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PROCYANIDIN METABOLISM—A HYPOTHESIS

EDWIN HASLAM*, CLIVE T. OPIE and LAWRENCE J. PORTER
Department of Chemistry, University of Sheffield, Sheffield S3 7HF, U.K.

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Abstract—The experimental evidence relating to the structure, chemical properties and biosynthesis of plant procyanidins is briefly reviewed. A theory is proposed to accommodate this evidence and to correlate the observed increase in polymeric character of the procyanidins as the plant tissue reaches maturity.

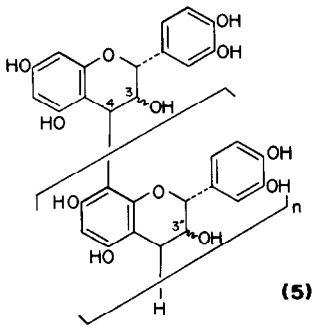
INTRODUCTION

Neither the physiological significance nor the precise role in higher plant metabolism of the many secondary metabolites such as alkaloids, terpenes and polyphenols is at all well understood although these subjects have been the topic of frequent discussion and comment [1,2]. Particularly intriguing in this respect is the function of those soluble polyphenols which have molecular weights in the range approximately of 500–3000 and which have the ability to precipitate proteins from aqueous media. Tissues which contain them are rendered characteristically astringent to the taste and it has often been contended that this particular property prevents damage to the growing plant by browsing animals and predators. Many observers have on this basis not surprisingly invoked the doctrine of final causes to explain the presence of such polyphenols in the plant.

The skins of immature fruit are often rich in procyanidins which are probably the most significant of these complex polyphenols to be found in the plant kingdom [3–6] (N.B. in the earlier literature prior to 1965 many of these substances often may have been referred to as leucoanthocyanins or leucoanthocyanidins [7,8]). The concentration of the different procyanidins usually changes upon ripening [9] and the attendant loss of astringency is of obvious economic importance. In some instances it is also thought that the changes in metabolism of these polyphenols may have some connection with the development of the characteristic anthocyanidin pigmentation of ripening fruit and of the autumnal colourations which precede leaf fall in the Northern hemisphere [10]. Although a great deal of the biochemistry which underlies these changes remains to be learnt an attempt is made here to correlate some of the features outlined above against the background of the present knowledge of the occurrence, structure and biosynthesis of the plant procyanidins [3,6,11,12].

DISCUSSION

Plant procyanidins are polyphenols which possess the general structural pattern of oligomeric flavan-3-ols [such as the B group procyanidins,(5)] and they occur almost invariably in association with one or both of the monomers (+)-catechin (**2a**) and (−)-epicatechin (**2b**). The principal exceptions to this simple generalisation are the procyanidins of the Palmae discovered by Marini-Bettolo and his collaborators [13,14] and those found in aquatic monocotyledons and related plant families [15]. Similarly monomers and simple oligomers occur free and unglycosylated [16]. Biosynthetic studies [11,12,17] strongly implicate the flav-3-en-3-ol (**3**), or a tautomeric form of this structure, as a key intermediate not only in



(5)

"Genetic character"	Procyanidin dimers (n = 1) Absolute configuration	Biosynthesis
W	B-1, 3R, 3"S, 4R	← **Ib + 2a**
X	B-2, 3R, 3"R, 4R	← **Ib + 2b**
Y	B-3, 3S, 3"S, 4S	← **Ia + 2a**
Z	B-4, 3S, 3"R, 4S	← **Ia + 2b**

the formation of the two monomers (+)-catechin (**2a**) and (−)-epicatechin (**2b**) but also in the biosynthesis of the oligomeric procyanidins. Stereospecific reduction of (**3**) thus gives either (**2a**) or (**2b**) and likewise stereospecific protonation gives either of the carbocations (**1a**) or (**1b**) which correspond in absolute stereochemistry at C-2 and C-3 to the flavan-3-ols, (+)-catechin (**2a**) and (−)-epicatechin (**2b**) respectively. The various distinctive patterns of procyanidins found in plants, and which may have some taxonomic value, are then thought to arise directly by reaction of one or both of the flavan-3-ols (**2a**,**2b**), utilising their nucleophilic character at C-6 or

* Author to whom all correspondence should be addressed.

C-8, with one or both of the carbocations (**1a**,**1b**). Each of the four possible reactions can be reproduced exactly in the laboratory by several procedures [12] and the products both qualitatively and quantitatively match those found in particular plants. Thus for example the pattern of procyanidins found in *Malus* sp., *Prunus* sp. and *Crataegus* sp. is given by reaction of (−)-epicatechin (**2b**) and the carbocation (**1b**), that of *Salix caprea* and *Fragaria* x *ananasa* by reaction of (+)-catechin (**2a**) and the carbocation (**1a**) and that of *Rubus* sp. by the reaction of (−)-epicatechin (**2b**) and the carbocation (**1b**). The laboratory reactions are thermodynamically controlled [12] and the close correspondence between the *in vitro* and *in vivo* situations raises the important question whether the reactions in Nature are under enzymic control or not. Thermodynamic factors determine that in (**2a**,**2b**) to yield procyanidins in a manner exactly parallel to the *in vitro* reactions already described. The four distinctive and biosynthetically homogeneous situations (*W*–*Z*) result as follows:

(i) *Cis* addition of the proton and hydride ion to (**3**)
  Y—addition to the α face gives **1a** + **2a**
  X—addition to the β face gives **1b** + **2b**

(ii) *Trans* addition of the proton and hydride ion to (**3**)
  Z—addition of the proton to the α and hydride ion to the β face gives **1a** + **2b**
  W—addition of the proton to the β and hydride ion to the α face gives **1b** + **2a**

Although for many plants their distinctive *in vivo* pattern of procyanidins is only satisfactorily reproduced *in vitro* by reaction together of both flavan-3-ols (**2a**,**2b**) and the two carbocations (**1a**,**1b**), examples of each of

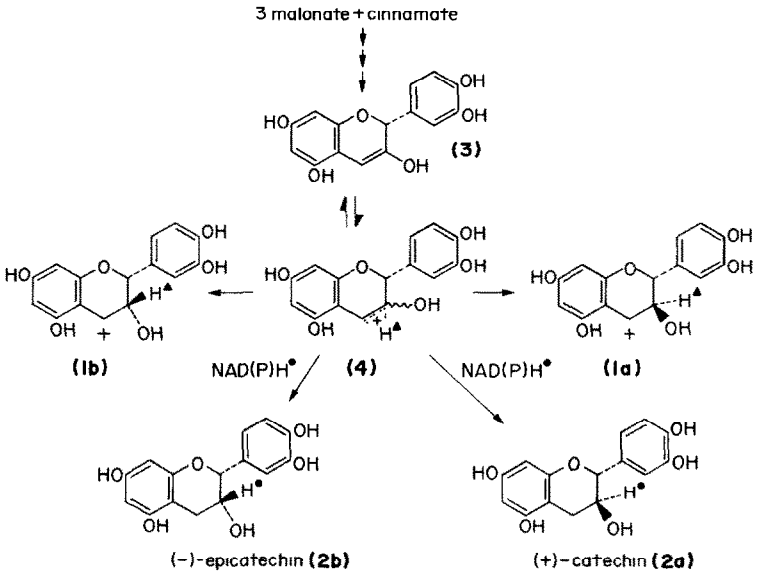


Fig. 1. Procyanidin metabolism. The biosynthesis of the flav-3-en-3-ol (3) is presumed to occur via the corresponding chalcone ⇌ dihydroflavone pair and from this point several pathways may be proposed (e.g. A. J. Birch, I.U.P.A.C., Munich, 1959). However, although it does not accord with a conventional interpretation of the isotopic tracer studies, a more direct route is possible. If the cinnamate is incorporated by conversion to the α-ketoacid (reversal of the PAL equilibrium and then equilibration of the amino acid and α-ketoacid) then the α-hydroxychalcone [D. G. Roux and D. Ferreira (1974) *Phytochemistry* **13**, 2039] would be the first $C_6.C_3.C_6$ intermediate. Reduction of this intermediate would give (3) directly [J. W. Clark-Lewis and D. C. Skingle (1967) *Australian J. Chem.* **20**, 2169].

each of the four possible reactions between flavan-3-ol (**2a** or **2b**) and carbocation (**1a** or **1b**) one major procyanidin dimer is produced (B-1 to B-4 respectively). The various higher oligomers and finally polymers which are also formed, and which it should be noted are often quantitatively of greatest significance in plants, can then be visualised to result from reaction of the appropriate dimer and further carbocation.

If the reduction of the flav-3-en-3-ol to flavan-3-ol is a two step process in which stereospecific proton addition to give the hybrid ion (**4**) precedes stereospecific (*cis* or *trans*) delivery of hydride ion [or its equivalent from say NAD(P)H] then the two carbocations (**1a**,**1b**) probably derive from a metabolic situation in which the supply of biological reductant is rate-limiting. The carbocations (**1a**,**1b**) would then be produced by leakage of the hybrid ion (**4**) from the active site of the enzyme and, it is postulated, react with the final reduction product the four 'genetically' homogeneous situations (*W* to *Z*) may also be readily identified in the plant kingdom. Some have been noted above. Most important from the biosynthetic point of view are those enumerated as *W* and *Z* in which the biosynthetic reaction occurs between carbocation and flavan-3-ol with *opposite* absolute stereochemistry at C-3. The theory, as outlined, provides a satisfactory explanation of these two situations. The status of flavan-3,4-diols in procyanidin biosynthesis [18–20] remains something of an enigma. In principle they may be envisaged to be in equilibrium with the carbocations (**1a**,**1b**). However to date no flavan-3,4-diol corresponding to the 5,7,3′,4′-phenolic hydroxylation pattern has been isolated from the vegetative tissues of a plant and similarly no *in vivo* experimental evidence has yet been obtained to support their presumed role [17].

The present hypothesis is based on a large number of structural and purely chemical observations and the

results of isotopic tracer experiments. A summary of these experiments is recorded in Fig. 2. The most significant of the results obtained are the retention in both flavan units of the proton (H_a) and the loss of the proton (H_c) in the cinnamate precursor, [11,17] and the differential extent of labelling of the two 'halves' of the procyanidin dimers (some 3 or 5 to 1) which leads to the concept of the derivation of the two halves from different metabolic sources. Tracer experiments also show that procyanidins. The concommitant formation of some of the characteristic anthocyanidin pigmentation of ripening fruits (e.g. apple, hawthorn etc.) is also understood if under these circumstances a fraction of the labile flav-3-en-3-ol (**3**) is also oxidatively metabolised to the anthocyanidin. This latter proposal is indeed a reminder of the percipient suggestion of Sir Robert Robinson [10] who commented in 1936 . . . "It is too early to attribute a predominant role to the leuco-anthocyanidins but it

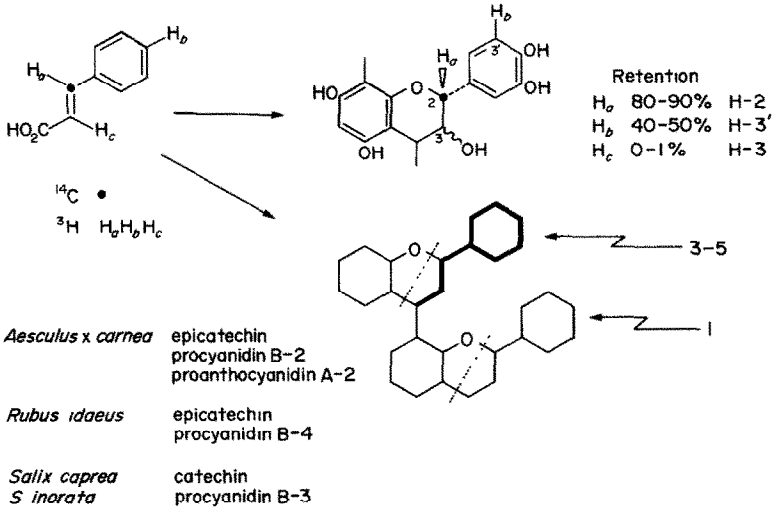


Fig. 2. Procyanidin biosynthesis—tracer studies.

synthesis is most active in the very early stages of growth but declines as the tissue matures. Analysis of the procyanidin profile in the male sallow willow catkin, *S. caprea*, (chromatography of the ethyl acetate soluble phenols on Sephadex LH-20 in ethanol monitored by UV [17]), before, during and after maturation shows that over this period there is a continuous change in the quantitative pattern of procyanidins present. The more highly condensed and polymeric forms increase steadily in concentration—a feature previously observed in other fruit by Goldstein and Swain [9]—and at maturation there is a concommitant slow decline in the concentration of the dimeric forms and of the flavan-3-ol, (+)-catechin (**2a**). In the context of the biosynthetic picture outlined (Fig. 1) these changes clearly correlate with the continued synthesis of the carbocation (**1a**) and hence the flav-3-en-3-ol (**3**) but with a discontinuity in the formation of (+)-catechin (**2a**) by reduction of (**4**). The carbocation (**1a**) presumably then captures and removes the lower oligomeric procyanidins and in these circumstances the balance of procyanidins moves to the higher polymeric forms. These changes are clearly compatible with the demonstrable loss of chlorophyll and hence of photosynthetic activity and reductive capacity of the plant tissue.

If, as has been suggested, [9,21] a critical minimum and maximum molecular weight of polyphenol is important in the association of polyphenols with proteins then such changes in the procyanidins of ripening tissues would correspond with the observable loss of astringency (as the fraction of procyanidins below the critical higher molecular weight falls) and with the decrease of readily extractable low molecular weight is already safe to assume that their modification represents an auxiliary pigmentation process. It is possibly operative in autumnal reddening . . ."

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