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THREE NEW THEAFLAVINS FROM BLACK TEA

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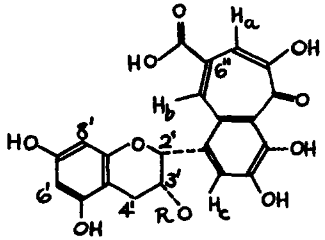
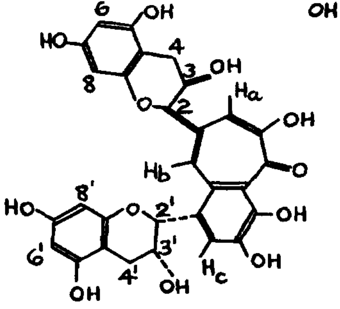
Five theaflavin pigments of black tea have so far been reported; theaflavin,[1-3] isotheaflavin,[2] theaflavin-3- and 3'-monogallates[2,3] and theaflavin digallate.[2,3] The pigments have been shown to be 1',2'-dihydroxy-3,4-benzotropolones formed by co-oxidation of pairs of catechins and gallocatechins occurring in green tea leaf.[1-3] We now report the isolation and synthesis of three further theaflavins, shown by NMR and mass spectrometry to be epitheaflavic acid (Ia),[2,3] epitheaflavic acid-3'-gallate (Ib),[4] and a third isomer (IIb) of theaflavin itself (IIa).

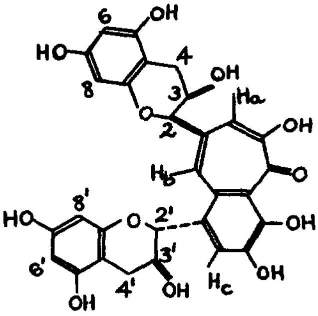


Ia, R=H

Ib, R=Galloyl



IIa



IIb

Table 1.

	Ia	Ib	Ic	IIa	IIb
2	-	-	-	5.02 [c]	5.00 [c]
$2'$	5.69 [c]	5.97 [c]	5.49 $J_{2'3'}$= 8.5	5.73 [c]	5.60 $J_{2'3'}$=8.0
3	-	-	-	4.45^{b}	4.35
$3'$	4.44	5.75	4.32	4.55^{b}	4.12
4 (2H)	-	-	-	2.96,2.86	2.85,2.90
[a] $4'^{*}$ $4'$	3.06 $J_{3'4'*}$=4.0 $J_{4'4'*}$=16.5 $J_{3'4'}$ =1.5 2.89	3.19 $J_{3'4'*}$=3.5 $J_{4'4'*}$=17.0 $J_{3'4'}$ =1.5 3.08	2.71 $J_{3'4'*}$=9.0 $J_{4'4'*}$=16.0 $J_{3'4'}$ =5.5 3.09	3.02 2.89	2.67 $J_{3'4'*}$=9.5 $J_{4'4'*}$=16.0 $J_{3'4'}$ = 5.5 2.96
$6,6'$ $8,8'$	6.00 $J_{6'8'}$ =2.0 6.07	6.11 $J_{6'8'}$ =2.0 6.14	5.96 $J_{6'8'}$ =2.0 6.11	6.03,6.10 $\left.\begin{matrix}J_{68}\\J_{6'8'}\end{matrix}\right\}$ =2.3 6.07,6.08	5.93,5.94 $\left.\begin{matrix}J_{68}\\J_{6'8'}\end{matrix}\right\}$ =2.3 6.02,6.06
a	7.83 J_{ab}= 1.3	7.83	7.89 J_{ab}= 1.3	7.54	7.64
b	8.74	8.75	9.20 J_{ab}= 1.3	7.97	8.23
c	8.05	8.02	7.82	8.03	7.74
Gallate (2H)		6.94			
Tropolone OH	15.0	14.85	14.90	14.93	14.98

Spectra were obtained from solutions in deuterioacetone using a Varian HA-220 spectrometer. All chemical shifts (except those for tropolone hydroxyl protons) refer to D_2O - exchanged solutions.

a) $4'^{*}$ refers to the quasi-axial proton.

b) Assignment based on decoupling from C_2H and $C_{2'}H$.

c) J_{23}, $J_{2'3'}$ <1.

Epitheaflavic acid, long suspected to occur in black tea,[4] has previously only been described as a synthetic (ferricyanide) oxidation product of gallic acid and (-)epicatechin.[2,3] Examination of crude theaflavin fractions of black tea by g.l.c. of their pertrimethylsilyl derivatives[5] indicated the presence of a species indistinguishable from Ia at levels in the range 0.03-0.1% of whole tea solubles. Mass spectral comparison of the appropriate trapped material with a silylated authentic sample of Ia confirmed that epitheaflavic acid (or less likely, the isomeric theaflavic acid[2]) is present in black tea. A pigment indistinguishable by NMR from epitheaflavic acid was also isolated in low yield from black tea solubles by chromatography on Sephadex LH20.[6]

A second pigment (Ib,$C_{28}H_{20}O_{14}$), identical to the principal product of alkaline ferricyanide oxidation of (-)epicatechin gallate and gallic acid, was similarly isolated. Its u.v., visible and i.r. spectra are very similar to those of epitheaflavic acid: both have $\lambda_{max.}$ (visible) at 400 nm., and i.r. bands at 1630 cm.$^{-1}$ (tropolone carbonyl) and 1700 cm.$^{-1}$ (ester and carboxyl carbonyl). Comparison of the intensities of tropolone and ester carbonyl bands suggests, as expected,[7] that Ib is epitheaflavic acid-3$'$-gallate. The appearance of molecular ions at m/e 932 (Ia) and 1228 (Ib) in the mass spectra of the pertrimethylsilyl derivatives supports this conclusion. The 220 MHz. NMR spectrum of Ib (Table 1) confirms this identification: the two gallate protons appear at 6.94 ppm (cf. theaflavin gallates at 6.90-7.00 ppm[3]), whilst the introduction of a 3$'$-gallate group shifts the $C_{3'}H$ and $C_{2'}H$ signals downfield by 1.33 and 0.29 ppm respectively from their positions in the spectrum of epitheaflavic acid (cf. 1.2-1.3 and 0.25-0.29 ppm for theaflavin and its gallates[3]). The low-field regions of the spectra of epitheaflavic acid and Ib are very similar and consistent with a 6"-carboxyl substituted tropolone ring structure.[3] The singlet $C_{2'}H$ signals and $C_{3'}H$-$C_{4'}H$ coupling constants show clearly that epitheaflavic acid and its 3$'$-gallate have the 2$'$,3$'$-cis configuration and similar chroman ring conformations.

A third pigment (IIb, $C_{29}H_{24}O_{12}$), isolated by preparative paper chromatography from crude theaflavin, was identical to the product of alkaline ferricyanide oxidation of (-) epigallocatechin and (+) catechin. Its u.v., visible and i.r. spectra are very similar to those of theaflavin (IIa), and the mass spectra of the pertrimethylsilyl ethers[3] are almost identical. Comparison of the NMR spectra of IIb, theaflavin[3] and isotheaflavin[2] shows clearly that IIb is a theaflavin isomer with the 2,3-*cis*-2',3'-*trans* configuration. Thus for IIb, as for (+) catechin (2,3-trans), $J_{2',3'} \sim 8$, $J_{3',4'*} \sim 9.5$ and $J_{3',4'} \sim 5.5$. In contrast, for 2,3-cis-flavan-3-ols $J_{23} < 1$, $J_{34*} \sim 4\text{-}5$ and $J_{34} \sim 3.5$[a]. It is interesting that in the spectra of 2',3'-*trans* configuration theaflavins (IIb and Ic, theaflavic acid[2]) the broad H_b signals and the H_c signals occur at lower and higher fields respectively than in those of the corresponding 2',3'-*cis* theaflavins (theaflavin and epitheaflavic acid). This implies that the configuration of the heterocyclic chroman ring affects the magnetic environment of H_b and H_c, presumably via its effect on the preferred conformation of the entire chroman group with respect to the benzotropolone ring system.

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a) Coupling constants obtained by first-order analysis of 220 MHz spectra of (-) epigallocatechin and (-) epicatechin.

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