Phenolic Hydroxy-group Reactivity in Catechin

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Summary N.m.r. and fluorescence spectra of the products of the reaction of catechin with ethyl bromoacetate show that the 3′- and 4′-phenolic hydroxy-groups become etherified before those at positions 5 and 7.

BARK polyphenolic fractions of western hemlock (*Tsuga heterophylla*) are believed to have the structure (I), composed of catechin-like units derived from leucocyanidin.[1,2] Knowledge of the relative reactivities of phenolic hydroxy-groups in catechin (II) to etherification should be illustrative of the reactivity of analogous groups in bark polyphenols. The reactivity of phenolic hydroxy-groups in certain flavones has recently been investigated;[3] however, no study of the relative reactivity of these groups in the flavan series, of which catechin is a member, has been made.

Molar equivalents (1—4) of ethyl bromoacetate and

catechin were reacted together in acetone containing anhydrous potassium carbonate for 6 h. Products were separated by t.l.c. (200:47:15:1; benzene–ethanol–water–acetic acid; I_2 visualization). At most six products were formed and each was isolated and purified by multiple elution preparative t.l.c. The compounds (III—VIII) are in order of increasing R_F. Integrated n.m.r. spectra indicated that two compounds contained one ethoxycarbonyl methyl-group each, one was disubstituted, two were trisubstituted, and one was tetrasubstituted. The structures of all, except for the monosubstituted compounds, were unambiguously determined.

Ferric chloride solution failed to give the typical strong green coloration produced by *o*-dihydroxy-groups in the catechol ring[4] thus indicating that the B-ring was substituted in all reaction products. The ferric chloride–ferricyanide spray[4] gave no colour with the tetrasubstituted compound (VIII).

The n.m.r. spectra of monosubstituted compounds (III—IV) were obtained in deuteriomethanol because of their low solubility in deuterioacetone, which was used otherwise, and gave poor detail because of solvent effects. Spectra of less pure monosubstituted compounds in deuterioacetone showed the 6- and 8-H as an AB quartet at τ 4 ($J_{6,8}$ 2 Hz, $J_{d,d}$ 9 Hz). An identical splitting pattern for (V) at τ 4·05 (6- and 8-H) indicated that ring B was disubstituted. A positive cinnamaldehyde–hydrochloric acid spray test (for resorcinol groups)[4] supported this assignment. Further conclusive proof was obtained by fluorescence spectroscopy.

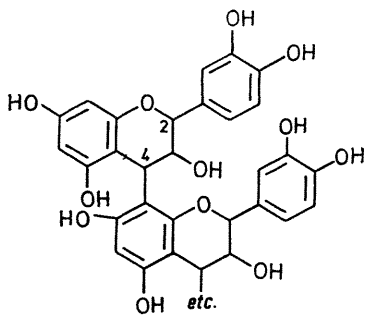
Fluorescence emission spectra of catechin and catechol (IX) are very similar (see Table); phloroglucinol does not fluoresce under identical conditions. It was concluded that the B-ring is the fluorescent chromophore of catechin with the A-ring being relatively inactive. Both catechin and catechol lose their fluorescent properties when ionized (pH 10) while, as expected, the fluorescence of the disubstituted catechol (X) does not vary with pH. The difference in fluorescent properties of the two monosubstituted catechins (III) and (IV) is of interest. Disubstitution causes the appearance of two emission bands: a more intense band at 360 and a weaker one at 320 nm. The occurrence of these same bands with the tetrasubstituted compound (VIII) supports assignment of substituents of the diethoxycarbonylmethylated product.

TABLE

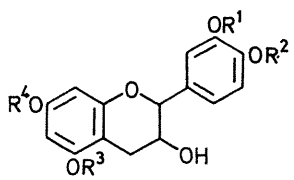
Fluorescent emission bands of substituted and unsubstituted catechol and catechin

Compound[b]	λ/nm[a] pH 6	pH 10
Catechol (IX)	315	
OO-Bisethoxycarbonylmethylcatechol (X)	360	360
Catechin (II)	315	
O-Ethoxycarbonylmethylcatechin (III or IV)	320	~360vw
O-Ethoxycarbonylmethylcatechin (III or IV)	360	360
OO-Bisethoxycarbonylmethylcatechin (V)	320, 360	310, 360
OOOO-Tetrakisethoxycarbonylmethylcatechin (VIII)	320, 360	310, 360

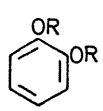
[a] λ (excitation) 280 nm.
[b] 8—12 p.p.m. in aqueous phosphate and borate buffers.



(I)



(II) $R^1 = R^2 = R^3 = R^4 = H$
(III) and (IV) $R^1, R^2 = H, CH_2 \cdot CO_2Et$; $R^1, R^3 R^4 = H$
(V) $R^1 = R^2 = CH_2 \cdot CO_2Et$; $R^3 = R^4 = H$
(VI) $R^1 = R^2 = R^4 = CH_2 \cdot CO_2Et$; $R^3 = H$
(VII) $R^1 = R^2 = R^3 = CH_2 \cdot CO_2Et$; $R^4 = H$
(VIII) $R^1 = R^2 = R^3 = R^4 = CH_2 \cdot CO_2Et$



(IX) R = H
(X) R = $CH_2 \cdot CO_2Et$

The structure of the trisubstituted products (VI—VII) was easily ascertained by n.m.r. Etherification of the 7-hydroxy-group should only slightly affect 6- and 8-H signals previously observed with compounds (III—V), since the symmetry has not been disrupted. Substitution at the 5-position should have a marked effect on the 6-H signal. A study of the spectra of flavanoids[5] confirms this assumption. It was clear that compound (VII) had its third substitutent located on the C-5 oxygen since the 6- and 8-protons were observed as a two proton singlet at τ 4·03. The spectrum of (VI), on the other hand, displayed these protons as a quartet. In the tetraethoxycarbonylmethylated compound (VIII) these protons form a compact AB quartet ($J_{d,d}$ 4 Hz).

Since monosubstitution occurs solely on the B-ring and only one disubstituted compound (V) was found (in 15—25% yields), it can be concluded that the B-ring phenolic hydroxy-groups are etherified prior to reaction with groups on ring A. Product-ratio analysis showed that both positions in ring B are almost equally reactive; this was also true for phenolic groups at C-5 and 7.

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