PLANT PHENOLS

III. SEPARATION OF FERMENTED AND BLACK TEA POLYPHENOLS BY CELLULOSE COLUMN CHROMATOGRAPHY

L. VUATAZ AND H. BRANDENBERGER

Research Laboratory of Nestlé's Products*, Vevey (Switzerland)

(Received May 20th, 1960)

INTRODUCTION

It is well known that the most important step in the manufacture of black tea is the fermentation during which the organoleptic qualities of the final product appear.

A chemical study of tea fermentation was undertaken by HARRISON AND ROBERTS in 1939[1]. It was rapidly recognised that the so-called fermentation was in fact an enzymic oxidation of some polyphenolic compounds followed by non-enzymic re-arrangements and polymerisations. In 1944 BRADFIELD AND PENNEY[2] found that the lower polymers, which are the most important from an organoleptic point of view, could be extracted from the infusion by ethyl acetate. Two years later, BRADFIELD[3], using dialysis, gave further evidence of the presence of substances of relatively high molecular weight in a tea infusion. Further work concerning the research in this field before 1952 is reported by ROBERTS[5].

Studying Assam tea extracts by paper chromatography, ROBERTS[4-6] has shown that the only polyphenols undergoing enzymic oxidation were (—)-epigallocatechin and its gallic ester. An increase in gallic acid during fermentation was noticed. More recently and still by paper chromatography, ROBERTS has detected a series of poly-phenolic substances which he called A, B, C, P, Q, S_I, S_{Ia}, S_{II}, X, Y and Z. He has shown that all these compounds are derived from the two aforementioned flavanols[7-9]. Tentative structures have been proposed for some of them and hypotheses made regarding their formation during fermentation[10-12].

The composition of the raw material used in our study is given in Table I (percentages calculated for dry weight) for:

    (a) fresh tea leaf as reference[13],

    (b) fermented but unfired tea,

    (c) black tea.

As in the case of tea leaf phenolics[13], black tea compounds may be divided into three fractions containing:

    (A) substances extracted from their aqueous solution by ethyl acetate,

---

* Manager: Dr. R. H. EGLI.

(B) substances remaining in the water layer and precipitating as lead salts in a weakly acidic medium (pH about 5.5),

(C) substances remaining in the water layer and precipitating as lead salts only in a weakly alkaline medium (pH about 8.5).

TABLE I

COMPOSITION OF THE RAW MATERIAL

Percentages calculated for dry weight; a = fresh tea leaf as reference;
b = fermented but unfired tea; c = black tea.

	a	b	c
Substances not extractable by 80% ethanol:			
proteins (Kjeldahl N × 6.25)	15.25	—*	—
fibres (by difference)	30.25	—	—
polyphenols lost during fermentation or firing, mainly by condensation with proteins	—	8.8	11.1
Substances extractable by 80% ethanol:			
insoluble in water			
pigments (chloroformic extract minus caffeine)	5.55	—	—
soluble in water			
caffeine (according to MSDA** 1939)	4.65	—	—
polyphenols soluble in ethyl acetate (A)	26.70	14.0	11.0
polyphenols precipitated by lead acetate at pH 5.5 (B)	3.60	7.4	8.1
polyphenols precipitated by lead acetate at pH 8.5 (C)	1.60	1.7	1.7
amino acids (Kjeldahl N × 6.25)	4.15	—	—
ash	4.40	—	—
sugars (by difference)	3.85	—	—

* These determinations have not been carried out because the substances do not take part in the fermentation.
** "Manuel Suisse des Denrées Alimentaires".

As fermented tea contains more complex polyphenolic mixtures than tea leaves, it is advisable to subject less complex fractions than (A) or (B) to the chromatographic runs. This can be achieved:

(i) by extracting decaffeinated tea extract with ethyl propionate or the mixture ethyl propionate–petroleum ether (9:1) prior to the treatment with ethyl acetate,

(ii) by separating the polyphenols in (A) and (B) according to their solubility in cold water.

This refined scheme gives rise to the following six groups:

(1) substances extracted with ethyl propionate,

(2) cold water-soluble substances extracted with ethyl acetate,

(3) cold water-insoluble substances extracted with ethyl acetate,

(4) cold water-soluble substances precipitated with lead acetate at pH 5.5,

(5) cold water-insoluble substances precipitated with lead acetate at pH 5.5,

(6) substances precipitated with lead acetate at pH 8.5.

In the following the term "group" of polyphenols will always refer to this fractionation.

EXPERIMENTAL SECTION

I. Isolation of fermented tea polyphenols

(a) *Raw material.* Part of the tea leaves mentioned previously[13] has been subjected to:

(i) withering (18 h at 23° and 60 % relative humidity, moisture loss about 40 %)

(ii) rolling (40 min)

(iii) fermentation (2 h at 28°).

This material is referred to as fermented tea.

(b) *Extraction.* Immediately after fermentation, 240 g of tea (containing 100 g solids) are homogenized with 560 ml ethanol (yielding with the tea moisture an 80 % ethanolic solution) and 1 l of 80 % ethanol. To avoid oxidation 100 mg $K_2S_2O_5$ in a 10 % aqueous solution are added. After homogenization the mixture is stirred (20 min, 40°), then filtered. The residue is extracted twice more with 750 ml of 80 % ethanol (20 min stirring at 40°). The three filtrates are combined and concentrated in a rotating evaporator under reduced pressure. The concentrated extract (about 500 ml) should contain at least 10 % ethanol in order to keep the polyphenol–caffeine complex, which is sparingly soluble in cold water, in solution. The caffeine and the pigments are extracted with $CHCl_3$ using a rotating flask to avoid emulsion formation.

(c) *Separation of the polyphenols.* The aqueous phase is freed of the residual $CHCl_3$ by vacuum evaporation, then extracted three times for 5 min with 1-l volumes of ethyl propionate, and three times for the same length of time with ethyl acetate. Both organic extracts are separately concentrated in a rotating evaporator under reduced pressure to 100 ml. About 100 ml of 20 % ethanol are added and the concentration is continued until organic solvents are removed. The first extract is freeze-dried. The second one is kept in about 100 ml water at 4° for several hours, then filtered. The precipitate is dried in a desiccator and the filtrate is freeze-dried after concentration to a few ml.

The aqueous phase remaining after ethyl acetate extraction is freed from dissolved organic solvent by vacuum evaporation. A few ml ethanol are added to dissolve the sparingly soluble polyphenols. The final pH is about 5.5. A saturated solution of $Pb(OAc)_2$ is added. The precipitate is filtered and washed with 3 l distilled H_2O and kept in suspension by continuous gentle stirring.

On gradually adding Dowex-50 (H-form) to the suspension, the Pb^{++} is bound to the cation exchanger and the free polyphenols go into solution. 0.2 vol. ethanol are added to dissolve the polyphenols. The mixture is filtered and the resin thoroughly washed with 20 % ethanol. The filtrate is concentrated to 100 ml under vacuum in a rotating evaporator, the concentrate kept at 4° for several hours, then filtered. The precipitate is dried in a desiccator and the filtrate freeze-dried after concentration to a few ml.

The polyphenols precipitated as lead salts at pH 8.5 are obtained as described previously[13] under I(d).

A quantity of fermented tea corresponding to 100 g dry weight gives the following yields for the six groups:

Group (1)	3.6 g		Group (4)	5.0 g
(2)	7.1 g		(5)	2.4 g
(3)	3.3 g		(6)	1.7 g

The substances present in each of these six groups are shown in Fig. 1. Those designated by numbers are identical with the main components of tea leaf[13]. The substances indicated by letters are formed during fermentation. When it is certain that a substance corresponds to one already detected by ROBERTS in Assam tea we have used the terminology of this author[8,9].

II. Isolation of black tea polyphenols

(a) Raw material. Part of the fermented tea described in Section Ia has been fired (20 min, 110°, air current). This material is referred to as black tea.

(b) Extraction. 105 g of black tea (containing 100 g solids) are homogenized with 1500 ml 80 % ethanol, then stirred (20 min, 40°) and filtered. The residue is extracted twice more with 750 ml of 80 % ethanol. The rest of the process is as in Section I.

The yields of the six groups of polyphenols are:

Group (1)	2.8 g		Group (4)	5.1 g
(2)	5.9 g		(5)	3.0 g
(3)	2.4 g		(6)	1.7 g

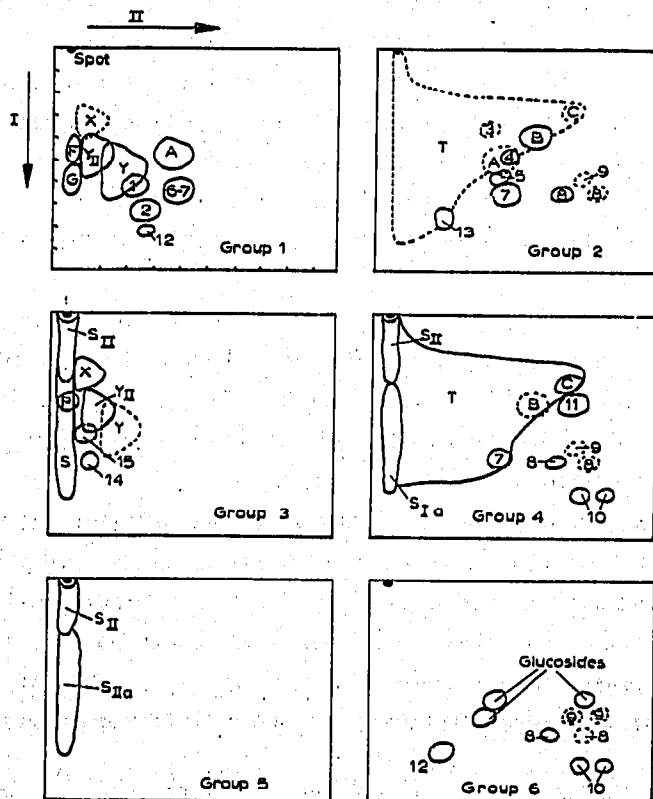The substances present in each of these six groups are shown in Fig. 1.

Fig. 1. Two-dimensional paper chromatograms. Paper: Whatman No. 1. Solvent systems for first dimension: butanol–acetic acid–water (4:1:2.2); second dimension: 2% acetic acid in water.

III. Chromatographic technique

(a) *Paper chromatography*. As described previously[13] under IId.

(b) *Adsorption column chromatography*. As described previously[13] under IIa to IIc, with exception of the following modifications:

(i) Washing the cellulose with 2 % citric acid (to remove traces of iron), then with alcohol and acetone prior to the treatment with distilled water.

(ii) Addition of substance mixture by blending the freeze-dried polyphenols with three to four times their weight of cellulose powder, moistening the mixture with water and placing it on the top of the column which is still covered with a small layer of water.

When the *total* polyphenols are subjected to adsorption chromatography, they are eluted according to Fig. 2. The end of Fraction VI corresponds to the arrival of
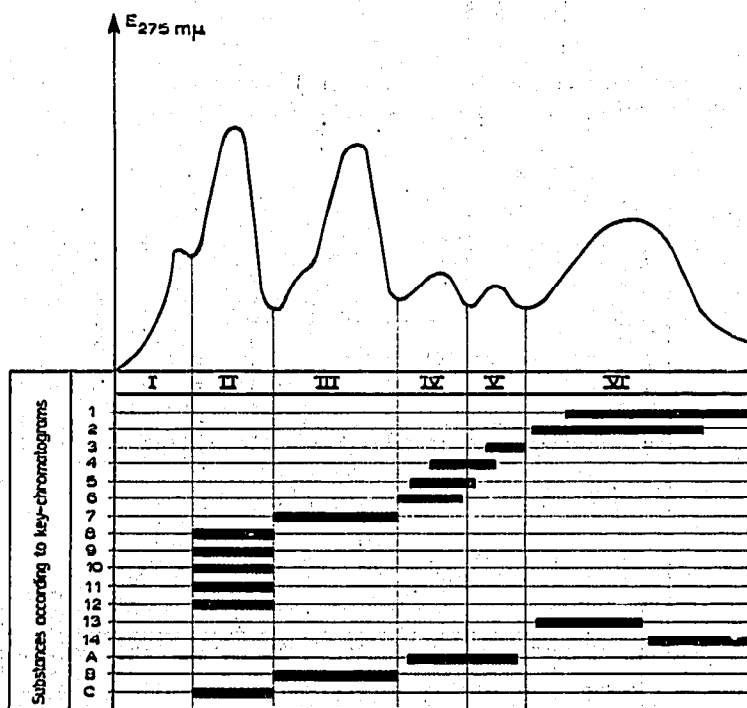

Fig. 2. Adsorption chromatography. Total polyphenols. Substances T which are present in each fraction are not indicated in this figure. Fraction I only contains substances which are not polyphenolic. These substances remain at the top of the partition columns.

the coloured substances at the bottom of the column. The approximate location of the substances remaining on the column is indicated in Fig. 3.

The cellulose may be removed from the glass column and cut into three pieces according to Fig. 3. Each piece is treated with 80 % ethanol. The extracts are concentrated under vacuum and freeze-dried.

By adsorption, the polyphenols are therefore divided into two groups, colourless and coloured compounds. With exception of T, 13 and 14, all the substances present in the six fractions of Fig. 2 are colourless.
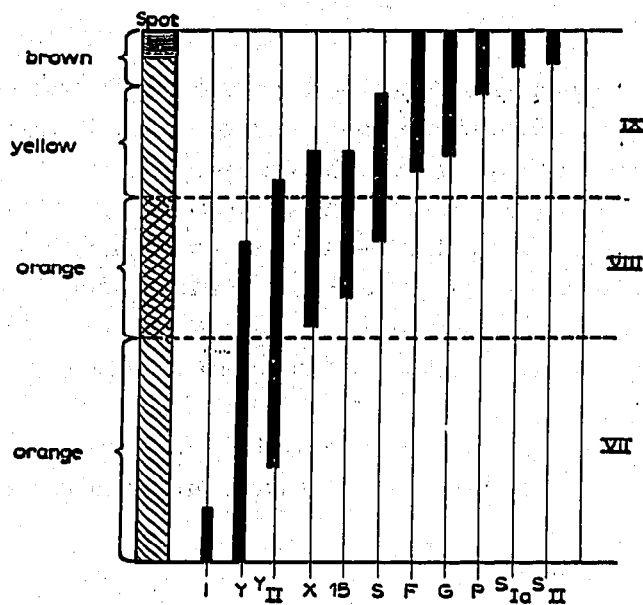
Fig. 3. Adsorption chromatography of the total polyphenols. Approximate location of the substances remaining on the column at the end of the elution.
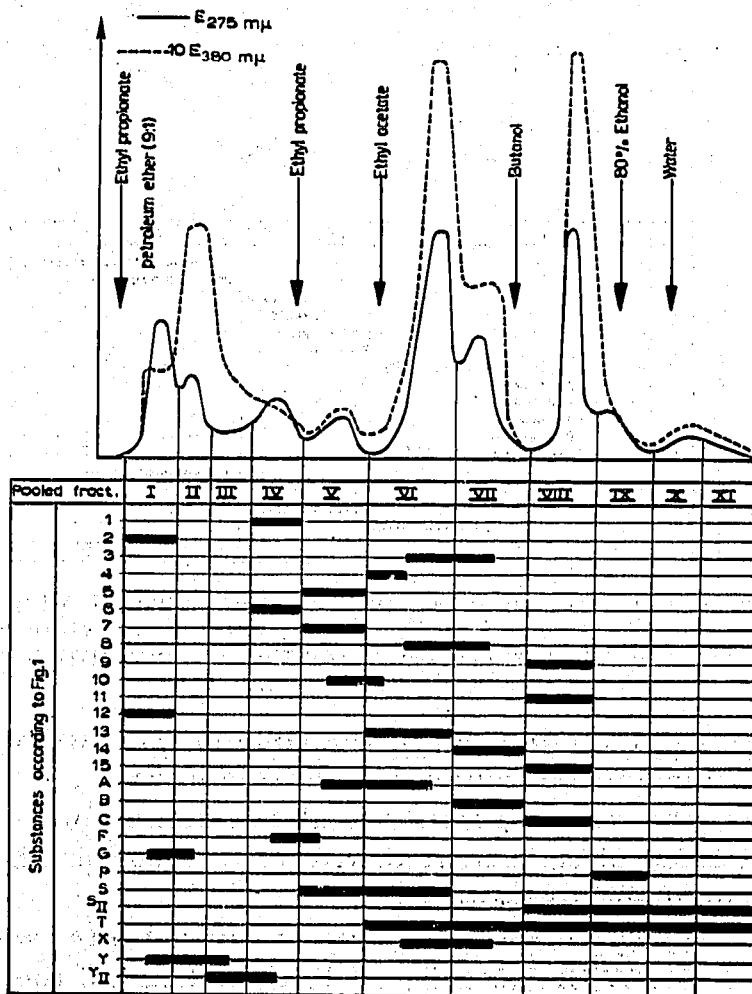

Fig. 4. Partition chromatography. Polyphenols soluble in ethyl acetate (Groups 1, 2 and 3).

(c) *Partition column chromatography*. Fig. 4 shows how the polyphenols extractable with ethyl acetate (Groups 1, 2 and 3) are eluted when the technique described previously[13] under IIIa to IIId is used.

Fig. 5 shows the behaviour of the substances precipitating as Pb salts at pH 5.5 (Groups 4 and 5).

Figs. 1 to 5 apply to fermented (unfired) tea polyphenols. The firing step does not have an important effect on the shape of the elution curves. With black tea
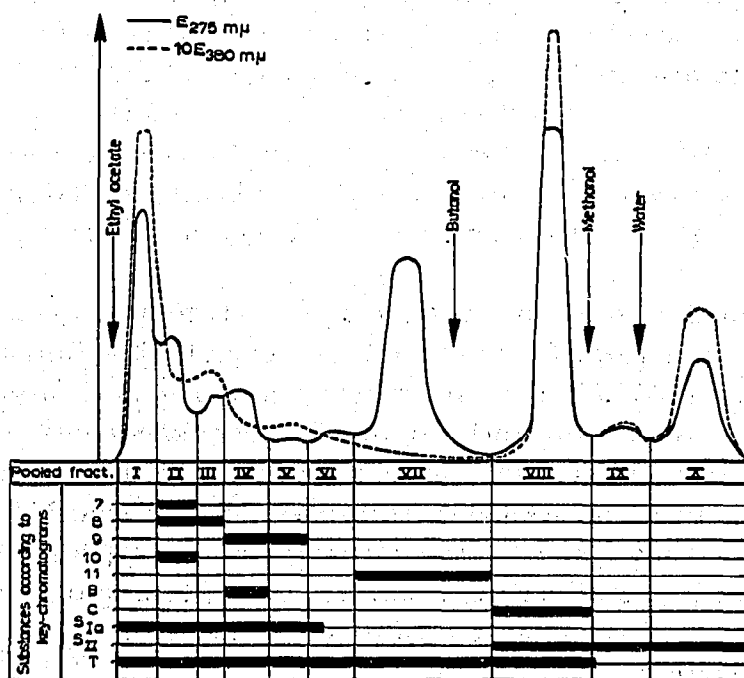

Fig. 5. Partition chromatography. Polyphenols precipitated as Pb salts at pH 5.5 (Groups 4 and 5).

polyphenols, Fig. 4 would show higher peaks for Fractions V and X, Fig. 5 for Fractions VIII and X.

Firing does not yield new substances, only quantitative changes occur (see Table I and the weights of the six groups of polyphenols).

IV. Isolation of pure polyphenols

(1) *Isolation of (—)-epigallocatechin gallate and (—)-epicatechin gallate*. If the polyphenols in Group 1 are subjected to adsorption chromatography at a very slow flow-rate, it is possible to have all the (—)-epigallocatechin gallate in Fraction VI. After partition chromatography with the mixture ethyl propionate–petroleum ether (9:1) the two flavanol gallates may be obtained quantitatively from this fraction.

(2) *Isolation of substance A*. Group I is subjected to adsorption chromatography. The fractions corresponding to Fractions IV and V of Fig. 2 are pooled and submitted to partition chromatography with ethyl propionate. Small amounts of (+)-catechin, (—)-epicatechin, gallic acid, (—)-epicatechin gallate and (—)-epigallocatechin gallate

may be present. However, they are eluted prior to substance A with ethyl propionate.

Substances T and some glucosides (not indicated in Fig. 1) remain at the top of the partition column.

Substance A has been crystallized from ether (see Table III for physical data).

(3) *Isolation of substance B*. Group II is subjected to adsorption chromatography. The two main constituents of Fraction III (Fig. 2) are readily separated with ethyl acetate. Fraction III should not be contaminated with the acids of Fraction II. However, small amounts of (+)-catechin, (—)-epicatechin and substance A do not interfere because they are eluted prior to substance B with ethyl acetate.

Substances T remain at the top of the partition column.

Substance B has been crystallized from water (see Table III for physical data).

(4) *Isolation of substance C*. Groups II and IV are pooled and subjected to adsorption chromatography. In addition to the acids mentioned in Fig. 2, Fraction II also contains some water-soluble glucosides, presumably of the 3-rhamnoglucoside type.

By partition chromatography with ethyl acetate the acids and most of the glucosides are separated from C which moves very slowly. Substances T remain at the top of the column. When the elution of the acids is complete, the cellulose is removed from the glass column and cut into 3 or 4 pieces, each of which is eluted with alcohol. In general, substance C is in the middle of the column.

Substance C has been crystallized from water (see Table III for physical data).

(5) *Isolation of coloured substances*. Substances Y, Y_{II} and X are obtained by pooling Groups 1 and 3, subjecting them to adsorption chromatography, pooling Fractions VII and VIII (Fig. 3) and submitting them to partition chromatography, using the gradient technique of Fig. 4.

From Fraction IX of the same adsorption chromatogram it is possible to get substances F and G which are eluted with ethyl propionate. With ethyl acetate substance P migrates very slowly (pink band). When this latter zone is completely separated from the brown substances S_{II} remaining at the top of the column, it may be eluted from the cellulose (removed from the glass column) with isoamyl alcohol

RESULTS AND DISCUSSION

The polyphenolic substances present in fermented or in black tea are listed in Table II. From the substances already present in tea leaves, only those acting as possible substrates in the enzymic oxidation are considered in the following discussion.

The substances formed during fermentation have been classified according to their visible and U.V. absorption spectra.

(a) *Colourless substances exhibiting only one absorption band (between 270 and 280 mμ)* [*]

---

[*] In the following we are dealing only with the spectrum range from 220 to 600 mμ.

TABLE II
POLYPHENOLIC SUBSTANCES PRESENT IN FERMENTED OR IN BLACK TEA

Code to Fig. 1	Name	Group where substance is to be found	Colour of substance
Main polyphenols already present in tea leaf[13]			
1	(—)-epigallocatechin gallate	1	white
2	(—)-epicatechin gallate	1	white
3	(—)-epigallocatechin	2	white
4	(+)-gallocatechin	2	white
5	(—)-epicatechin	2	white
6	(+)-catechin	1,2	white
7	gallic acid	1,2,4	white
8	chlorogenic acid	2,4,6	white
9	neochlorogenic acid	2,4,6	white
10	*p*-coumaryl-quinic acid	4,6	white
11	theogallin	2,4	white
12	caffeic acid	1,6	white
13	kaempferol-3-glucoside	2	yellow
14	quercetin-3-glucoside	3	yellow
15	myricetin-3-glucoside	3	yellow
Polyphenols formed during fermentation			
A	bisflavanol[12]	1,2	white
B	bisflavanol[12]	2,4	white
C	bisflavanol[12]	2,4	white
X	theaflavin[8,11,12]	1,3	orange
Y	theaflavin gallate[8,11,12]	1,3	orange
Y_{II}		1,3	orange-red
S		3	orange
F		1	orange-yellow
G		1	orange-yellow
P		3	pink
T		2,4	brown
S_{Ia}	thearubigin[8,11,12]	2,5	brown
S_{II}	thearubigin[8,11,12]	3,4,5	brown

(1) *Monomers.* (—)-Epigallocatechin gallate and (—)-epicatechin gallate can be obtained quantitatively as described in Section IV 1. On the basis of dry weight, the two substances are present in the following percentages:

	Tea leaves[13]	Fermented tea
(1) (—)-Epigallocatechin gallate	10.55 %	0.5 %
(2) (—)-Epicatechin gallate	2.15 %	0.3 %

95 % of (1) and 89 % of (2) disappear in 2 hours of fermentation. We do not know what happens to (2) which is reported by ROBERTS as not taking part in the fermentation of Assam tea. It might be possible that the enzymic systems of Assam and Ceylon

Substance A
(from ether)

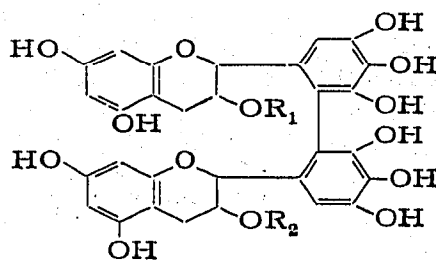Substance B
(from water)

Substance C
(from water)

Fig. 6. The figures on the scale indicate mm.

teas are different. Furthermore, it has been reported by DZHEMUKHADZE AND MILESHKO[14] that (—)-epicatechin gallate is oxidized during processing of Georgian tea leaf.
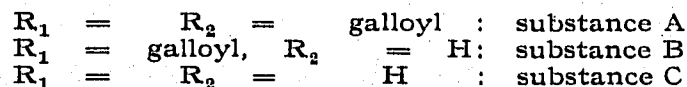
Although we do not have any quantitative results with regard to the other four flavanols (which are much more difficult to isolate from fermented tea than from fresh tea leaves) we can say that (—)-epigallocatechin is almost completely oxidized whereas (+)-catechin, (+)-gallocatechin and (—)-epicatechin seem to escape oxidation.

(2) *Presumed dimers (biflavanols).* Substances A, B and C have been obtained in a crystalline state, A from ether, B and C from water (see Fig. 6).

ROBERTS suggests[12] that substances A, B and C have the structures (I).

(I)

R₁	=	R₂	=	galloyl	:	substance A

R₁ = R₂ = galloyl : substance A
R₁ = galloyl, R₂ = H : substance B
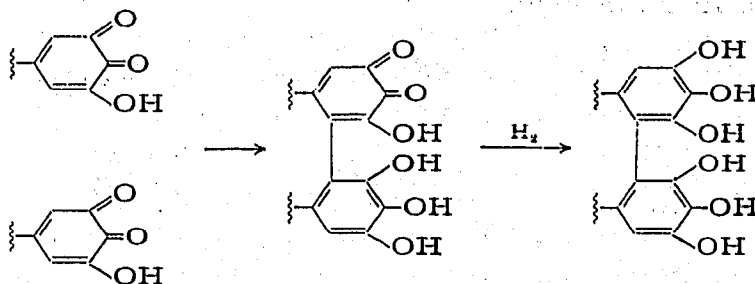R₁ = R₂ = H : substance C

They would be formed by dimerisation of:

2 molecules of (—)-epigallocatechin gallate
1 molecule of (—)-epigallocatechin gallate and 1 molecule of (—)-epigallocatechin
2 molecules of (—)-epigallocatechin,

respectively, in the quinone form, the dimer being reduced in the course of a coupled reaction.

Physical data are reported in Table III.

The surprisingly high hydrogen content of substance A could be explained by the assumption that this substance crystallizes with two molecules of ether. This is supported by the fact that at 215° the crystals decompose very violently. From the carbon and hydrogen contents of B, it cannot be ascertained whether this substance crystallizes with one molecule of water or not.

TABLE III

PHYSICAL DATA

		Substances		
		A	B	C
Crystallized from		ether	water	water
Formula*		$C_{44}H_{34}O_{22}$	$C_{37}H_{30}O_{18}$	$C_{30}H_{20}O_{14}$
		914.71	762.61	610.50
Analysis				
calc. for anhydrous product	% C	57.90	58.27	59.01
	% H	3.75	3.97	4.29
calc. with 2 molecules cryst. ether	% C	58.76	56.93	
for A and with 1 molecule cryst.	% H	5.12	4.13	
H₂O for B				
found (for A and B two different	% C	58.24 59.61	56.98 57.45	59.06
crystalline preparations were analysed)	% H	5.74 5.40	4.22 4.06	4.71
M.p.**		decomp. at around 215° without melting	decomp. at around 200° without melting	can be heated to 300° without decomp. or melting
$[\alpha]_D^{25°}$ ***		—247° ± 2°	—183° ± 2°	—66° ± 2°
λ_{max} ***		277 mμ	274 mμ	270 mμ
ε_{max}†		20,500	15,500	4,500

* According to ROBERT's assumption[12].
** The substances have been dried over P_2O_5–NaOH under 0.01 mm Hg at 60° for 4 h. The crystals turned slightly brown. M.p. measured with Kofler block.
*** For the determination of $[\alpha]_D$ and for the U.V. spectra we have used white crystals dried over P_2O_5–NaOH under 0.01 mm Hg at room temperature for 1 h. $[\alpha]_D$ determined in ethanol.
† Calculated on the basis of the molecular weights given under "Formula". The spectra have been taken in ethanol with a Beckman DK-2 spectrophotometer.

If we compare the $[\alpha]_D$, λ_{max} and ε_{max} with the corresponding values of (—)-epigallocatechin and of its gallic ester[13]:

	(—)-Epigallocatechin gallate	(—)-Epigallocatechin
$[\alpha]_D^{25°}$	—185° ± 2°	—59.5° ± 2°
λ_{max}	275 mμ	271 mμ
ε_{max}	11,500	1,450

we see that ROBERTS' assumption is consistent with our physical data.

In addition to that, the I.R. spectra (to be published elsewhere) show a strong ester band at 5.9 μ for A. This band is reduced in B and absent in C.

It is interesting to note that A cannot be crystallized from water because its aqueous solutions become too viscous on cooling (for instance a 1.5 % solution at 4°).

Substances A and B are very sensitive. The dry crystals turn slightly brown within a few days, even at 4°. Substance C is stable.

(b) Coloured substances

(1) *Polyphenols with three absorption bands**. Fig. 7 shows the spectra of substances Y, Y_{II} and X. Our $E_{1\,cm}^{0.002\,\%}$ values for X and Y do not agree too well with ROBERTS' values[11,12].

Substance Y_{II} has not been reported by ROBERTS in Assam tea. In the dry state, it is a light red powder, whereas X and Y are orange.

These three substances have a great tendency to precipitate from their solution in an amorphous state (although these precipitates seem to be sometimes microcrystalline). So far all our preparations have consisted of approximately 50 % of crystalline and 50 % of amorphous material, as can be seen with the polarising microscope.

(2) *Polyphenols with two absorption bands**. We have been able to isolate four compounds belonging to this class.

It is certain that our substance P corresponds to that of ROBERTS[10]: their paper-chromatographic behaviour is the same and both have an E_{max} at 520 mμ.

Substances F, G and S have not been obtained chromatographically pure. They all have an E_{max} between 370 and 380 mμ. G is bright yellow whereas F and S are rather orange.

Our substances F and G do not seem to correspond to ROBERTS' substances Q and Z.

(3) *Polyphenols with one absorption band**. This class is made up of substances S_{Ia} and S_{II} which are brown and have been called thearubigins by ROBERTS[8]. According to this author[11], they are oxidation products of the theaflavins. They have an E_{max} at around 275 mμ and a more or less pronounced shoulder at about 370 mμ.

Thearubigins (which must be viewed as a mixture of different substances) make up the whole Group 5 of polyphenols (see Fig. 1). Our thearubigins contain 0.55 % N which cannot be accounted for either by caffeine or amino acids. Hydrolysis with 5 N HCl at 120° for 12 hours (sealed tube containing 250 mg thearubigin and 10 ml 5 N HCl) gives rise to the following free amino acids which have been detected by paper chromatography: alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, valine.

This could indicate that thearubigin is partly composed of substances related to humic acids.

The *o*-quinone forms of the 3′,4′-dihydroxy- and the 3′,4′,5′-trihydroxyflavan derivatives are known to be highly reactive. One of their fates is to dimerise to substances A, B, C and theaflavins. But they might also condense with amino acids or with proteins, yielding humic acid-like substances. It is likely that the polyphenols condensing with proteins lose their extractability by 80 % alcohol. This would account for the drop in total polyphenols occurring during fermentation (see Table I). The polyphenols condensing with amino acids could give rise to the N-containing substances present in Group 5.

---

* See the footnote on p. 24.

Partition chromatography of Group 5 according to Fig. 5 yields four fractions. S_{Ia} which is eluted with ethyl acetate does not contain N, whereas the three other fractions (eluted with butanol, methanol and water respectively) all contain N.

About 50 % of the N-containing substances remain adsorbed on the cellulose and can be eluted with alkaline solutions.
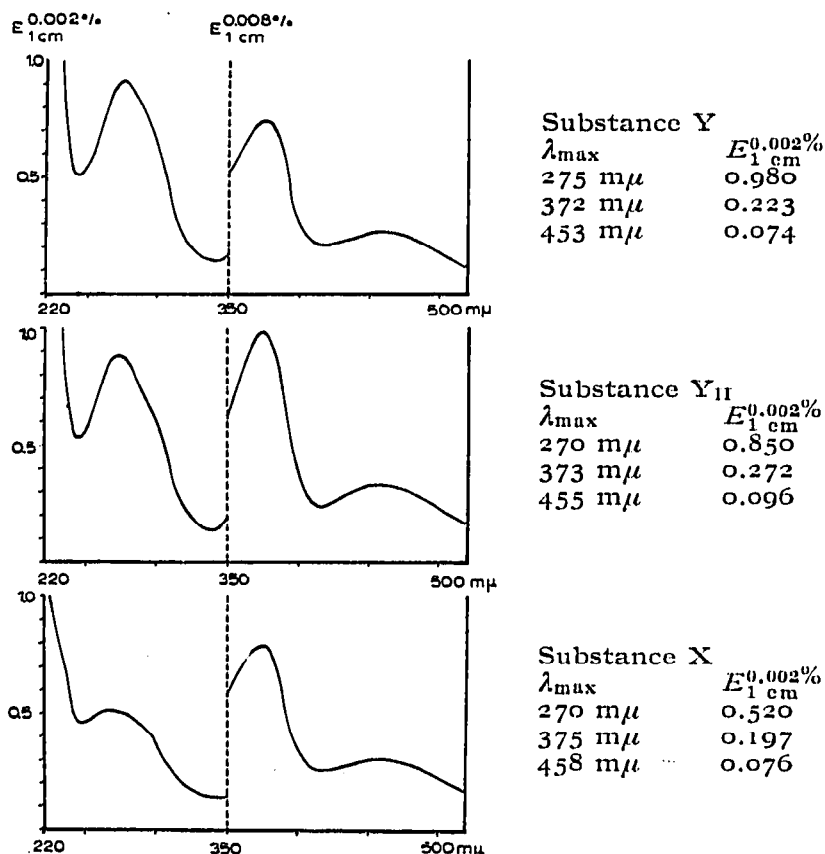
Substance Y

λ_{max}	$E_{1\ cm}^{0.002\%}$
275 mμ	0.980
372 mμ	0.223
453 mμ	0.074

Substance Y_{II}

λ_{max}	$E_{1\ cm}^{0.002\%}$
270 mμ	0.850
373 mμ	0.272
455 mμ	0.096

Substance X

λ_{max}	$E_{1\ cm}^{0.002\%}$
270 mμ	0.520
375 mμ	0.197
458 mμ	0.076

Fig. 7. Substances Y and Y_{II} are paper-chromatographically pure. Substance X is contaminated with very small amounts of glucoside 15 and substance S.

(4) *Substances without absorption bands**. Substances T seem to be oxidative breakdown products of thearubigin. They have lost their polyphenolic characteristics and do not even possess an E_{max} at about 270 mμ.

SUMMARY

The polyphenolic constituents of fermented (unfired) and black (fermented and fired) Ceylon tea have been fractionated by column chromatography, using procedures

---

* See the footnote on p. 24.

recently described for the preparation of (unfermented and unfired) tea leaf poly-phenols[13].

(—)-Epigallocatechin gallate and (—)-epicatechin gallate, the two main poly-phenols of tea leaf, have been isolated quantitatively. The amount of both compounds is much lower than in tea leaves, which indicates that not only (—)-epigallocatechin gallate but also (—)-epicatechin gallate disappear during enzymic oxidation.

The fermentation products have been classified according to their absorption spectra in the U.V. and visible region. Some of them have been purified chromatog-raphically and characterised physically:

(a) Three colourless substances possessing one U.V. maximum only, which had already been detected paper-chromatographically by ROBERTS[12], could be prepared in a crystalline state. Their analytical data support the view[12] that these compounds are biflavanols resulting from oxidative dimerisation of (—)-epigallocatechin and/or its gallic ester.

(b) Three coloured substances possessing three absorption maxima have been prepared paper-chromatographically pure. Two of them, orange in colour, seem to be identical with the compounds described by ROBERTS[8,11,12]. The third one is light red and has, to our knowledge, not yet been reported.

(c) Four coloured substances possessing two absorption maxima have been obtained. Only one of them has been detected by ROBERTS in Assam tea.

(d) The fraction called thearubigin by ROBERTS[8,11,12] possesses only one maximum in the U.V. This brown fraction is a very complex mixture. It has been found to yield 14 amino acids on hydrolysis with HCl.

NOTE ADDED IN PROOF

Since this paper was submitted, substance C has been subjected to alkaline degrada-tion. The presence of phloroglucinol and ellagic acid as main degradation products clearly indicates that the two molecules of (—)-epigallocatechin are linked by a $2'-2'$ C–C linkage as was postulated by ROBERTS[12]. This work which is to be published else-where, has been carried out by Mr. J. MONNIN.

REFERENCES

[1] C. J. HARRISON AND E. A. H. ROBERTS, *Biochem. J.*, 33 (1939) 1408.
[2] A. E. BRADFIELD AND M. PENNEY, *J. Soc. Chem. Ind.*, 43 (1944) 306.
[3] A. E. BRADFIELD, *Chem. & Ind. (London)*, (1946) 242.
[4] E. A. H. ROBERTS AND D. J. WOOD, *Biochem. J.*, 49 (1951) 414.
[5] E. A. H. ROBERTS, *J. Sci. Food Agr.*, 3 (1952) 193.
[6] E. A. H. ROBERTS AND D. J. WOOD, *Biochem. J.*, 53 (1953) 332.
[7] E. A. H. ROBERTS, *J. Sci. Food Agr.*, 8 (1957) 72.
[8] E. A. H. ROBERTS, *J. Sci. Food Agr.*, 9 (1958) 212.
[9] E. A. H. ROBERTS, *Chem. & Ind. (London)*, (1957) 1354.
[10] E. A. H. ROBERTS AND D. M. WILLIAMS, *J. Sci. Food Agr.*, 9 (1958) 217.
[11] E. A. H. ROBERTS, *J. Sci. Food Agr.*, 9 (1958) 381.
[12] E. A. H. ROBERTS, *J. Sci. Food Agr.*, 10 (1959) 167.
[13] L. VUATAZ, H. BRANDENBERGER AND R. H. EGLI, *J. Chromatog.*, 2 (1959) 173.
[14] K. M. DZHEMUKHADZE AND L. F. MILESHKO, *Doklady Akad. Nauk S.S.S.R.*, 114 (1957) 959.