Biosynthesis of Plant Proanthocyanidins

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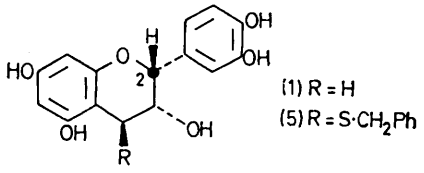
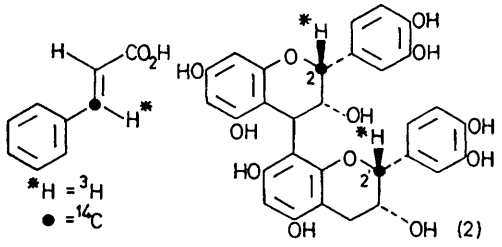
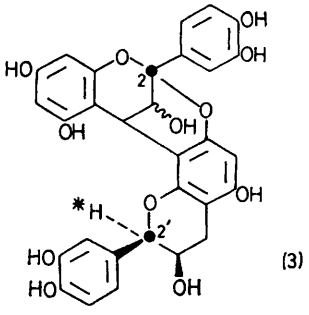
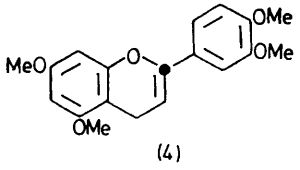
Summary Studies of the biosynthesis of (—)-epicatechin and related plant proanthocyanidins have been made in *Aesculus carnea* and *Rubus* sp., these are interpreted in terms of a new scheme of biogenesis.

THREE proposals have been variously made to account for the biosynthetic formation of the interflavan linkages of the



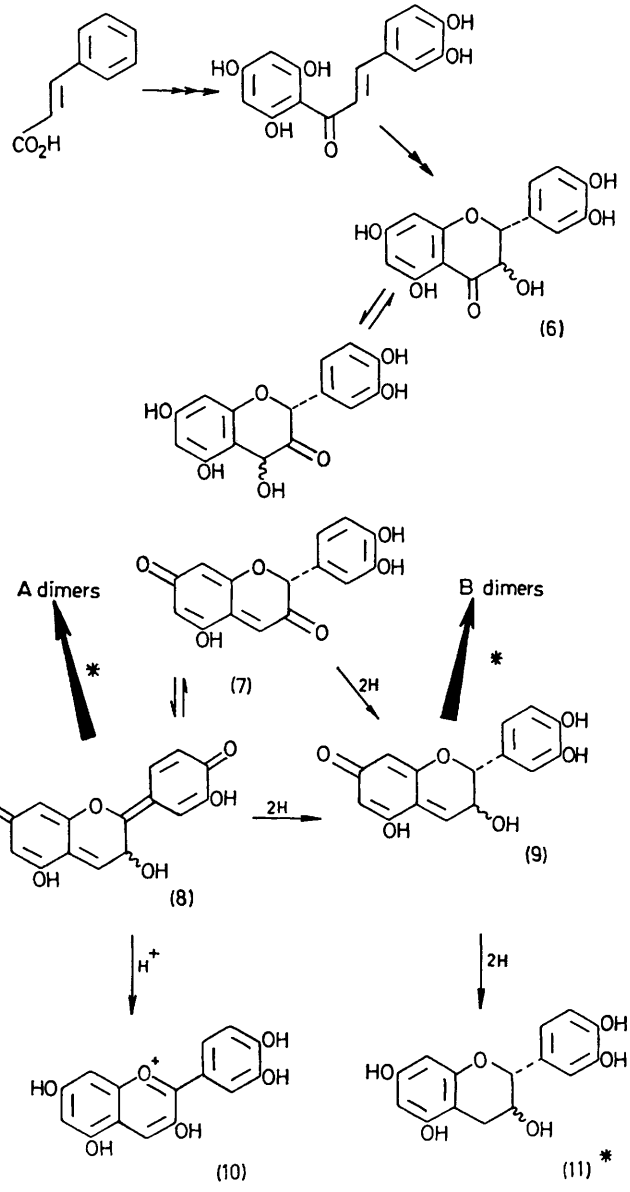






condensed proanthocyanidins. They are respectively (i) a simulated acid-catalysed condensation between phenolic flavan-3,4-diol and flavan-3-ol precursors,[1] (ii) the condensation of a phenolic flavan-3-ol and the anhydro-base of an anthocyanidin[2] and (iii) the oxidative dimerisation of two phenolic flavan-3-ol molecules.[3,4] Evidence in favour of pathways analogous to (i) and (ii) has now been obtained.

[3-^{3}H,^{14}C]-Cinnamic acid was administered to the embryo fruit of *Aesculus carnea* and (—)-epicatechin (**1**), procyanidin B-**2** [**2**; (—)-epicatechin-(—)-epicatechin] and proanthocyanidin A-2 (**3**) were isolated[5] after 6 days. Purification to constant activity was effected for (**1**) and (**2**) by conversion into their phenolic methyl ethers and for (**3**) by formation of its nona-acetate.[6] The ^{3}H:^{14}C ratios



SCHEME
The biosynthesis of the proanthocyanidins

found in the products in two separate experiments are shown in the Table. Both radioactive labels in (—)-epicatechin were shown to reside at the predicted position 2 to an extent greater than 0·90 of the total by transformation

into veratric acid and the flav-2-ene (**4**).[7] Degradation of procyanidin B-2 (**2**) with toluene-α-thiol[5] gave (−)-epicatechin (**1**; lower half) and the thioether (**5**; upper half). The position of the radioactive labels were similarly located at C-2 (*ca.* 0·85 of the total) after conversion of (**5**) into (−)-epicatechin (**1**) by treatment with Raney nickel. Measurements of the specific activities showed that the incorporation into the 'upper half' of the dimer was always in excess (three to four times) of that in the 'lower half'. Degradation of proanthocyanidin A-2 (**3**) has not been possible[8] but the observed $^{3}H:^{14}C$ ratios (Table) may be rationalised on the reasonable assumption that the radioactive labels are located as shown (C-2,2′) but with a similar difference of incorporation into the 'upper' as opposed to the 'lower' half of the dimer. Analogous measurements have been made of the biosynthesis of (−)-epicatechin (**1**) and procyanidin B-4 [(+)-catechin-(−)-epicatechin] in *Rubus fructicosus* (Table) and in addition [*G*-^{14}C]epicatechin[8] has been shown to label almost exclusively the 'lower half' of this dimer. These results clearly demonstrate that the two halves of the condensed proanthocyanidin dimers are assembled from quite different metabolic pools and that the biosynthesis does not involve a straightforward dehydrogenation process (iii).

TABLE

$^{3}H:^{14}C$ ratios in (−)-epicatechin and proanthocyanidins

3-[^{3}H,^{14}C]-Cinnamic acid	5·0	14·0	10·2
(−)-Epicatechin (**1**)[a,b]	4·9 (3·3)	13·5 (1·0)	9·2 (0·5)
Procyanidin B-2 (**2**)[a]	5·5 (0·3)	13·9 (0·23)	
(−)-Epicatechin (**1**)	4·6 (0·06)	12·4 (0·06)	
(2*R*,3*S*,4*S*)-4-Benzylthioflavan-3,3′,4′,5,7-pentaol (**5**) ..	—	12·8 (0·17)	
Procyanidin A-2 (**3**)[a]	0·97 (0·5)	3·50 (0·3	
Procyanidin B-4[b]			9·8 (0·3)
(−)-Epicatechin (**1**)			8·6 (0·06)
(2*R*,3*S*,4*RS*)-4-Benzylthioflavan-3,3′,4′,5,7-pentaol ..			9·2 (0·24)

[a] *Aesculus carnea.* [b] *Rubus fructicosus.*
Figures in parentheses show percentage incorporations of radioactivity into the metabolite.

The reddening of proanthocyanidin containing vegetative tissues as they ripen (fruit) or at senescence (leaves) has now been correlated experimentally with a change from proanthocyanidin to anthocyanidin metabolism[8] and these observations have been combined with the biosynthetic data discussed above to derive the working hypothesis shown in the Scheme. Pathways are proposed from the known dihydroflavonol intermediate (**6**)[9] to both cyanidin (**10**) and the flavan-3-ols [**11**; (+)-catechin or (−)-epicatechin]. It is suggested that in proanthocyanidin-producing tissues the reductive sequence to (**11**) predominates and that the proanthocyanidins are formed when the flavan-3-ol (**11**) captures the quinone methide derivatives (**8**,**9**) which are intermediates in its own biogenesis. The postulated intermediate (**9**) in its protonated form is equivalent to the 4-carbonium ion derived from the related flavan-3,4-diol and thus formation of the B-dimers is analogous to the pathway (i) proposed earlier. Failure to locate the flavan-3,4-diols in vegetative tissues[3,5] may mean that they do not survive the conditions for their isolation. Formation of the A type dimers is analogous to the known condensation of phloroglucinol with flavylium salts.[10]

At senescence, or when the tissue ripens, it is envisaged that the reductive processes decline in importance and the intermediate (**7**) is then transformed directly into cyanidin (**10**).

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