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ISOTHEAFLAVIN. A NEW BLACK TEA PIGMENT

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The green leaf used in the manufacture of black tea contains a number of flavanols and flavanol gallates including (-)-epigallocatechin gallate (9-13%), (-)-epicatechin gallate (3-6%), (-)-epigallocatechin (3-6%), (-)-epicatechin (1-3%), and other flavanols (1-2%).[1] These precursors are transformed into the theaflavin[2-5] and thearubigin[6] type pigments of black tea during the fermentation step, which involves an enzyme-catalysed oxidative coupling process. Thus, theaflavin (I) is considered to be formed from (-)-epicatechin (IV) and (-)-epigallocatechin (VI) as precursors. However, the enzyme-catalysed oxidative coupling seems rather unspecific towards substrates, so this encouraged the search for isomers of theaflavin (I). We now report the isolation of an isomer of theaflavin (I) from aqueous infusions of black tea which has been called isotheaflavin (III).

Column chromatography of the theaflavin fraction, as described in the preceding communication,[5] yielded a mixture of theaflavin (I) and isotheaflavin (III) which was separated by thin layer chromatography [cellulose – n-butanol : formic acid : water (100 : 25 : 60)]. It was then found that isotheaflavin could be isolated preparatively from the mother liquors remaining after the crystallisation of theaflavin[3] obtained by tannase hydrolysis of theaflavin gallates.[7] This preparative procedure involved the sequence (i) isolation of a mixture of theaflavin and isotheaflavin by column chromatography [Sephadex LH 20 – propan-2-ol : acetic acid : water (4 : 1 : 4)], (ii) paper chromatography [3MM papers – n-butanol : acetic acid : water (4 : 1 : 5)], and (iii) column chromatography [Sephadex LH 20 – 60% aqueous acetone]. This detail is given because the two-dimensional chromatographic behaviour of isotheaflavin is similar to that of the theaflavin gallates, and this probably explains why isotheaflavin has not been identified previously.

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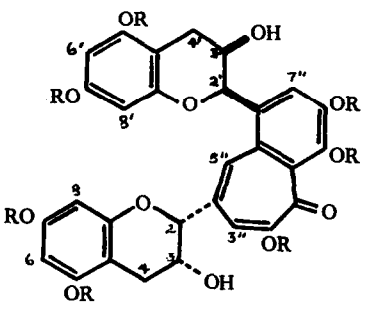
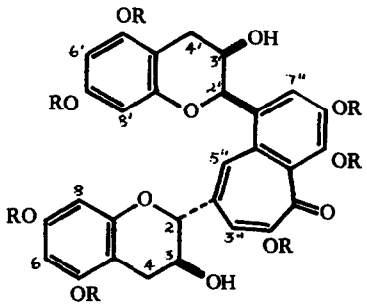
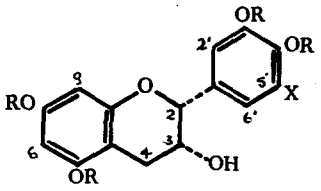


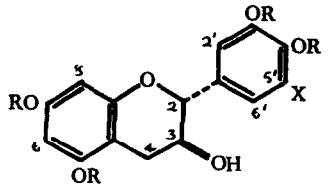
Theaflavin (I) R = H
(II) R = Me



Isotheaflavin (III) R = H
(IV) R = Me



(-)-Epicatechin (IV) R = X = H
(V) R = Me; X = H
(-)-Epigallocatechin (VI) R = H; X = OH
(VII) R = Me; X = OMe



(+)-Catechin (VIII) R = X = H
(IX) R = Me; X = H
(+)-Gallocatechin (X) R = H; X = OH

TABLE 1

Absorption spectra λ_{max} nm (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)
(III)	207 (93,500)	231 sh (28,600)	270 (18,800)	297 sh (14,700)	378 (7,500)	462 (2,700)
(II)	207 (111,600)		268 (20,800)	315 (9,990)	378 (3,290)	
(IV)	207 (97,500)		267 (15,600)	312 (7,600)	375 (2,430)	

The IR and UV spectra (Table 1) of theaflavin (I) and isotheaflavin (III) were very similar and, although their NMR spectra (Table 2) showed some common features, they also showed some useful differences. The high resolution mass spectrum of isotheaflavin heptamethyl ether (IV) established its molecular formula [Found: M, 662.2354. $C_{29}H_{17}O_5(OMe)_7$ requires M, 662.2363]. The mass spectral fragmentation patterns of theaflavin heptamethyl ether (II) and isotheaflavin heptamethyl ether (IV) were extremely similar. These results indicated that isotheaflavin was a diastereomer of theaflavin (I) and the relative configuration (III) was then deduced for isotheaflavin by detailed comparison of the NMR spectra summarised in Tables 2 and 3.

An important study[8] has examined the general relationship between the NMR spectra of flavan derivatives and their relative configuration. The four protons (H-2, H-3, H-4, and H-4*) associated with the heterocyclic ring of flavan-3-ols constitute an ABXY system and the spectra (Table 3) of the 2, 3-cis- (IV–VII) and the 2, 3-trans-flavan-3-ols (VIII–X) show informative differences. The 2, 3-cis-flavan-3-ols show singlet signals for H-2 ($J_{2,3} \sim 0$) and signals of deceptively simple multiplicities for H-4 and H-4*. In contrast, the 2, 3-trans-flavan-3-ols give detailed spectra amenable to first order analysis ($J_{2,3} \sim 8$; $J_{3,4} \sim 6$; $J_{3,4^*} \sim 9$, and $J_{4,4^*} \sim 16$ Hz). Comparison of the information given in Tables 2 and 3 provides a remarkably satisfying correlation and clearly demonstrates that theaflavin has the 2, 3-cis-2', 3'-cis- and isotheaflavin the 2, 3-trans-2', 3'-cis-relative configuration.

This is the first direct deduction of 2, 3-cis-2', 3'-cis-configuration of theaflavin; this relative configuration is compatible with the absolute configuration (I) proposed on the basis of its formation from (-)-epicatechin (IV) and (-)-epigallocatechin (VI). Any possibility considered previously of a change of configuration during the oxidative coupling leading to theaflavin (I) is now removed. Similarly, it seems probable that isotheaflavin (III) is formed from (-)-epicatechin (IV) and (+)-gallocatechin (X) as precursors and experiments related to this hypothesis are in progress. The possibility that isotheaflavin (III) could have arisen as an artefact derived from theaflavin (I) during isolation procedures involving acidic solvents is most unlikely. Theaflavin did not isomerise when kept in acidic solvents for several months. An independent correlation of the absolute configuration of theaflavin (I) and isotheaflavin (III) is provided by the similarity of their ORD curves. This indicates that they have the corresponding absolute configurations at the benzylic chiral centres, 2 and 2'.

TABLE 2. NMR Spectra of theaflavin, isotheaflavin, and their heptamethyl ethers

	2	3	4	4*[c]	2'	3'	4'	4*'[c]	6, 8; 6', 8'	3''	5''	7''	Tropolone OH	Methoxyl groups
(I)[a]	4.97 s	5.46 m	7.07 m		4.25 s	5.54 m	7.07 m		3.97-3.99 m	2.46 s	2.02 s	2.00 s	-4.88 s	-
(II)[b]	5.38 s	5.55 m	7.19 m		4.51 s	5.77 m	7.11 m		3.77 d 3.87 d 3.90 d 3.94 d $J_{6,8} = J_{6',8'} = 2.5$	4.04 s	2.79 s	2.31 s	-	6.07, 6.12, 6.23, 6.23, 6.23, 6.31, 6.31 All s
(III)[a]	5.28 d $J_{2,3} = 8$	5.95 m	6.91 dd $J_{3,4} = 5.5$ $J_{4,4^*} = 16$	7.44 dd $J_{3,4^*} = 9$ $J_{4,4^*} = 16$	4.30 s	5.58 m	7.10 m		3.94-4.07 m	2.60 s	2.22 s	1.98 s	-4.83 s	-
(IV)[b]	5.45 d $J_{2,3} = 7$	~6.0[d] m	7.17 dd $J_{3,4} = 5.5$ $J_{4,4^*} = 16$	7.44 dd $J_{3,4^*} = 8$ $J_{4,4^*} = 16$	4.66 s	5.81 m	7.13 m		3.80 d 3.88 d $J_{6,8}$ or $J_{6',8'} = 2.5$ 3.92 s (two H)	4.02 s	3.08 s	2.34 s		6.04, 6.06, 6.22, 6.24, 6.24, 6.24, 6.28 All s

Chemical shifts are given on the τ scale. Multiplicities and coupling constants (J Hz.) have been derived by first order analysis: s = singlet (broadened in some cases by additional coupling); d = doublet; dd = double doublet; m = multiplet (either the range of the multiplet or the centre of the multiplet is quoted). The symbols "d" and "t" are used to refer to "doublets" and "triplets" associated with deceptively simple spectra. Deuteriation established the presence of signals assignable to hydroxyl groups; these are not included in the Table.

a 100 MHz. spectrum, solvent (CD_3COCD_3).

b 220 MHz. spectrum, solvent ($CDCl_3$).

c 4* and 4*' refer to the 4- and 4'-quasi-axial protons.[8]

d Partly obscured by methoxyl group signals.

TABLE 3. NMR Spectra (100 MHz.) of flavan-3-ols and their methyl ethers

	2	3	4	4*	6	8	2'	5'	6'	Methoxyl groups
(IV)	5.15 s	5.80 m	7.23 "t"	7.23 "t"	4.01 d $J_{6,8}$ = 2.5	4.11 d $J_{6,8}$ = 2.5	2.98 s	3.24 s	3.24 s	-
(VI)	5.18 s	5.79 m	7.20 "t"	7.20 "t"	3.99 d $J_{6,8}$ = 2.5	4.09 d $J_{6,8}$ = 2.5	3.44 s	-	3.44 s	-
(V)	5.10 s	5.78 m	7.13 "d"	7.13 "d"	3.84 d $J_{6,8}$ = 2.5	3.92 d $J_{6,8}$ = 2.5	2.95 (H_A) d ABX system J_{AX} = O;	3.15 (H_X) d J_{AB} = 2;	2.99 (H_B) dd J_{BX} = 8	6.14, 6.16, 6.26, 6.28 s s s s
(VII)	5.10 s	5.73 m	7.10 "d"	7.10 "d"	3.82 d $J_{6,8}$ = 2.5	3.90 d $J_{6,8}$ = 2.5	3.17 s	-	3.17 s	6.15, 6.15, 6.18, 6.33, 6.26 s s s s s
(VIII)	5.43 d J = 7.5	5.99 m	7.06 dd $J_{3,4}$ = 5.5 $J_{4,4*}$ = 16	7.47 dd $J_{3,4*}$ = 9 $J_{4,4*}$ = 16	3.98 d $J_{6,8}$ = 2.5	4.12 d $J_{6,8}$ = 2.5	3.10 s	3.24 s	3.24 s	-
(X)	5.48 d J = 7.5	6.01 m	7.08 dd $J_{3,4}$ = 5.5 $J_{4,4*}$ = 16	7.48 dd $J_{3,4*}$ = 8 $J_{4,4*}$ = 16	3.98 d $J_{6,8}$ = 2.5	4.12 d $J_{6,8}$ = 2.5	3.55 s	-	3.55 s	-
(IX)	5.36 d J = 8.0	~6.1 m[d]	6.96 dd $J_{3,4}$ = 5.5 $J_{4,4*}$ = 16.5	7.45 dd $J_{3,4*}$ = 9 $J_{4,4*}$ = 16.5	3.87 d $J_{6,8}$ = 2.5	3.91 d $J_{6,8}$ = 2.5	3.06 (H_A) d ABX system J_{AX} = O;	3.16 (H_X) d J_{AB} = 2;	3.04 (H_B) dd J_{BX} = 8	6.16, 6.16, 6.23, 6.28 s s s s

For footnotes refer to Table 2. Solvents: (IV), (VI), (VIII), and (X), CD_3COCD_3; (V), (VII), and (IX), $CDCl_3$.

It has already been recognised that it is not possible to determine the preferred conformations of flavan-3-ols[8] and isoflavans[9] on the basis of coupling constant information. Proposals[4] for the preferred conformations of the heterocyclic ring of theaflavin derivatives should also probably be treated with reserve.

REFERENCES

(1) D. J. Millin and D. W. Rustidge, Process Biochem. 2 (6), 9 (1967); D. J. Millin, D. J. Crispin, and D. Swaine, J.Agr.Food Chem. 17, 717 (1969).

(2) Y. Takino, A. Ferretti, V. Flanagan, M. A. Gianturco, and M. Vogel, Tetrahedron Letters 4019 (1965); Canad.J.Chem. 45, 1949 (1967); A.Ferretti, V. P. Flanagan, H. A. Bondarovich, and M. A. Gianturco, J.Agr.Food Chem. 16, 756 (1968).

(3) A. G. Brown, C. P. Falshaw, E. Haslam, A. Holmes, and W. D. Ollis, Tetrahedron Letters 1193 (1966).

(4) T. Bryce, P. D. Collier, I. Fowlis, P. E. Thomas, D. Frost, and C. K. Wilkins, Tetrahedron Letters 2789 (1970).

(5) D. T. Coxon, A. Holmes, W. D. Ollis, and V. C. Vora, Tetrahedron Letters (1970).

(6) A. G. Brown, W. B. Eyton, A. Holmes, and W. D. Ollis, Nature 221, 742 (1969); Phytochemistry 8, 2333 (1969).

(7) E. A. H. Roberts and M. Myers, J.Sci.Food Agric. 10, 172 and 176 (1959); E. Haslam and J. E. Stangroom, Biochem.J. 99, 28 (1966).

(8) J. W. Clark-Lewis, L. M. Jackman, and T. M. Spotswood, Austral.J.Chem. 17, 632 (1964).

(9) K. Kurosawa, W. D. Ollis, B. T. Redman, I. O. Sutherland, O. R. Gottlieb, and H. Magalhães Alves, Chem.Comm. 1265 (1968).