodn. Soedin., Akad. Nauk Uz.S.S.R.* **5**, 350 cited in *Chem. Abstr.* (1966), **64**, 3961.
 Nakagawa, M. & Torii, H. (1964a). *Agric. biol. Chem., Tokyo*, **28**, 160.
 Nakagawa, M. & Torii, H. (1964b). *Agric. biol. Chem., Tokyo*, **28**, 497.
 Roberts, E. A. H., Wight, W. & Wood, D. J. (1958). *New Phytol.* **57**, 211.
 Roux, D. G. (1958). *Nature, Lond.*, **181**, 1454.
 Roux, D. G. & Evelyn, S. R. (1958). *Biochem. J.* **70**, 344.
 Roux, D. G. & Maihs, A. E. (1960). *J. Chromat.* **4**, 65.
 Roux, D. G. & Paulus, E. (1962). *Biochem. J.* **82**, 320.
 Saayman, H. M. & Roux, D. G. (1965). *Biochem. J.* **97**, 794.
 Seakins, J. W. T. (1959). *Nature, Lond.*, **183**, 1168.
 Somers, T. C. (1966). *Nature, Lond.*, **209**, 368.
 Swain, T. & Hillis, W. E. (1959). *J. Sci. Fd Agric.* **10**, 63.
 Vuataz, L., Brandenberger, H. & Egli, R. H. (1959). *J. Chromat.* **2**, 173.
 Zaprometov, M. N. (1963). *Soviet Pl. Physiol.* **10**, 58.