CHROMATOGRAPHIC BEHAVIOUR AND CHEMICAL STRUCTURE

II. THE TEA CATECHINS

by

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By partition chromatography on silica gel columns the components of the so-called "white tannin" of green tea have been separated and subsequently identified as catechins, gallocatechins (hydroxy-catechins) and their galloyl esters (BRADFIELD, PENNEY, AND WRIGHT[1], BRADFIELD AND PENNEY[2]). An examination of the chromatographic behaviour of these substances on paper has now been made, employing the technique described previously (BATE-SMITH AND WESTALL[3]). Chromatography was carried out at 20° C, butanol-acetic acid-water (4:1:5) being used as solvent, and the position of the spots on the developed chromatogram was revealed by spraying with ammoniacal silver nitrate.

Values of R_F and R_M ($R_M = \log (\frac{1}{R_F} - 1)$) are shown in Table I. *d*-Catechin has not been found in tea, but values for a specimenof this substance isolated from gambir are included for comparison. Gallocatechin-a-gallate is the "substance 2A" of BRADFIELD AND PENNEY[2].

TABLE I

	R_F	R_M		R_F	R_M
d-catechin	0.76	—0.50	*l-epi*catechin gallate	0.86	—0.79
dl-catechin	0.74	—0.45	*l*-gallocatechin gallate	0.72	—0.41
*l-epi*catechin	0.65	—0.27	gallocatechin-a-gallate	0.71	—0.39
dl-gallocatechin	0.57	—0.12			
l-gallocatechin	0.47	+0.05			

Examination of these figures brings to light certain interesting regularities. Comparing the diastereoisomers *d*-catechin and *l-epi*catechin, the effect of the differing spatial arrangements of the groups about the pyran ring results in a difference in the partition coefficients of these substances between water and butanol-acetic acid which is reflected in the differences in the R_F and R_M values, ΔR_F and ΔR_M being 0.11 and — 0.23 respectively. Closely similar figures $\Delta R_F = 0.10$, $\Delta R_M = -0.17$, are obtained by subtracting the values for *l*-gallocatechin from those for *dl*-gallocatechin.

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This similarity strongly suggests a diastereoisomeric relationship between *dl*-gallocatechin and *l*-gallocatechin, the configuration of the former, apart from mirror-image relationships, corresponding to *d*-catechin and of the latter, to *l-epi*catechin.

In further support of the configurational identity of *l-epi*catechin and *l*-gallocatechin, reference may be made first to the effect of galloylation of these substances on the R_F and R_M values. The increments $\Delta R_F = 0.21$ and $\Delta R_M = -0.52$ obtained by subtraction of the figures given in Table I for *l-epi*catechin gallate and *l-epi*catechin agree well with the increments $\Delta R_F = 0.25$ and $\Delta R_M = -0.46$ for galloylation of *l*-gallocatechin. Secondly, the observed molecular rotations of *l-epi*catechin, *l*-gallocatechin and their galloyl esters in alcohol show a regularity to be expected if the suggested stereochemical relationship exists, as may be seen from Table II.

TABLE II

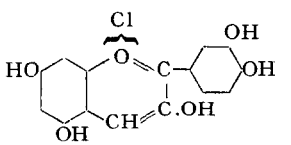
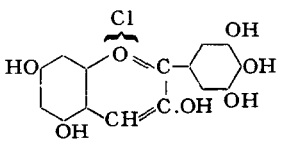
	$[\alpha]_D$		$[\alpha]_D$
*l-epi*catechin	— 69°	*l*-gallocatechin	— 60°
*l-epi*catechin gallate	—190°	*l*-gallocatechin gallate	—179°

It follows that *l*-gallocatechin of the tea would be more suitably designated *l-epi*-gallocatechin, reserving the name *l*-gallocatechin for the optically active diastereoisomer of which the *dl*-gallocatechin of tea is the optically inactive form. It is possible that gallocatechin-α gallate is the galloyl ester of this *l*-gallocatechin, but further investigation of this substance is required.

In connection with the effect of constitutional changes on chromatographic behaviour (BATE-SMITH AND WESTALL[3]), the effect of the introduction of a third hydroxyl in a vicinal position in a benzene ring already carrying two *ortho* hydroxyl groups is shown for various pairs of substances in Table III. In each case the additional hydroxyl group diminishes R_F and increases R_M but the numerical value of ΔR_M is dependent on the constitution of the molecules as a whole.

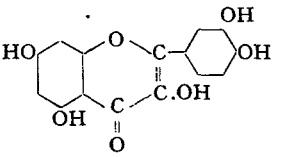
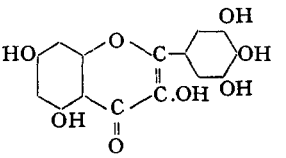
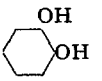
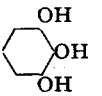
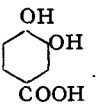
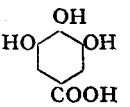
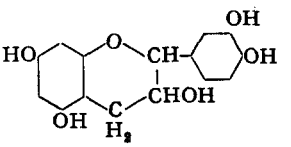
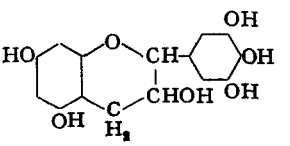
TABLE III

R_F, R_M, AND ΔR_M IN ACID BUTANOL 20° C

Substance	Acid	R_F	R_M	ΔR_M
Cyanidin 	2*N*HCl	0.69	—0.36	— 0.62
Delphinidin 	2*N*HCl	0.35	0.26	

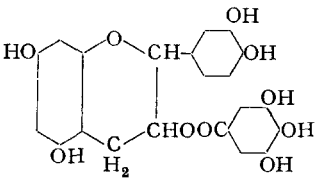
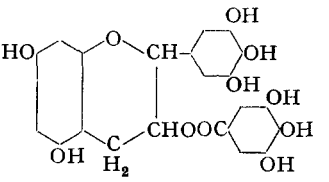
References p. 444.

TABLE III (continued)

Substance	Acid	R_F	R_M	ΔR_M
Quercetin 	Acetic	0.74	—0.46	— 0.58
Myricetin 	Acetic	0.43	0.12	
Catechol 	Acetic	0.91	—1.02	— 0.49
Pyrogallol 	Acetic	0.77	—0.53	
Protocatechuic acid 	Acetic	0.85	—0.76	— 0.43
Gallic acid 	Acetic	0.68	—0.33	
Catechin 	Acetic	0.76	—0.50	— 0.38
Gallocatechin 	Acetic	0.57	—0.12	
*Epi*catechin (formula as catechin)	Acetic	0.65	—0.27	— 0.32
*Epi*gallocatechin (formula as gallocatechin)	Acetic	0.47	0.05	

References p. 444.

TABLE III (continued)

Substance	Acid	R_F	R_M	ΔR_M
*Epi*catechin gallate 	Acetic	0.86	−0.79	−0.38
*Epi*gallocatechin gallate 	Acetic	0.72	−0.41	

As regards one of the authors (E. C. BATE-SMITH), this work forms part of the programme of the *Food Investigation Organisation of the Department of Scientific and Industrial Research.*

SUMMARY

Specimens of catechins and gallocatechins and their gallate esters, isolated from green tea, have been examined by the method of filter-paper chromatography. Regularities are observed between chromatographic behaviour, molecular rotation and chemical constitution, and these suggest that the *l*-gallocatechin of tea would be more suitably designated by *l*-epigallocatechin.

RÉSUMÉ

Nous avons examiné par la méthode de chromatographie sur papier-filtre des spécimens de catéchines et de gallocatéchines et de leurs esters galliques isolés du thé vert. Les régularités observées entre le comportement chromatographique, la rotation moléculaire et la constitution chimique suggèrent que la *l*-gallocatéchine du thé serait désignée de façon plus adéquate par le nom de *l*-epigallocatechine.

ZUSAMMENFASSUNG

Aus grünem Thee isolierte Proben von Catechin, Gallocatechin und ihren Gallensäureestern wurden unter Anwendung der Filterpapier-Chromatographie untersucht. Zwischen dem chromatographischen Verhalten, der Molekularrotation und der chemischen Konstitution wurden Regelmässigkeiten beobachtet, die darauf hinweisen, dass *l*-Gallocatechin besser als *l*-Epigallocatechin bezeichnet werden könnte.

REFERENCES

1 BRADFIELD, PENNEY, AND WRIGHT, *J. Chem. Soc.* (1947) 32.
2 BRADFIELD AND PENNEY, *ibid.* (1948) 2249.
3 BATE-SMITH AND WESTALL, *Biochim. Biophys. Acta*, 4 (1950) 427.

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