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THE STRUCTURES OF THE THEAFLAVINS OF BLACK TEA

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Theaflavin, a pigment of black tea, has recently been shown to be a disubstituted 1'2'-dihydroxy-3,4-benzotropolone formed from epigallocatechin (EGC) and epicatechin(EC).[1-3] Consideration of the mechanism of formation of 1',2'-dihydroxy-3,4-benzotropolones from catechols and pyrogallols[4] suggests that similar pigments formed from other pairs of flavanols should occur in tea.[5] We wish to report the isolation and synthesis of 3 such pigments, which are shown by NMR and mass spectrometry to be gallate esters of Theaflavin.

Two crystalline pigments were isolated from tea: one was identical in all respects to Roberts' Theaflavin (TF1),[5] the other (TF3) was identical to the product of alkaline ferricyanide oxidation of EGCG and ECG. A third amorphous fraction (TF2) gave acetate and trimethylsilyl derivatives chromatographically identical to those of the products of ferricyanide oxidation of EGCG + EC (TF2A) and EGC + ECG (TF2B). TF2A and B and TF3 display u.v. and visible absorption spectra similar to that of TF1. Their i.r. spectra are also very similar and all include a band at 1630 cm.$^{-1}$ attributed to tropolone carbonyl groups. The i.r. spectra of TF2A and B and TF3 also include bands at 1698 cm.$^{-1}$ attributed to ester carbonyl groups: comparison of the intensities of the tropolone and ester bands indicates, as expected,[4] that TF3 is a digallate and that TF2A and B are monogallates of TF1. The appearance of molecular ions at m/e 1508 (TF2A and B) and 1804 (TF3) in the mass spectra of the trimethylsilyl derivatives supports this conclusion.

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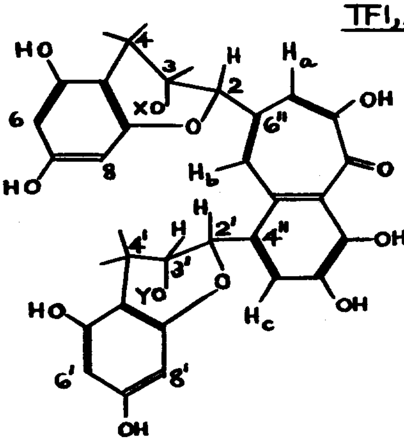
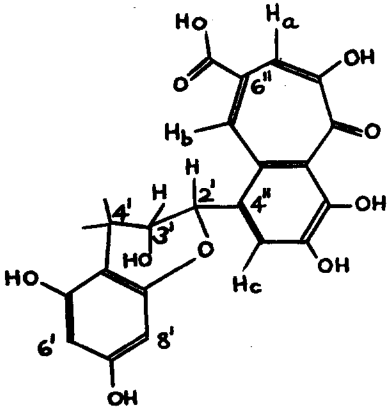
TABLE 1

TF1,2,3



TF4



	TF1	TF2A	TF2B	TF3	TF4
X	H	Galloyl	H	Galloyl	
Y	H	H	Galloyl	Galloyl	
2	5.02	5.31	5.08	5.48	–
2'	5.73	5.76	5.98	6.12	5.69
3	4.45	5.70	4.40	5.76	–
3'	4.55	4.54	5.75	5.80	4.44
4 } (4H)	3.02,2.86	2.8 – 3.2 mult.	2.8 – 3.2 mult.	3.31,3.05	2.89,3.06 (2H) J_{AB}, 16.5
4'	2.96,2.89			3.10,3.01	J_{AX}, $J_{BX} \simeq 2$
6,6'	6.03,6.10 J, 2.3	6.03,6.08 J, 2.2	5.98,6.02 J, 2.3	6.13,6.16 J, 2.3	6.00 J, 2.3
8,8'	6.07,6.08	6.09,6.11	6.07,6.12	6.15,6.18	6.07
a	7.54	7.62	7.58	7.78	7.83 (d,J=2.5)
b	7.97	7.97	8.00	8.00	8.74
c	8.03	8.02	8.03	8.10	8.05
gallate(2H)	–	6.91	6.93	{7.00(2H), {7.04(2H)	–
Tropolone OH	14.93	14.82	14.95	14.89	15.0

Spectra were obtained from solutions in deuterioacetone using a Varian HA-220 spectrometer. Our thanks are due to Mr. Verwiel of the TNO Laboratory, Delft, The Netherlands, for the use of this instrument, and for running many of the spectra. All δ-values quoted (except those for tropolone hydroxyl protons) refer to D_2O-exchanged solutions.

The 220 MHz NMR spectra of the Pigments (Table 1) confirm these identifications. Comparison of the gallate regions (ca. 7 ppm) shows that TF3 (4 gallate protons) is a digallate ester and that TF2A and B are isomeric monogallate esters (2 gallate protons) of TF1. Comparison of the spectra of TF2, TF2A and TF2B shows that TF2 is a mixture of TF2A and B in the ratio 1.75:1. The gallate groups shift the C_3H and $C_{3'}H$ lines downfield by 1.2-1.3 ppm and the C_2H and $C_{2'}H$ lines downfield by 0.22-0.46 ppm from their positions in the spectrum of TF1. The lines due to $C_{2'}H$ in TF2B and TF3 cannot be precisely located due to superposition of the 6,8,6',8' AB patterns.[a] The 3 protons of the benzotropolone ring (H_a, H_b, H_c) absorb in the region 7.5-8.1 ppm, and in all 4 spectra the centre line is broad. The assignments of these protons in Table 2 differ from those previously published for TF1,[1,2] and are based on the detection in double resonance experiments of (a) long-range benzylic couplings[6] of C_2H with H_a and of $C_{2'}H$ with H_c; and (b) mutual nuclear Overhauser effects (NOE)[7] between H_b and $C_{2'}H$. The NOEs and the H_b line width are symptoms of the efficient mutual relaxation [b] and close approach of H_b and $C_{2'}H$: inspection of Dreiding models confirms that of the 3 benzotropolone aromatic protons, H_b can approach closest to $C_{2'}H$.

The NMR spectrum of the ferricyanide co-oxidation product of gallic acid and EC (TF4) provides further support for these assignments. The $C_{2'}H$ line appears at 5.69 ppm, thus confirming the assignment of lines at 5.73 and 5.76 ppm in the spectra of TF1 and TF2A to $C_{2'}$ protons. Comparison of the low-field regions of the spectra of TF1 and TF4 shows that introduction of a carboxyl group at the 6″-position of the tropolone ring shifts the two broader lines (assigned to H_a and H_b in TF1) downfield, whilst the chemical shift of the sharpest line (H_c) remains unchanged. Decoupling experiments with TF4 demonstrate (a) a meta-coupling of 2.5 Hz between H_a and H_b, (b) benzylic coupling of $C_{2'}H$ with both H_b and H_c, and (c) no NOE between $C_{2'}H$ and H_b. This atypical behaviour is attributed to the fact that TF4 has a less crowded structure than TF1-3. In TF1-3, the avoidance of serious interactions between the 2

(a) Integration of these multiplets confirms that they arise from 5 protons instead of the expected 4, and expanded scale spectra show the superposition of a broad $C_{2'}H$ line on the high-field end of the multiplet.

(b) Progressive saturation experiments confirm that H_b and $C_{2'}H$ saturate less readily than the other protons.

bulky flavan groups probably restricts their rotation about the bonds joining them to the benzotropolone ring system: this results in small time-averaged H_b-$C_{2'}H$ separations, and very efficient mutual relaxation of H_b and $C_{2'}H$. This accounts for relaxation-broadening of H_b lines, the NOEs, and for the failure to detect long-range couplings with H_b. In contrast, TF4 has only one flavan substituent: its rotation about the $C_{2'}$-$C_{4''}$ bond is therefore less restricted than in TF1-3, and the time-averaged $C_{2'}H$-H_b separation is greater. The influence of the consequently less efficient mutual relaxation of H_b and $C_{2'}H$ on the populations of their spin states is insufficient to produce NOEs, and their relaxation rates are not fast enough to cause collapse of long-range couplings.

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