Biosynthesis of Proanthocyanidins

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Summary The incorporation of 4',5,7-trihydroxydihydroflavonol into the dimeric procyanidin B-4 in *Rubus idaeus* indicates that both flavan-3,4-diols and flavan-3-ols are derived biogenetically from dihydroflavonols.

THE commonly occurring dimeric procyanidins, *e.g.* (**1**), are likely condensation products of a flavan-3,4-diol with a catechin.[1] Haslam and his co-workers[2] have recently shown that cinnamic acid was incorporated intact into the 'upper' and 'lower' flavan portions of procyanidin B-4 (**1**), whereas epicatechin (**2**) was incorporated only into the lower portion. Dihydroflavonols have been shown to be precursors of catechins,[3] and flavan-3,4-diols have been postulated to be intermediates in this transformation.[2,4] No experimental study of the biosynthetic relationship of flavan-3,4-diols to dihydroflavonols however, has so far been made. We now report results of *in vivo* feeding experiments in which the incorporation of a dihydroflavonol into the two flavan portions of a procyanidin was studied.

[*U*-^{3}H]Aromadendrin (**3**)† (3·1 μmol; 53·0 μCi μmol^{-1}) in aqueous solution was fed *via* a cotton wick to a mature new raspberry stem (*Rubus idaeus*). [1-^{14}C]Phenylalanine

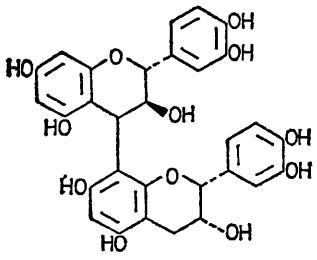
† Obtained by tritiation of aromadendrin by the Wilzbach method, carried out by the Radiochemical Centre, Amersham. We thank Dr. H. E. Hillis for the gift of the aromadendrin.

TABLE

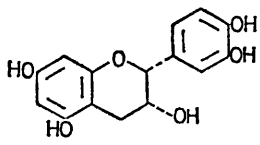
Incorporation of radioactivity into epicatechin and procyanidin B-4

Precursor	Specific activity/10^4 dpm μmol^{-1} 2×P	3×P	4×P	Dilution	% Incorporation
[1-^{14}C] Phenylalanine					
epicatechin	2·96	2·67		326	1·13
procyanidin B-4	3·36	3·24		269	0·45
[G-^{3}H] Aromadendrin					
epicatechin	21·72	20·88		563	0·44
procyanidin B-4	24·42	22·86		515	0·19
[G-^{3}H] Aromadendrin					
epicatechin	4·50				
procyanidin B-4	5·45		1·39[a]		
4-benzylthioflavan-3,3′,4′,5,7-pentaol			0·80		
epicatechin			0·50		

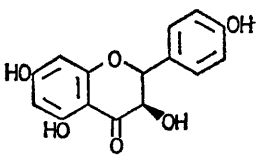
[a] After addition of B-4 carrier. An ϵ value of $4{\cdot}09 \times 10^3$, based on that determined for epicatechin at λ280 nm, was used for the spectrophotometric determination of the amount of thiol (**4**) obtained. An ϵ value double that for epicatechin was used for the estimation of B-4.



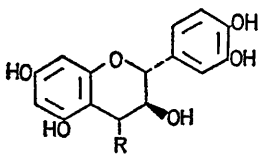
(1)



(2)



(3)



(4); R = SCH_2Ph
(5); R = OH

(5·1 μmol; 3·93 μCi μmol^{-1}) was similarly administered in a separate parallel experiment. Flavan constituents were extracted[5] after 48 h and procyanidin B-4 (**1**) and epicatechin (**2**) were isolated and purified by successive paper chromatography. Radioactivity and incorporation data for the two parallel experiments are given in the Table.

In a third experiment, [^{3}H]-labelled B-4 obtained after feeding of [^{3}H] aromadendrin was further purified after the addition of carrier material. This was then degraded[5] with toluene-α-thiol into 4-benzylthioflavan-3,3′,4′,5,7–pentaol (**4**) and epicatechin (**2**), representing the 'upper' and 'lower' halves respectively of the original B-4 dimer. These products were purified by paper chromatography and their specific activities determined (Table).

The incorporation results show that aromadendrin is about as good a precursor for the monomeric and dimeric flavanols in *R. idaeus* as phenylalanine, comparable to results previously obtained[3] for the biosynthesis of catechins in tea. The distribution of labelling into both halves of the B-4 molecule, as indicated by the relative specific activities of the degradation products (Table), clearly shows that the presumed flavan-3,4-diol precursor (**5**) of B-4 is derived at least as readily as epicatechin from the dihydroflavonol (**3**). The results thus lend experimental support for the belief that the probable sequence of biosynthesis for these classes of compounds is: dihydroflavonol ⟶ flavan-3,4-diol ⟶ flavan-3-ol.

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