

THE CONSTITUTION AND CONFIGURATION OF THE THEAFLAVIN PIGMENTS OF BLACK TEA

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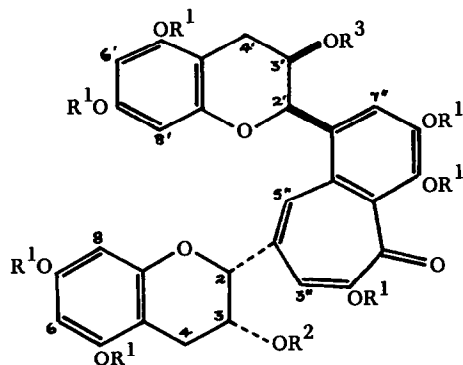
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The pigments characteristic of black tea infusions are of two general types¹ associated with the theaflavin fraction and the thearubigin fraction. The thearubigins have been recognised as polymeric proanthocyanidins.² The theaflavin fraction contains a considerable number of compounds which are benzotropolone derivatives including theaflavin^{3,4} (I; ca. 8%), theaflavin-3-gallate (II; ca. 30%), theaflavin-3'-gallate (III; ca. 20%), theaflavin-3,3'-digallate (IV; ca. 40%), isotheaflavin⁵ (4%), and theaflavic and epitheaflavic acids;⁶ the approximate relative proportions given by our present fractionation procedures are indicated. We now report upon the elucidation of the constitutions and configurations of the galloyl esters (II, III, and IV) by spectroscopic methods, and the confirmation of these configurations by synthesis.

Chromatography [Sephadex LH 20; propan-2-ol, acetic acid, water (4 : 1 : 4)] of the theaflavin fraction⁷ gave three well separated bands. The first consisted mainly of theaflavin (I) and isotheaflavin,⁵ the second yielded a mixture of the monogallates (II and III), and the third gave the digallate (IV). The IR spectra of the galloyl esters were very similar to that of theaflavin, but there were additional bands ($\nu_{\text{CO}} \sim 1690 \text{ cm}^{-1}$) which could be assigned to gallates derived from alcoholic hydroxyl groups. The absorption spectra (Table 1) of the compounds (II, III, and IV) were extremely similar to that of theaflavin (I) and their ORD curves closely corresponded with the ORD curve of theaflavin (I).

The molecular formulae of the compounds (II), (III), and (IV) were established by high resolution mass spectrometric examination of their methyl ethers (methylation with dimethyl sulphate and potassium carbonate in boiling acetone). The monogallates (II and III) gave the decamethyl ethers (VI and VII) [Found: M, 856.2939. $\text{C}_{36}\text{H}_{18}\text{O}_6(\text{OMe})_{10}$ requires M, 856.2943]; the digallate (IV) was similarly characterised as the tridecamethyl ether (VIII) [Found: M, 1050.353. $\text{C}_{43}\text{H}_{19}\text{O}_7(\text{OMe})_{13}$ requires M, 1050.352]. Their absorption spectra (Table 1) and their mass spectral fragmentation patterns summarised in the Scheme showed a striking relationship to that of theaflavin heptamethyl ether³ (V) and indicated particularly that the gallates (II, III, and IV) were galloyl derivatives involving either the 3- or the 3'-hydroxyl groups of theaflavin (I). The fragmentations were clearly dominated by (i) cleavage (-212 a.m.u.) involving the trimethoxybenzoyloxy residues and (ii) retro-Diels-Alder reactions (-166 a.m.u.).

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	$R^1 =$	$R^2 =$	$R^3 =$
(I)	H	H	H
(II)	H	X	H
(III)	H	H	X
(IV)	H	X	X
(V)	Me	H	H
(VI)	Me	Y	H
(VII)	Me	H	Y
(VIII)	Me	Y	Y

X = Galloyl (3,4,5-trihydroxybenzoyl); Y = 3,4,5-trimethoxybenzoyl

TABLE 1
Absorption spectra λ_{\max} (ϵ_{\max}) in ethanol

(I)	207 (93,000)	227 sh (26,600)	268 (21,400)	294 sh (17,900)	379 (10,100)	467 (3,700)
(II)	207 (110,000)		275 (30,500)		378 (9,150)	462 (3,300)
(III)						
(IV)	207 (118,000)		278 (35,200)		378 (9,200)	460 (3,160)
(V)	207 (111,600)		268 (20,820)	315 (9,990)	378 (3,290)	
(VI)	207 (142,000)		268 (29,300)	306 (11,700)	374 (3,040)	
(VII)						
(VIII)	208 (158,000)		269 (37,200)	306 (14,800)	368 (2,930)	

Comparison of the NMR spectra given in Table 2 clearly supported the constitutions (II), (III), and (IV). Although the monogallates were isolated by chromatography as a single fraction, the NMR spectra obtained on this mixture (II + III; approximate ratio 3 : 2) and the derived mixture of decamethyl ethers (VI + VII) permitted the assignments given in Table 2. Having established the constitutions of these three galloyl esters, the indication from their ORD curves that they had corresponding absolute configurations (II, III, and IV) was settled by synthesis. Theaflavin heptamethyl ether (V) and 3,4,5-trimethoxybenzoyl chloride in pyridine gave, after chromatographic fractionation, theaflavin-3,3'-digallate tridecamethyl ether (VIII) and a mixture of theaflavin-3- and -3'-monogallate decamethyl ethers (VI and VII).

The theaflavin pigments do not occur in the green leaf, but are formed by an enzyme-catalysed oxidative process during the fermentation of green leaf to give black tea. If hydrolysis of gallate esters is not involved, then the probable precursors⁵ are as indicated: theaflavin 3-gallate (II) [(-)-epicatechin and (-)-epigallocatechin gallate], theaflavin-3'-gallate (III) [(-)-epicatechin gallate and (-)-epigallo-

TABLE 2. NMR Spectra (100 MHz.)^a

Compounds ^b	2	3 m ^a	2'	3' m ^a	4, 4' m ^a	6, 8; 6', 8' m ^a	3''	5''	7''	Tropolone -OH	H _g ^c	Methoxyl
(I)	4.97	5.46 ^d	4.25	5.54 ^d	7.07	3.97-3.99	2.46	2.02	2.00	-4.88	-	-
(II)	4.66	4.20	4.20	5.36	6.92	3.85-4.01	2.41	1.99	1.98	-4.84	3.12	-
(III)	4.90	5.54	4.01	4.20	6.92	3.85-4.01	2.41	1.99	1.98	-4.76	3.05	-
(IV)	4.52	4.17	3.90	4.17	6.91	3.82-3.89	2.26	2.01	2.01	-4.81	2.96, 3.01	-
(V)	5.38	5.52	4.50	5.76	7.12	3.79-3.96	4.05	2.79	2.35	-	-	6.04, 6.12, 6.27-6.30
(VI)	5.04	4.66	4.30	5.86	7.09	3.72-3.91	4.05	2.96	2.36	-	2.92	6.04-6.24, 6.45
(VII)	5.33	5.86	4.30	4.66	7.09	3.72-3.91	4.05	2.96	2.43	-	2.92	6.04-6.24, 6.45
(VIII)	4.96	4.48	4.27	4.48	7.02	3.74-3.87	4.02	2.95	2.52	-	2.88, 2.90	6.14-6.22, 6.35, 6.50

^a Chemical shifts are given on the τ scale. Unless otherwise indicated, the signals given in Table 2 are singlets. Multiplets = m, and either the range of the multiplet or the centre of broad multiplets is quoted.

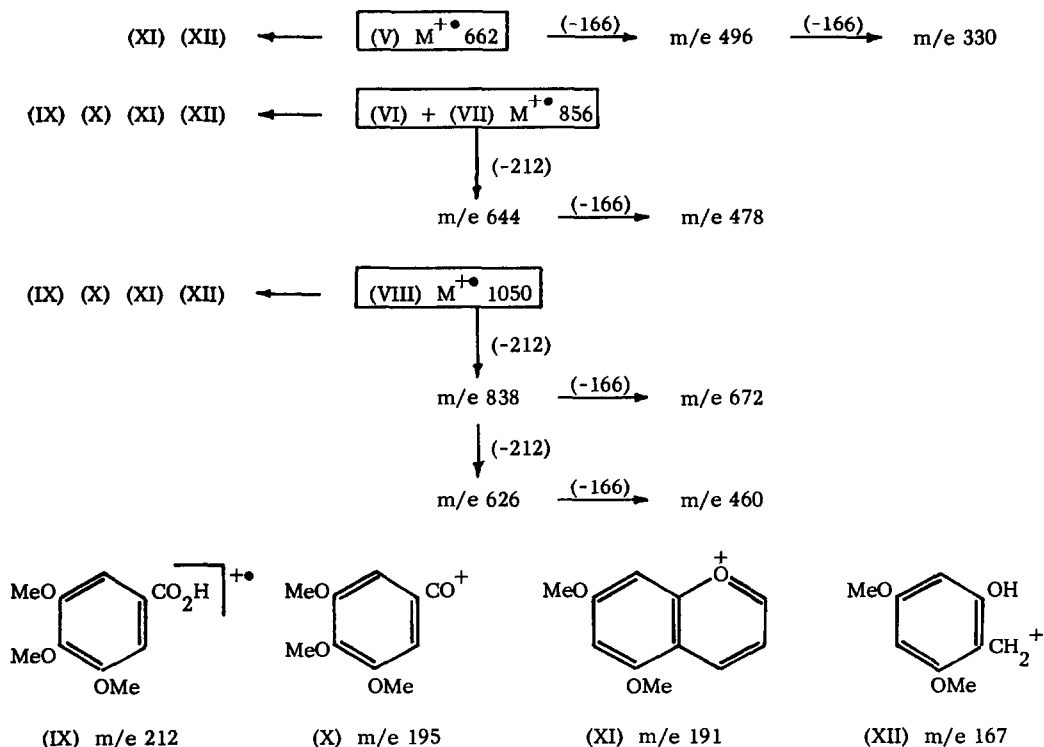
^b Solvents: (CD₃)₂CO for compounds (I) - (IV); CDCl₃ for compounds (V) - (VIII).

^c H_g refers to the 2, 6-hydrogen atoms of either galloyl or 3, 4, 5-trimethoxybenzoyl groups

^d These assignments differ from those of P. D. Collier *et al.*⁸

catechin] and theaflavin-3,3'-digallate (IV) [(-)-epicatechin gallate and (-)-epigallocatechin gallate].

Our configurations (II, III, and IV) are different from those already reported,⁸ but Dr. Collier has kindly informed us that these earlier formulations⁸ are in error and that they should be amended to become identical with ours.



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