Phytochemistry, 1977, Vol. 16, pp. 1625–1640. Pergamon Press. Printed in England.

REVIEW

SYMMETRY AND PROMISCUITY IN PROCYANIDIN BIOCHEMISTRY

EDWIN HASLAM

Department of Chemistry, The University, Sheffield S3 7HF, England

(*Received* 6 *June* 1977)

Key Word Index—Procyanidins; structure; stereochemistry; helical conformations; biosynthesis.

Abstract—The occurrence and significance of procyanidins in the plant kingdom are reviewed. Stages in the elucidation of the structure and the stereochemistry of the procyanidins are outlined. Preferred helical conformations for the procyanidins, arising from the steric hindrance to rotation about the interflavan bond, are described. Biosynthetic studies are discussed and a theory of procyanidin metabolism is proposed.

INTRODUCTION

I have spent a fair proportion of my scientific career investigating the chemistry of plant polyphenols and it is one area of the chemistry of these substances that I wish to discuss in this review*. For a chemist, structure is all important and knowledge of structure is, I believe, the only secure basis upon which advances in biological chemistry can be made. However I hope that I can also convey my broader interests in plant polyphenols and how these impinge upon certain areas of plant biochemistry. My attitudes and aspirations are reflected in a statement T. A. Geissman [1] made just a few years ago:

"Certainly structures are important—but the determination of structure in itself is ceasing to be of much interest or importance, and often turns out to be an exercise in the manipulative and interpretative skill of the investigator. Some syntheses are in the same class.

My own tendency (in which of course, I am not alone) is to look at biological relationships: taxonomy, phylogeny, biosynthesis and biotransformations.... The future of phytochemistry is to use the chemical information as the starting point for inquiry into questions that lie in the realms of biology".

Plant polyphenols are a fascinating group of substances whose chemistry has successively attracted the attentions of distinguished chemists for a century or more. Structurally, they represent a very diverse group of plant products; they are widely distributed in the plant kingdom and often they are present in surprisingly high concentrations. Consider, for example, the tea plant (*Camellia sinensis*) in which up to 40% of the dry matter of the leaves may be composed of polyphenols of one form or another. A large part of the continuing fascination of these substances as objects for study derives from the fact that by and large chemists and biologists have been unable to explain satisfactorily the metabolic function of these lavishly accumulated materials.

For a few polyphenols, well defined biological effects can be ascribed. Thus anthocyanidin pigmentation has an obvious function in making fruit and flowers attractive to birds and insects and scientists have subsequently used such relationships as the basis of *post hoc* rationalisations for the presence of these polyphenolic secondary metabolites in the plant in the first place. As a consequence the view that these polyphenols, attractive as many may be to the eye, are waste products of metabolism is not I think a fashionable one. However I must personally confess to a serious and nagging fear that a part at least of my scientific career has been spent inspecting the loot in the garbage bin of plant metabolism!

If we do not know what function they perform, that does not stop our appreciation of their presence whenever they do occur in plant materials. One class of particular significance to man in this respect are those polyphenols which complex with proteins. This association may reduce the nutritive value of foodstuffs and the interaction, when it is with proteins of the palate, gives rise to a characteristic astringent taste. As such their presence frequently determines our enjoyment of particular fruits such as the apple, the cranberry, the strawberry and the blackberry and of a whole range of drinks such as tea, beer and wine. Without sufficient polyphenols a wine, for example, will be flat and insipid whilst too much gives a harsh, rough quality. Red wines generally have a higher polyphenol content than white ones because the skins of the grape are used in the production of red wine to give it its colour and the polyphenols which are also concentrated in the skin are extracted as well as the pigments. Evidence has also been obtained to show that polyphenols such as these substantially influence the ways in which soil organic matter is produced. Indeed R.L.M. Synge and others have invoked this property as a rationalisation of their metabolism by the plant in the first place. It is suggested that these polyphenols have no immediate function physiologically in the plant but they determine that when the plant dies a soil should become established more suitable for the growth of a subsequent generation of plants. A good example of this effect may be seen on the heaths and moorlands of Northern England where plants such as heather (*Calluna*

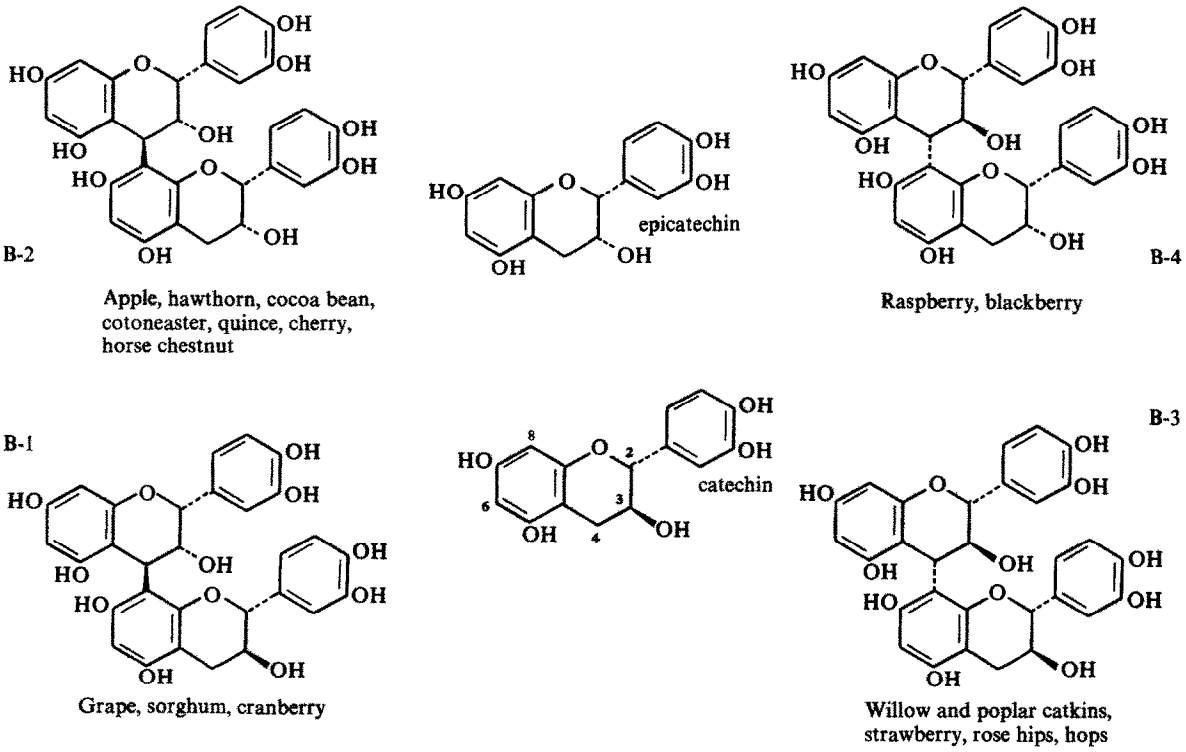
* Based on a lecture given to the Phytochemical Society of Europe on April 5th during the symposium *Biochemical Aspects of Plant and Animal Coevolution* by the winner of the Tate and Lyle Award in Phytochemistry for 1977.

vulgaris) mountain cranberry (*Vaccinium vitis-idaea*) and bearberry (*Arctostaphylos uva-ursi*) dominate the vegetation. It may be significant that all of these plants metabolise substantial quantities of polyphenols and amongst them those which strongly associate with proteins. When these plants die, it is assumed that the complexing of proteins with phenols in the plant litter helps produce a soil in which only the progeny of these particular plants can thrive.

These polyphenols are widely referred to as *tannins* and it is about one class of these—the *condensed tannins*—that I wish to discuss here. The structure of these compounds has remained a classical unsolved problem of organic chemistry for three quarters of a century and we have been fortunate in Sheffield in being able to provide a solution to part of this problem. I am not enamoured by the word tannin* since it cannot be precisely defined in the chemical sense and for this reason it has been widely misapplied and abused in both the botanical and biochemical literature. A chemically much more accurate description of many of the condensed tannins found in the vegetative tissues of plants is *procyanidins* [2] and it is on this group that I shall concentrate.

A great measure of national pride attaches to the first recorded observations on procyanidins. The names of Willstätter and Tswett take pride of place in the German literature and that of other mid-European countries, whilst the French nominate a third party the viticulturist Laborde. However the English with their customary strong sense of history seem easily to win this particular episode in the shape of one Robert Boyle in 1840. All these workers noted that very many plant tissues gave rise to a deep red colour—later shown to be that of the pigment cyanidin—when treated with acid. Rosenheim in 1920 began the first systematic investigation of these substances which he called *leucoanthocyanins* and in the 1930's Sir Robert and Lady Robinson conducted the first surveys of plant materials in order to define their occurrence. In the 1950's further and much more extensive analytical work was developed by Bate-Smith who noted that leucoanthocyanins were confined mainly to plants with a woody habit of growth and with Swain he drew attention for the first time to the very close similarity in systematic distribution in plants of these compounds and those substances rather indefinitely defined in the literature of botany as tannins. Bate-Smith and Swain concluded that leucoanthocyanins were most commonly responsible for the broad range of reactions (precipitation of gelatin and alkaloids, astringent taste and the formation of amorphous phlobaphens with acid) generally attributed to tannins in plants.

About this time the first definitive structural work in this area commenced and since the molecules in question appeared to contain no carbohydrate the nomenclature took a subtle turn to *leucoanthocyanidin*. Forsyth in the late 1950's was the first investigator to show that the characteristic leucoanthocyanidin reactions of many plant tissues were probably due to flavan-3-ol oligomers. In the 1960's the nomenclature was changed to the present one following proposals of Freudenberg and Weinges and in this review I want to broadly summarise the various aspects of work which has been carried out in



Scheme 1. Major dimeric procyanidins found in plants. Occurrence and structure.

* The original definition of a tannin is a substance which converts an animal hide to leather.

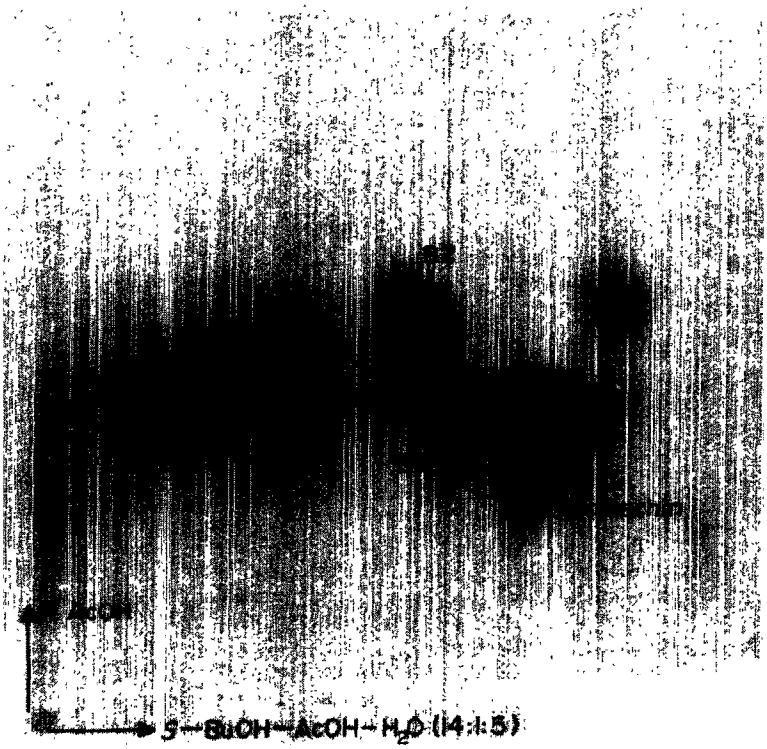


Fig. 1. Procyanidin fingerprint—hawthorn (*Crataegus monogyna*).

Sheffield on the plant procyanidin problem over the last ten years. In doing so it is entirely appropriate to mention the very elegant contributions to the solution of this problem by Freudenberg and Weinges in the Heidelberg school [3] and also the excellent work on related proanthocyanidins from the woods and barks of trees by Roux [4] and his collaborators in South Africa.

PROCYANIDIN CHEMISTRY

Our own work [5–8] has concentrated upon the procyanidins found in the vegetative tissues of plants and for reasons of supply these have almost invariably turned out to be fruit-bearing plants. Initially we carried out a large scale survey of plants using two dimensional paper chromatography. In all cases where procyanidins were found so also were one or other or both of the flavan-3-ols: (+)-catechin and (−)-epicatechin. The survey also revealed 'procyanidin fingerprints' which could be classified into four principal categories. Plants were grouped according to their 'procyanidin fingerprint' (Scheme 1).

One highly characteristic fingerprint, and probably the most commonly encountered one, is that found in hawthorn (*Crataegus monogyna*). Co-occurring with (−)-epicatechin is one major procyanidin dimer (B-2, formally two (−)-epicatechin units C-4 to C-8 linked), a minor procyanidin dimer (B-5, two (−)-epicatechin units C-4 to C-6 linked), a major trimer and tetramer and various higher oligomers (Fig. 1). An alternative and again highly characteristic pattern is found in the sallow willow catkin (*Salix caprea*) where (+)-catechin, a major procyanidin dimer (B-3, two (+)-catechin units C-4 to C-8 linked), a minor procyanidin dimer (B-6, two (+)-catechin units C-4 to C-6 linked), a trimer, tetramer and higher oligomers constitute the fingerprint (Fig. 2). The other two characteristic fingerprints were found in raspberry and blackberry (*Rubus idaeus*, *R. fruticosus*)—(−)-epicatechin, procyanidin B-4 and associated procyanidins and in the cereal sorghum (*Sorghum vulgare*)—(+)-catechin, procyanidin B-1 and associated oligomeric procyanidins. In passing it may be noted that these latter two categories are the most significant from a biosynthetic point of view. Thus it should be remarked that the two flavan-3-ol fragments which formally compose the major procyanidin dimer (B-4 or B-1) are of opposite absolute stereochemistry at C-3.

It was possible using this form of analysis to classify most procyanidin-bearing plants according as to whether they belong to one or other of the four 'pure' forms or to any particular combination. For isolation purposes the most fruitful sources of the various procyanidins are indicated in Scheme 1. Using a variety of isolation procedures (principally chromatography on Sephadex LH-20 and counter-current distribution) it has been possible to isolate all the dimeric and the trimeric procyanidins for chemical investigation. In some cases the yields are quite impressive. Thus one kilogram of fresh horse chestnut shells (*Aesculus hippocastanum* or *A.* × *carnea*) yields upwards of one gram of procyanidin B-2.

Comparatively little is yet known concerning the higher oligomeric forms but the principles which govern the way in which they are constructed are thought to be the same as for the simple dimers and trimers. Hence it

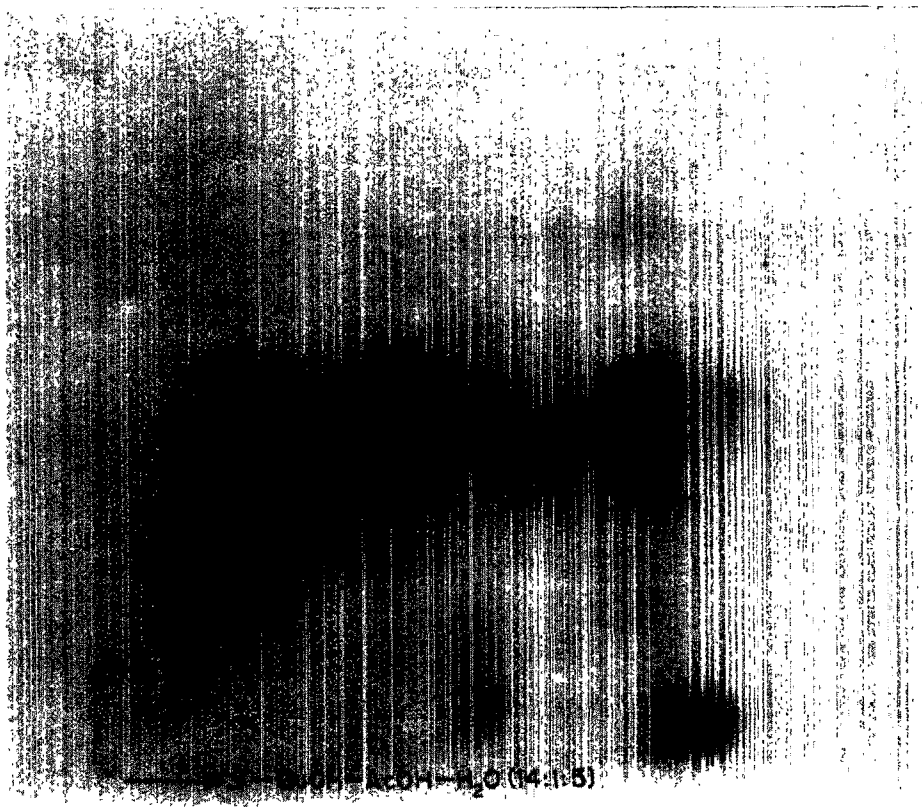


Fig. 2. Procyanidin fingerprint—willow catkin (*Salix caprea*).

is assumed that their structures are essentially extensions of these simpler structures in which the molecular size is increased by the addition of further flavan units by mainly C-4 to C-8 linkage. From a physical point of view however this increase in molecular weight causes a decrease in solubility and special techniques may be necessary to isolate them. In terms of their ability to precipitate proteins and hence their astringency to the palate the dimeric procyanidins have a relative astringency of some 10% compared to Chinese gallotannin (tannic acid) [9, 10]. As the molecular size of the procyanidin increases so also does its astringency but as yet no detailed study has been carried out to determine which are the important components of the procyanidin complex in determining the astringent taste of a plant material.

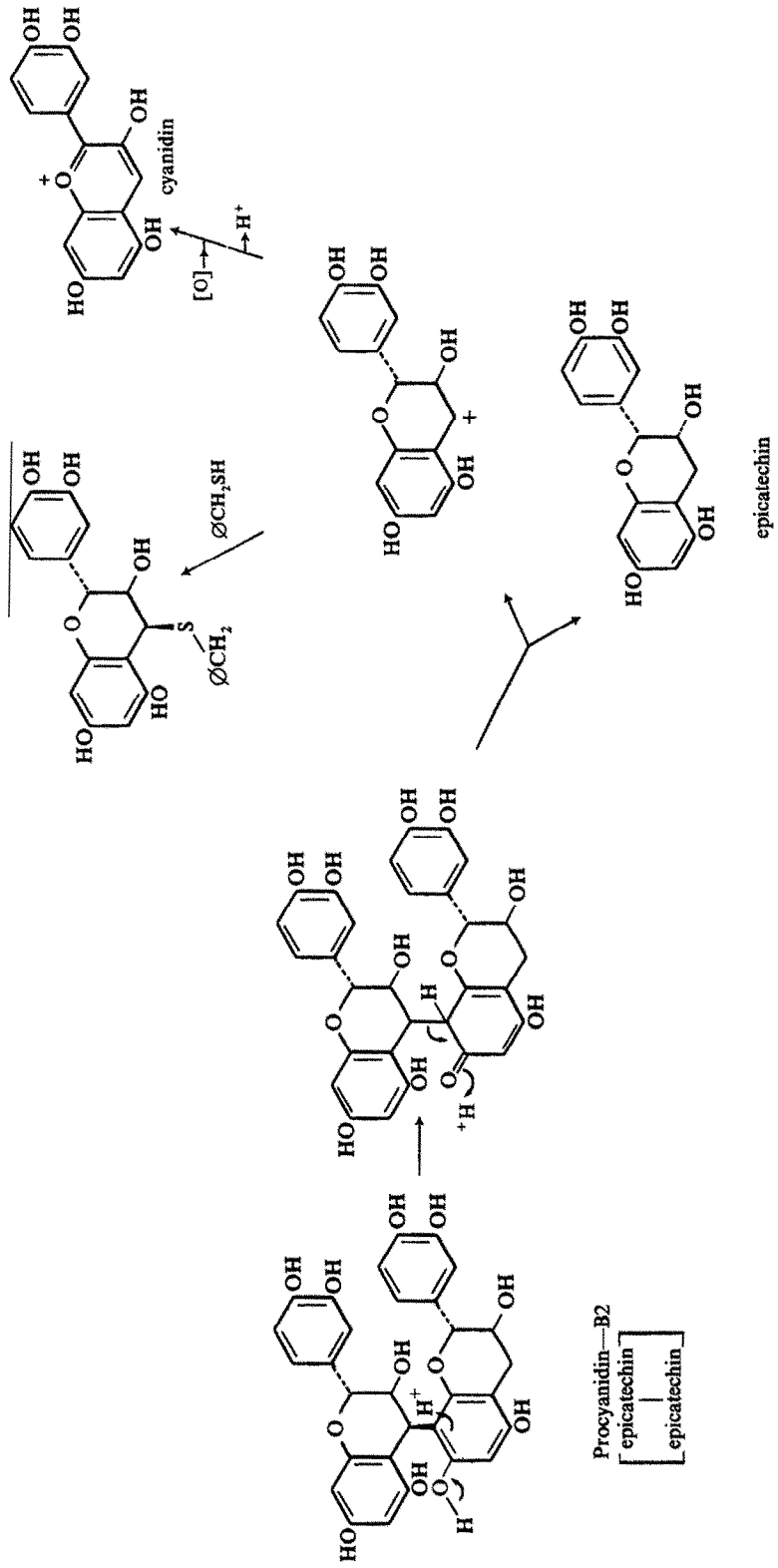
Determination of the chemical structure of the procyanidins proved to be a challenging problem particularly since the armamentarium of spectroscopic methods to which the organic chemist has recourse these days to solve structural problems proved, in the early stages, to have limited value. Instead chemical procedures to deduce structure had to be evolved and although this often proves to be a more time consuming procedure it has the invaluable bonus that one learns inevitably a great deal more about the properties and the chemistry of the substance in question. A few aspects of this work are noted below.

Procyanidins are so named because they are chemically degradable to give the pigment cyanidin. This property arises from the lability towards acids of the interflavan bond (Scheme 2). In a procyanidin dimer, acid catalysed fission of this bond gives the flavan-3-ol from the 'lower half' and the flavan-4-yl carbocation from the 'upper half'. This extremely reactive intermediate, unless it is trapped, decays by loss of a proton and a hydride ion to give the pigment cyanidin. This chemical reaction forms the basis of the highly distinctive reaction by which the compounds are recognised. The carbocation may, however, be trapped by a reactive nucleophile —such as a thiol, toluene-α-thiol, and this reaction forms the basis of the chemical degradation of procyanidins which has been utilised for structural purposes (Schemes 2 and 3). The flavan-4-yl-thioethers may be isolated and characterised and hence the structure and composition of the procyanidin dimers determined. This fundamental reaction of procyanidins thus allows our description to be advanced one stage further (Scheme 4).

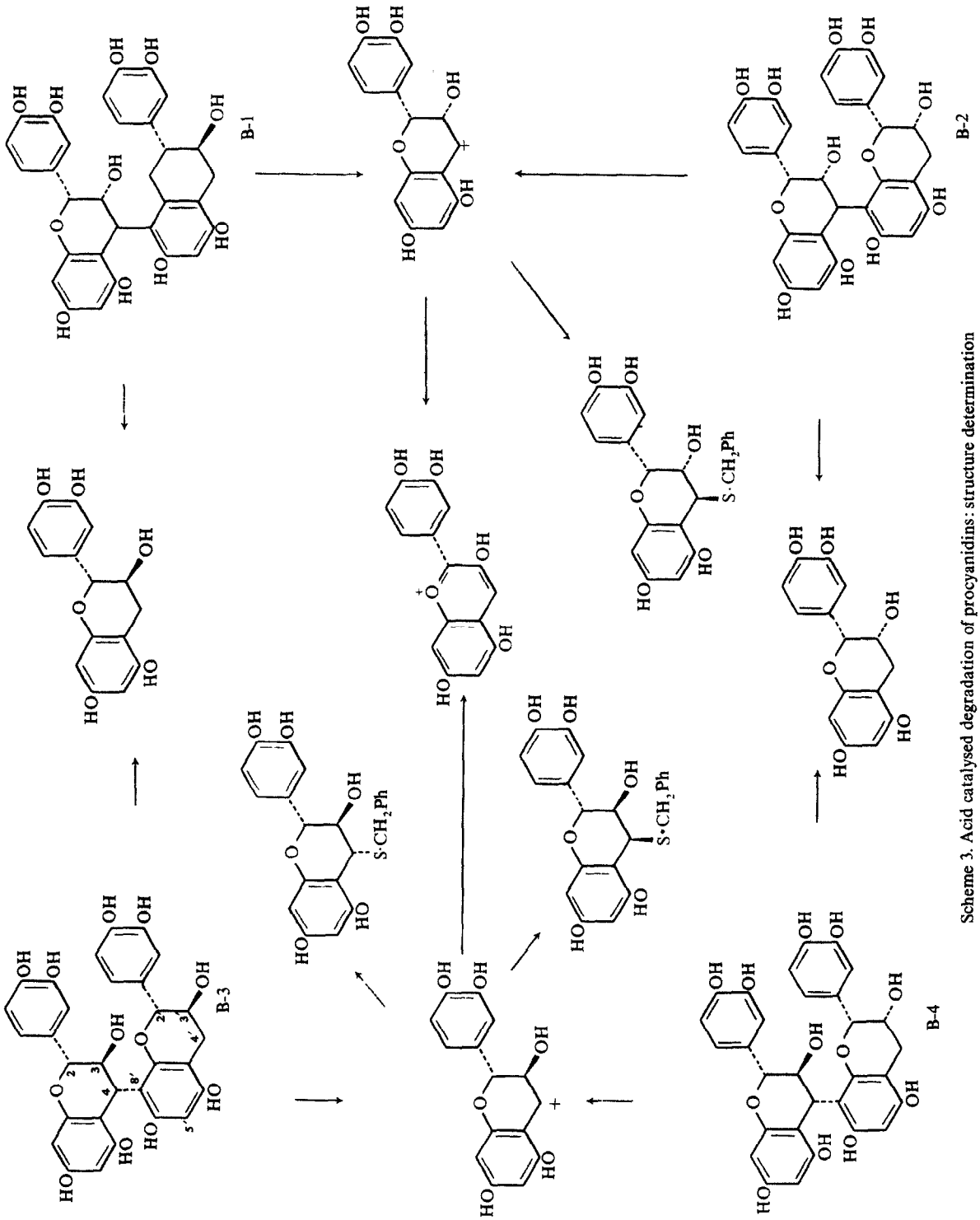
PROCYANIDIN STEREOCHEMISTRY

Nor seeks nor finds he mortal blisses,
But feeds on the aerial kisses
Of shapes that haunt thought's wildernesses.
He will watch from dawn to gloom
The lake-reflected sun illume
The yellow bees in the ivy bloom,
Nor heed, nor see, what things they be;
But from these create he can
Forms more real than living man,
Nurslings of immortality!

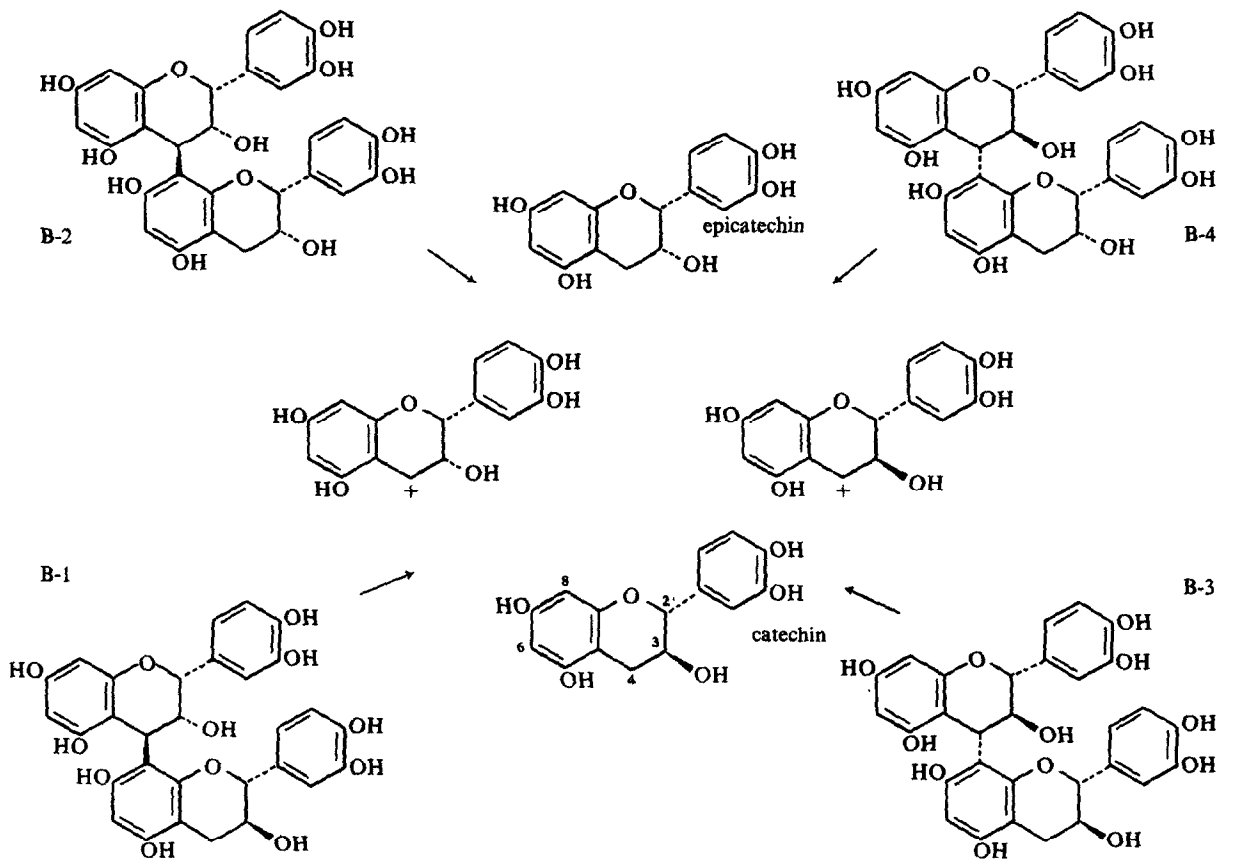
So spoke the poet Shelley—but not about a chemist! It is however an appropriate introduction to some thoughts and flights of fancy which we have had concerning the shapes which these procyanidin molecules adopt.



Scheme 2. Acid catalysed degradation of procyanidin B-2.



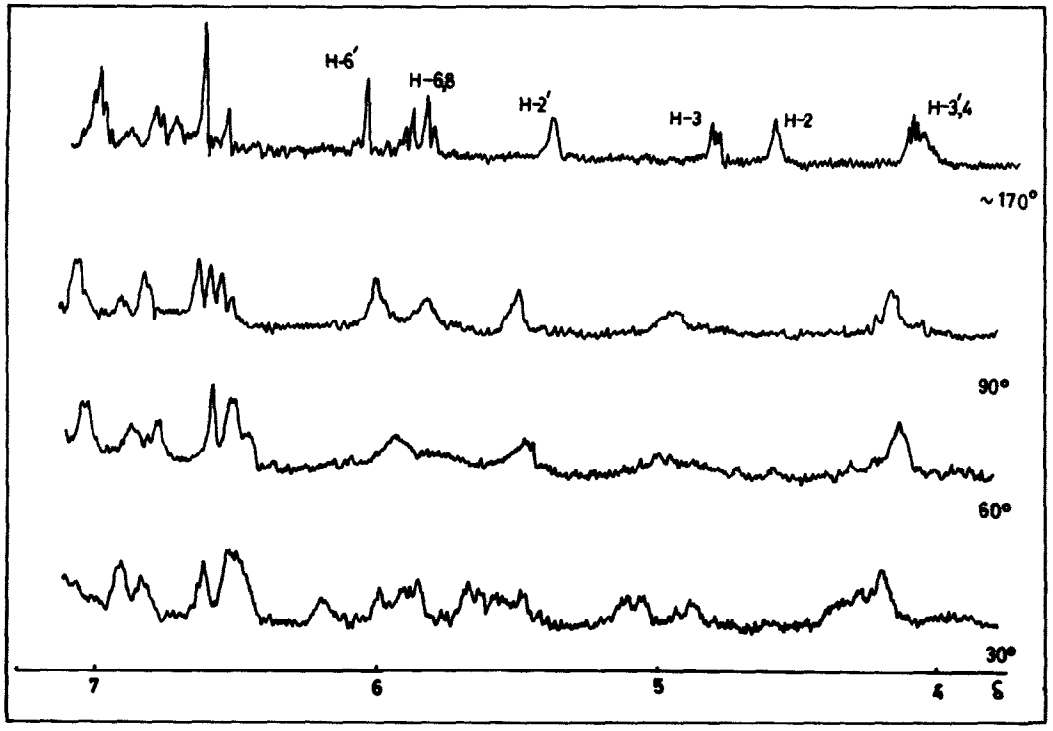
Scheme 3. Acid catalysed degradation of procyanidins: structure determination

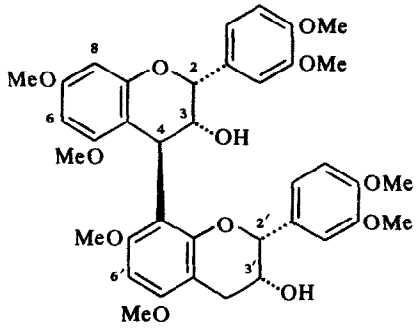


Scheme 4. Procyanidins: degradation.

One of the most significant developments for biology over the last twenty-five years has been the realisation of the crucial importance of the interplay between shape and conformation of molecules and their biological function. So far we have dealt with the chemistry of these procyanidin compounds in flatland. Let us add another dimension and consider their shape.

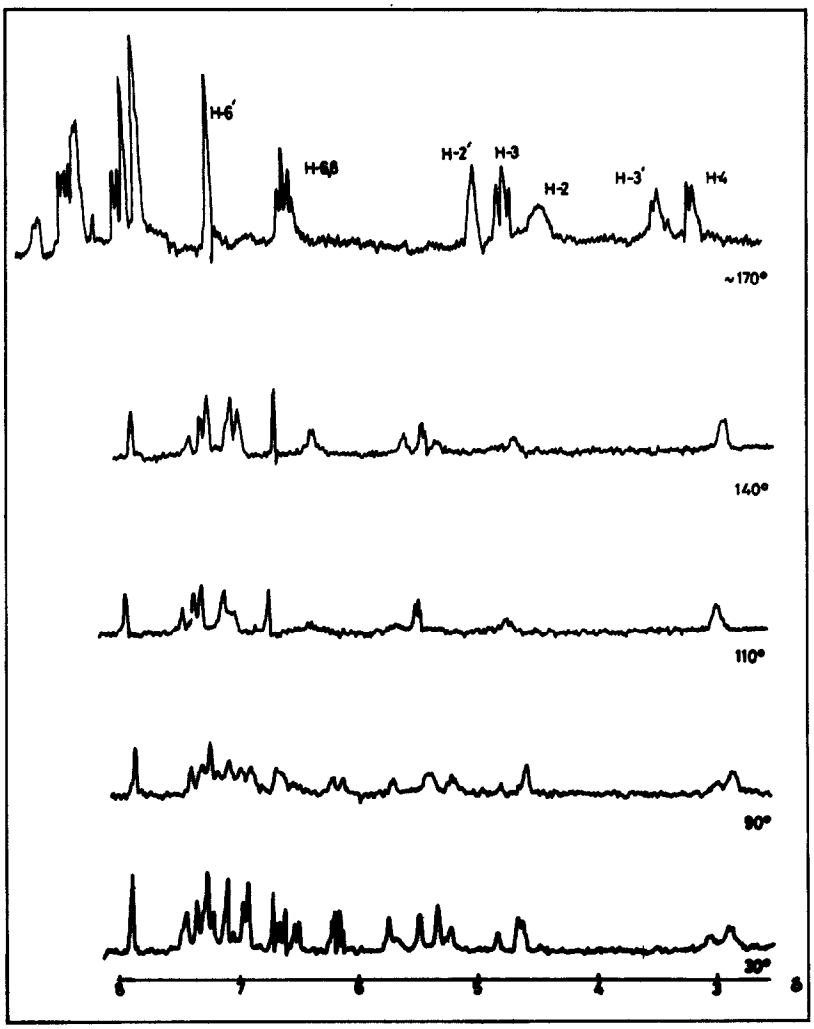
During the early stages of our investigations, structure determination had, for many of the procyanidins, been hindered but not helped by the application of spectroscopic techniques. Typical observations made at that time are exemplified by the ^{1}H NMR spectra of procyanidin B-2 and its derivatives. That of the free phenol was first order and readily analysed but at room temperature that of the octamethyl ether (Fig. 3) and deca-acetate (Fig. 4) were inexplicably complex and not directly capable of analysis. For other procyanidins such as B-3 and B-4 the spectra of the free phenol and also the derivatives were all unexpectedly complex and defied a simple analysis. It took some 3–4 years to solve the mystery and we were then able to show that the phenomena and our difficulties arose from restricted rotation around the interflavan bond. Thus for procyanidin B-2 methyl ether and acetate derivatives the molecules exist in solution as a mixture of at least two major conformations. The rate of interconversion of these conformers at 30° is sufficiently slow to permit these conformations to be separately observed by the ^{1}H NMR measurement; hence the complexity of these spectra. As one raises the temperature at which the ^{1}H NMR observations are carried out, then one provides more molecules with sufficient energy to overcome the barrier to rotation about the interflavan bond and the rate of interconversion increases such that at about 170° the ^{1}H NMR measurement is unable to distinguish between the rapidly interconverting conformations and an average first order spectrum is observed. This phenomenon is noted for all the procyanidins and their derivatives and details are summarised in Fig. 5 for the two situations as exemplified by procyanidins B-2 and B-3. Rotation about the interflavan bond is restricted in both cases but because of the differing stereochemistry at C-4 the factors which give rise to this property are different in the two examples. Molecular models show that for procyanidin B-1, procyanidin B-2 and their derivatives this hindered rotation is caused primarily by steric interference between the proton at C-2 and the bulky substituents (—OR where R = H, Ac or Me) *ortho* to the interflavan bond in the 'lower' flavan-3-ol unit. The stereochemical situation in procyanidins B-3 and B-4 is analogous to that of the 9-arylfluorene system and models indicate that the interference between the oxygen substituents at C-3 and C-5 in the 'upper' flavan-3-ol unit and those *ortho* to the interflavan bond in the 'lower' flavan-3-ol is primarily responsible for the barrier to rotation. Determination of the energy barriers to rotation showed these to be generally in the range

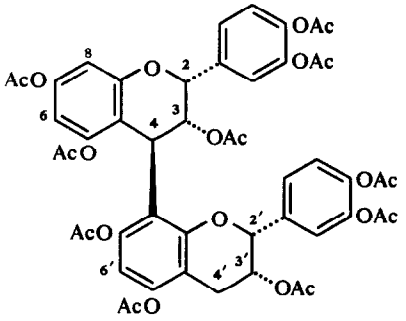




Procyanidin B2 Octamethyl ether $\Delta G^{\ddagger}_{rot}$ 18.7 kcal mol^{-1}
d_5 nitrobenzene

Fig. 3. Variable temperature ^{1}H NMR spectra of procyanidin B-2 octamethyl ether.





Procyanidin B-2
Deca-acetate
$\Delta G^{\ddagger}_{rot} = 19.5$ kcal mol^{-1}
d_5 nitrobenzene

Fig. 4. Variable temperature ^{1}H NMR spectra of procyanidin B-2 deca-acetate.

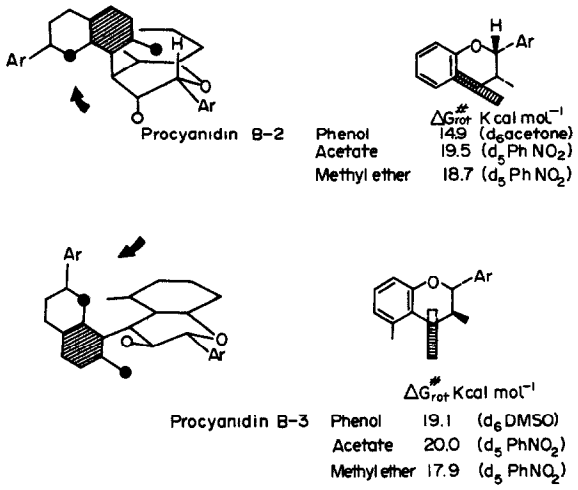


Fig. 5. Procyanidins: restricted rotation and preferred conformations.

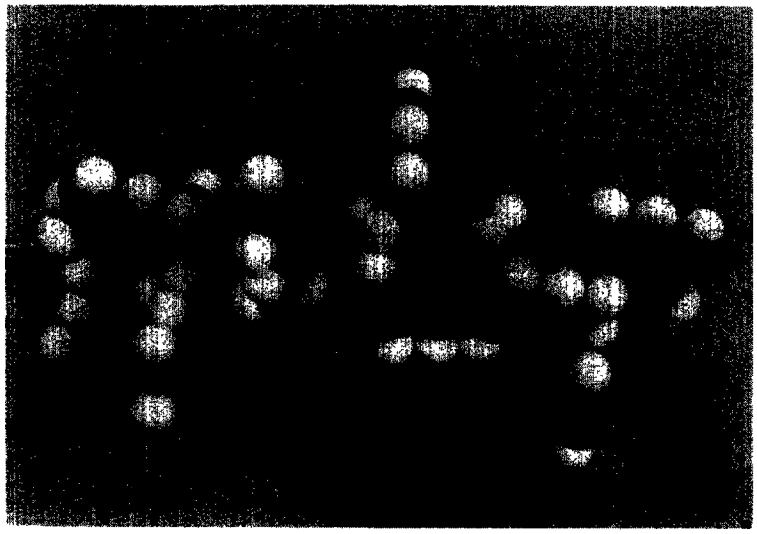


Fig. 6. Oligomeric procyanidins: the left hand helix of $[(-)\text{-epicatechin}]_n$.

15–20 kcal mol^{-1}—too low to permit the isolation of different conformational forms of the procyanidins by conventional preparative means, but sufficiently high to observe and measure in the ^1H NMR experiment and to suggest that the procyanidins very probably exist in preferred shapes or conformations. Inspection of molecular models allows a prediction of these preferred shapes to be made (Fig. 5).

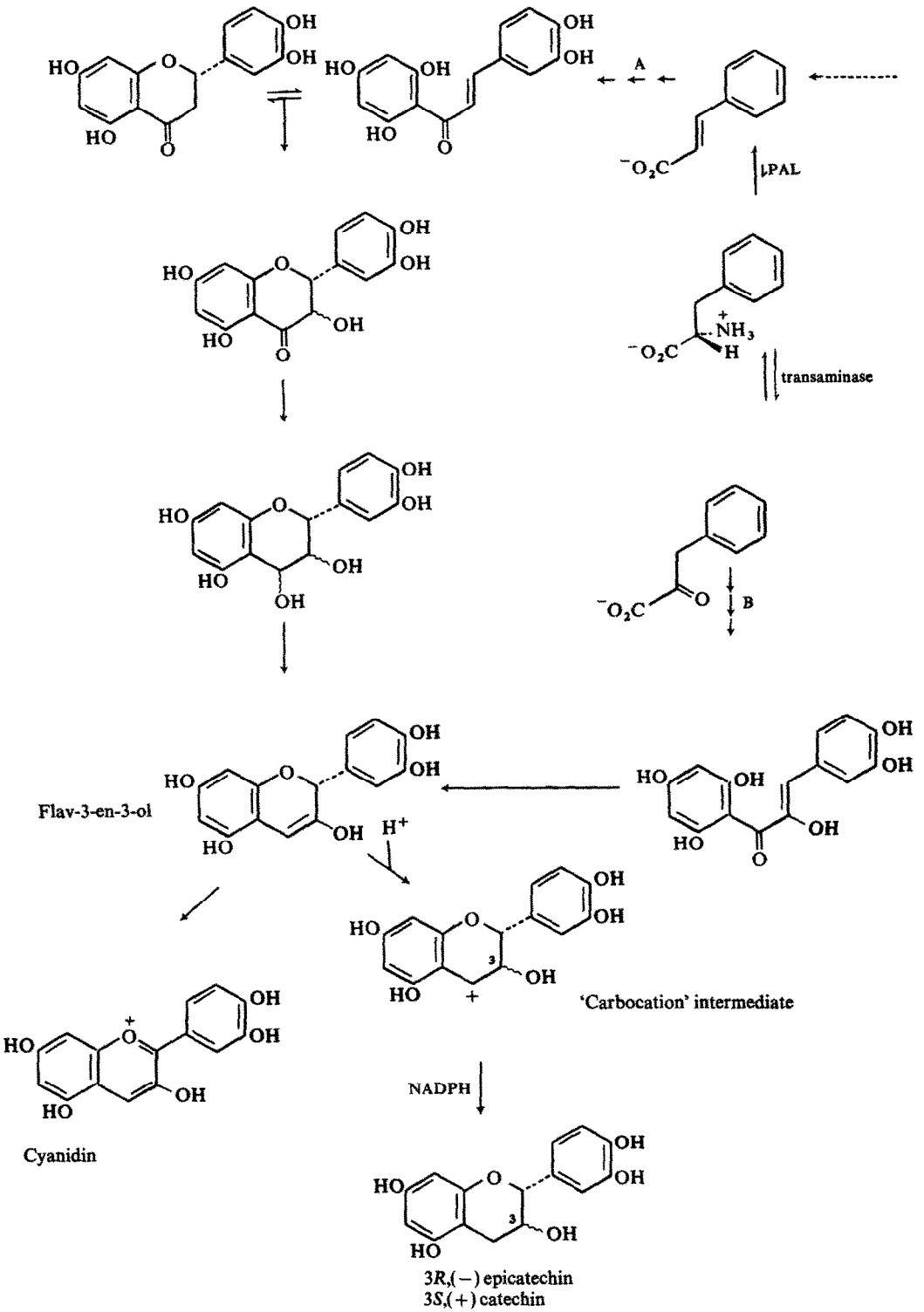
Two interesting features emerge from these observations: (a) Examined from quite different perspectives (Fig. 5, arrows) models of procyanidins B-2 and B-3 emerge as molecules related *almost* as object to mirror image, and (b) elaboration of the oligomeric forms of procyanidins by the addition of further flavan-3-ol units, bearing in mind the type of conformational restraint about the interflavan bond which has been shown to exist, leads to two helical structures. The central core of these linear polymers is composed of rings A and B of the flavan repeat unit and ring C (the *ortho* dihydroxyphenyl ring) projects laterally from this core. Significantly perhaps those formed from units related to (−)-epicatechin (e.g. extension of the procyanidin B-2 type) are *left hand* helices (Fig. 6) whilst those of the type related to procyanidin B-3 and formed from units related to (+)-catechin are *right hand* helices. These conformational projections are based on a detailed study both in the crystal form and in solution of the flavan ring system whose conformation is slightly unusual and is not quite as has been predicted by previous workers. Ring A, the heterocyclic oxygen, C-4 and C-2 are virtually in the same plane whilst C-3 lies some distance (~0.7 Å) below that plane.

At the moment we are unable to put forward a hypothesis to explain what meaning if any these intriguing observations upon the shape of the procyanidins may have. However it is interesting to note that what little evidence is available suggests that it is the exposed 'catechol' type units (ring C) on the periphery of the helices which are of importance in the interaction and complexing with proteins. These brief glimpses of the structural properties of the procyanidins illustrate I hope the inherent symmetry in the properties of the procyanidins. Now let us take a closer look at what is known of their biochemistry and lift the veil on the more promiscuous aspects of our subject.

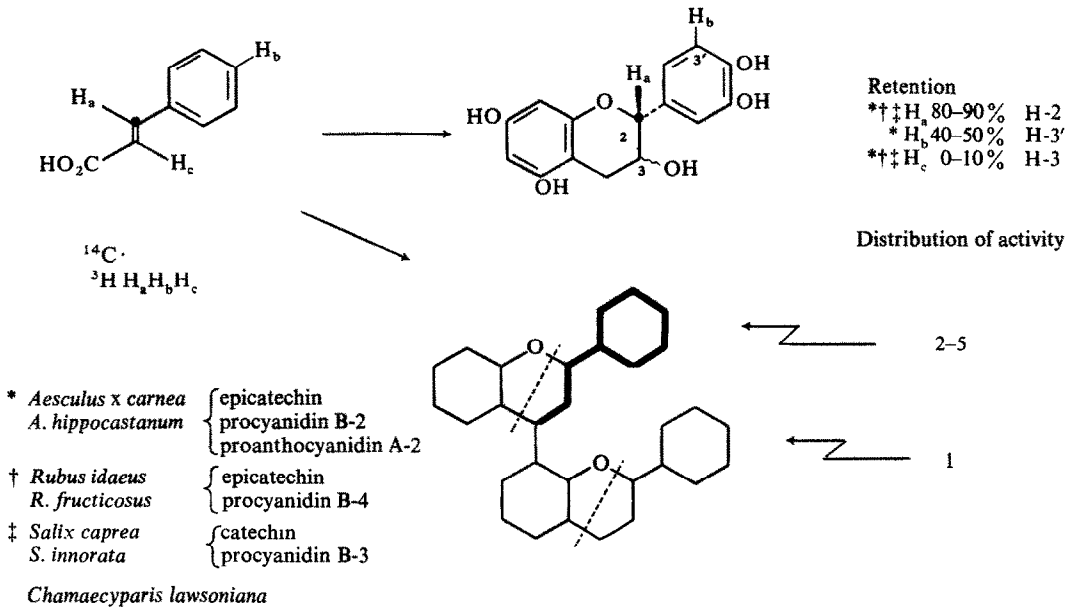
PROCYANIDIN BIOSYNTHESIS

Procyanidins are flavonoids and a great deal has already been adumbrated elsewhere concerning the general pathways of flavonoid biosynthesis. Ring A of the flavonoid carbon skeleton is derived from acetate (malonate) and ring C and the three carbon atoms of the heterocyclic ring originate from cinnamate. Evidence for this theory comes predominantly from isotopic tracer studies and from enzymic work in tissue cultures. The chalcone–dihydroflavone pair are the first recognisable intermediates on the pathway but steps from this stage to the various distinctive classes of flavonoid are somewhat uncertain. This 'credibility gap' is to a large extent inherent in the nature of the biosynthetic tracer method dealing with sequences of reactions which are essentially limited to oxidation and reduction and reactions involving loss of water. Scheme 5 shows one speculative pathway (route A), based on the conventional intermediacy of the chalcone–dihydroflavone pair, to the flavan-3-ols and anthocyanidins which are the two principal groups of flavonoids which lack an oxygen atom at position 4 on the heterocyclic ring. The flav-3-en-3-ol is a key intermediate on this pathway; reduction would give the flavan-3-ol and oxidation the anthocyanidin. However because of the continuing uncertainty regarding the status of the dihydroflavonol in the biosynthesis of *all* the flavonoids it is possible to speculate also on alternative biosynthetic routes. An alternative pathway (Scheme 5, route B) to the flav-3-en-3-ol might for example be via the α-hydroxychalcone derived from the α-keto acid as the C_6—C_3 precursor.

The study of the biosynthesis of the procyanidins has been conducted with a range of fruit-bearing plants —horse chestnut (*Aesculus hippocastanum*, *A.* × *carnea*), raspberry and blackberry (*Rubus*), male willow catkin (*Salix caprea*, *S. innorata*). The results using a variety of labelled cinnamate precursors are summarised in Scheme



Scheme 5. Suggested pathways of biosynthesis of cyanidin, (+)-catechin and (−)-epicatechin.



Scheme 6. Tracer experiments on procyanidin biosynthesis.

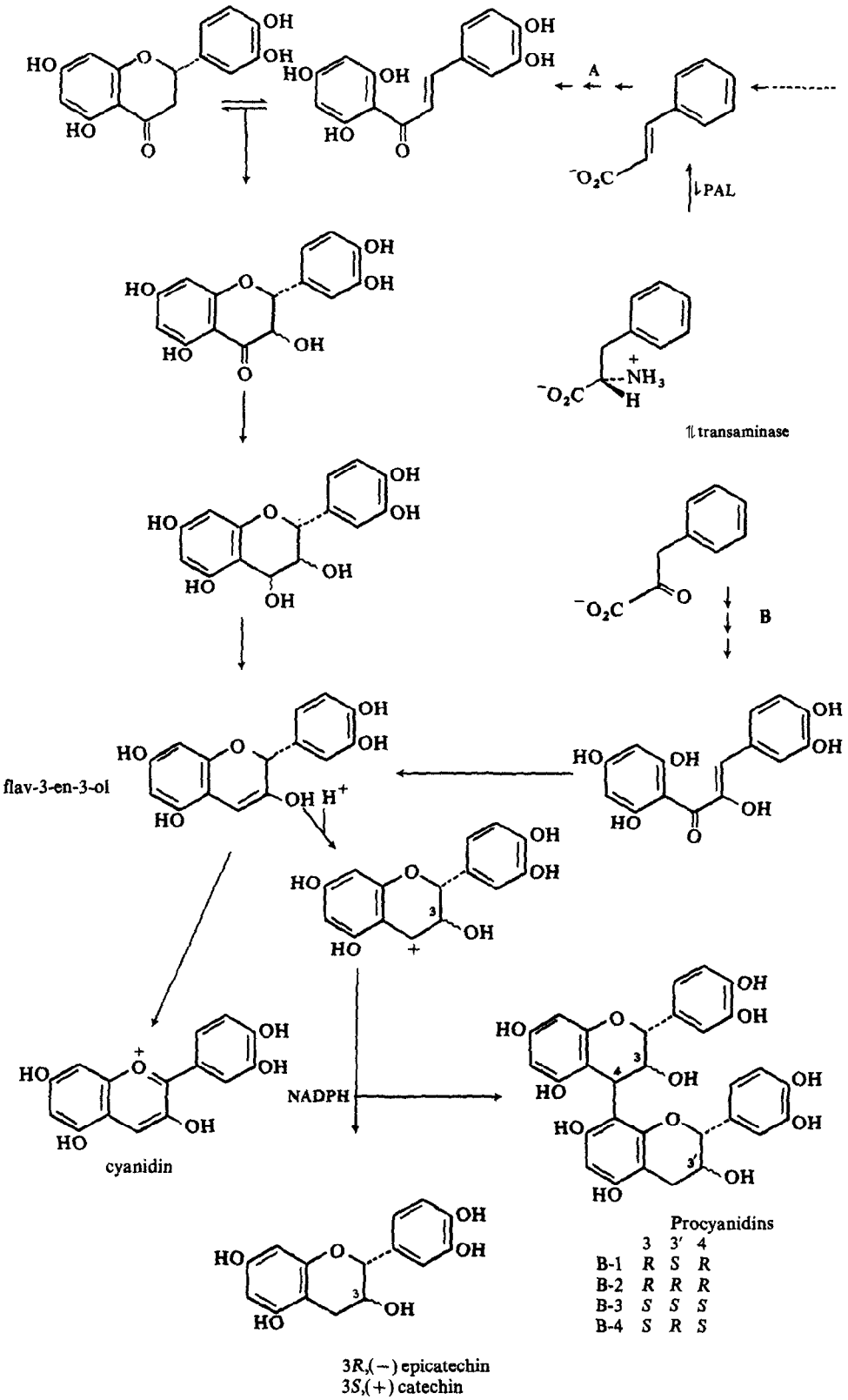
6 and were broadly similar for all the plant species examined. A study of procyanidin metabolism in the cereal sorghum showed that when the coat of the grain was etiolated procyanidins were not metabolised but when this took on the green colour of unripe grain there also occurred a rapid phase of procyanidin synthesis, which was followed by a steady state situation in which procyanidin synthesis was small or zero. No such clear distinction was made with other plants but it was observed during the biosynthetic studies that the time of administration of the isotopically labelled precursors was critical. Tissue which was relatively mature showed little evidence of incorporation of the intermediates.

The results (Scheme 6) show that the C_6—C_3 carbon skeleton of the cinnamate precursor is incorporated intact into the flavan units, H_a was retained (although significantly only 80–90%), H_b was lost and H_c was retained (50% retention, one NIH shift occurring on the introduction of the *ortho* dihydroxy orientation of phenolic groups). A further significant observation was that the two identical structural fragments which go towards the overall procyanidin dimer structure were labelled to different extents. This crucial feature of the evidence has been interpreted to show that the two flavan-3-ol type units of the procyanidin molecule are derived from *different* metabolic entities. Coupled with the strong circumstantial evidence which implies a very close connection to flavan-3-ol biosynthesis itself, this evidence has been formulated to give a projected scheme of biosynthesis for procyanidins as indicated in Scheme 7.

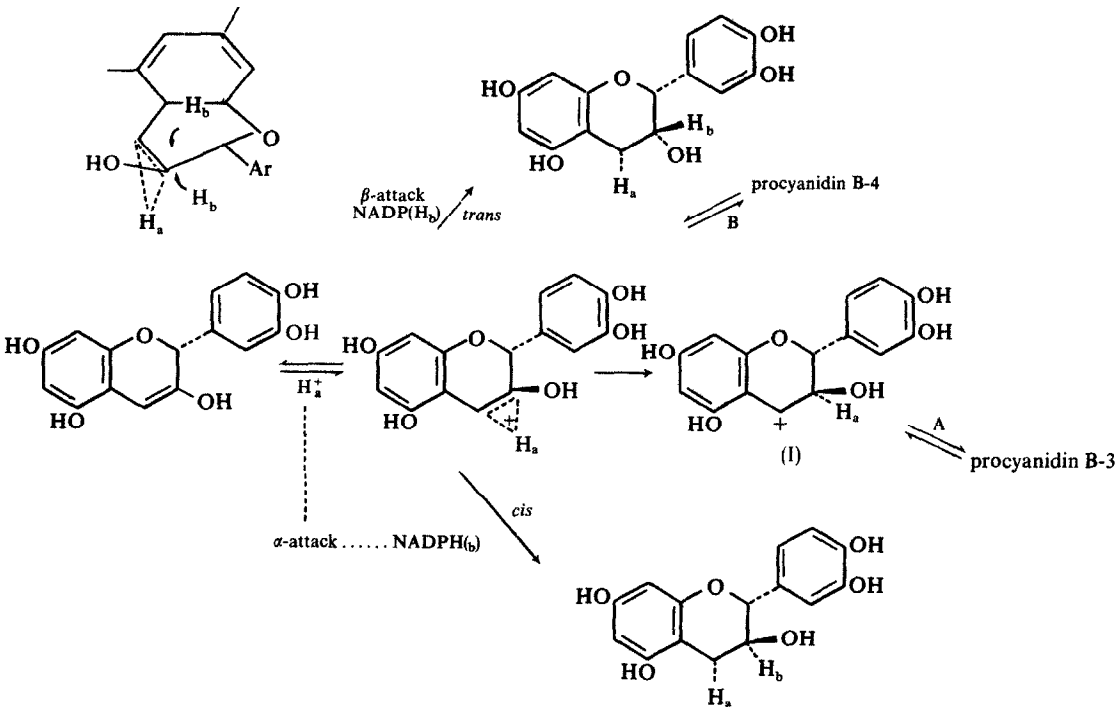
If the reduction of the flav-3-en-3-ol to flavan-3-ol is envisaged as a two-step process in which stereospecific proton addition to give the hybrid protonated species precedes stereospecific (*cis* or *trans*) delivery of hydride ion (or its equivalent from say NADPH), then the carbocations (3*S* or 3*R* absolute stereochemistry) probably may derive from a situation in which the supply of biological reductant is rate-limiting. The carbocations would then be formed by leakage of the hybrid ion from the active site of the enzyme and, it is postulated, would then react with the final reduction product, the flavan-3-ol, still remaining in the vicinity of the enzyme to yield procyanidins. The trimers, tetramers and various higher oligomers and finally polymers which are also formed can then be visualised to result from reactions of the appropriate dimer and further carbocation.

The hypothesis [11] is elaborated in greater detail for two of the possible metabolic situations in procyanidin biosynthesis in Scheme 8. Proton addition to the flav-3-en-3-ol from the α face gives the bridged hybrid ion and hydride addition can then be visualised to occur from the same α face (*cis* addition) to give (+)-catechin or from the opposite β face (*trans* addition) to give (−)-epicatechin. Should however the hybrid protonated species escape from the active site then the product would be the highly reactive carbocation (I) corresponding in stereochemistry at C-3 to (+)-catechin. The two metabolic situations described would then correspond to metabolism (A) of (+)-catechin, procyanidin B-3 etc. as in *Salix caprea* or (B) of (−)-epicatechin, procyanidin B-4 etc. as in *Rubus*. The two alternative situations would result from entirely analogous arguments but the initial proton addition would be to the β-face of the flav-3-en-3-ol.

If this hypothesis is correct, then reacting one or other of the postulated carbocation intermediates with the appropriate flavan-3-ol metabolite [(+)-catechin or (−)-epicatechin] should give rise to a situation *in vitro* very similar to that predicted to occur in the plant. There are several ways in fact in which this situation can be set up in the laboratory. Probably the simplest method is to utilise a procyanidin dimer as a source of the carbocation. Thus procyanidin B-2 in acid media releases the carbocation related in stereochemistry at C-3 to (−)-epicatechin and similarly procyanidin B-3 gives the carbocation related analogously to (+)-catechin. With the acidity correctly adjusted a solution of procyanidin B-2 and (−)-epicatechin should be a model for procyanidin metabolism in *Malus*, *Prunus* and *Crataegus* (Fig. 1). The progress of the model biosynthetic experiment can be followed by paper chromatography (Fig. 7) and it can



Scheme 7. Procyanidin biosynthesis.



Scheme 8. Procyanidin biosynthesis: procyanidins B-3 and B-4.

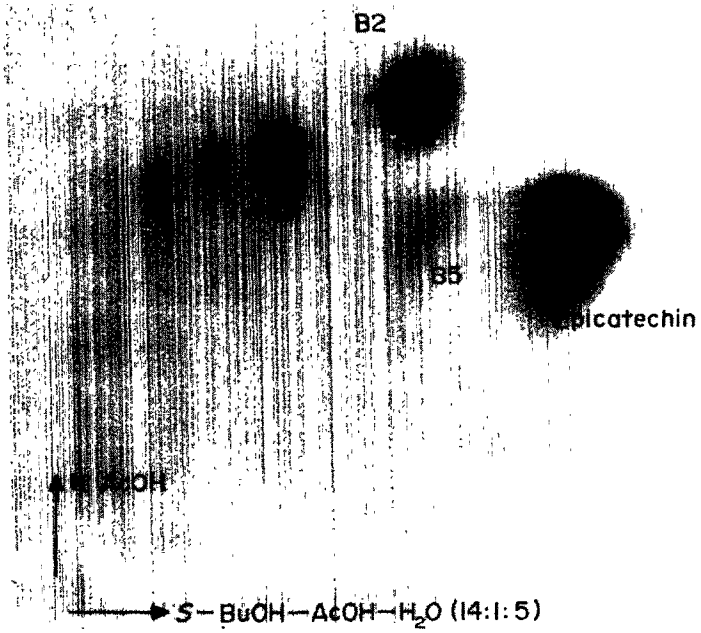
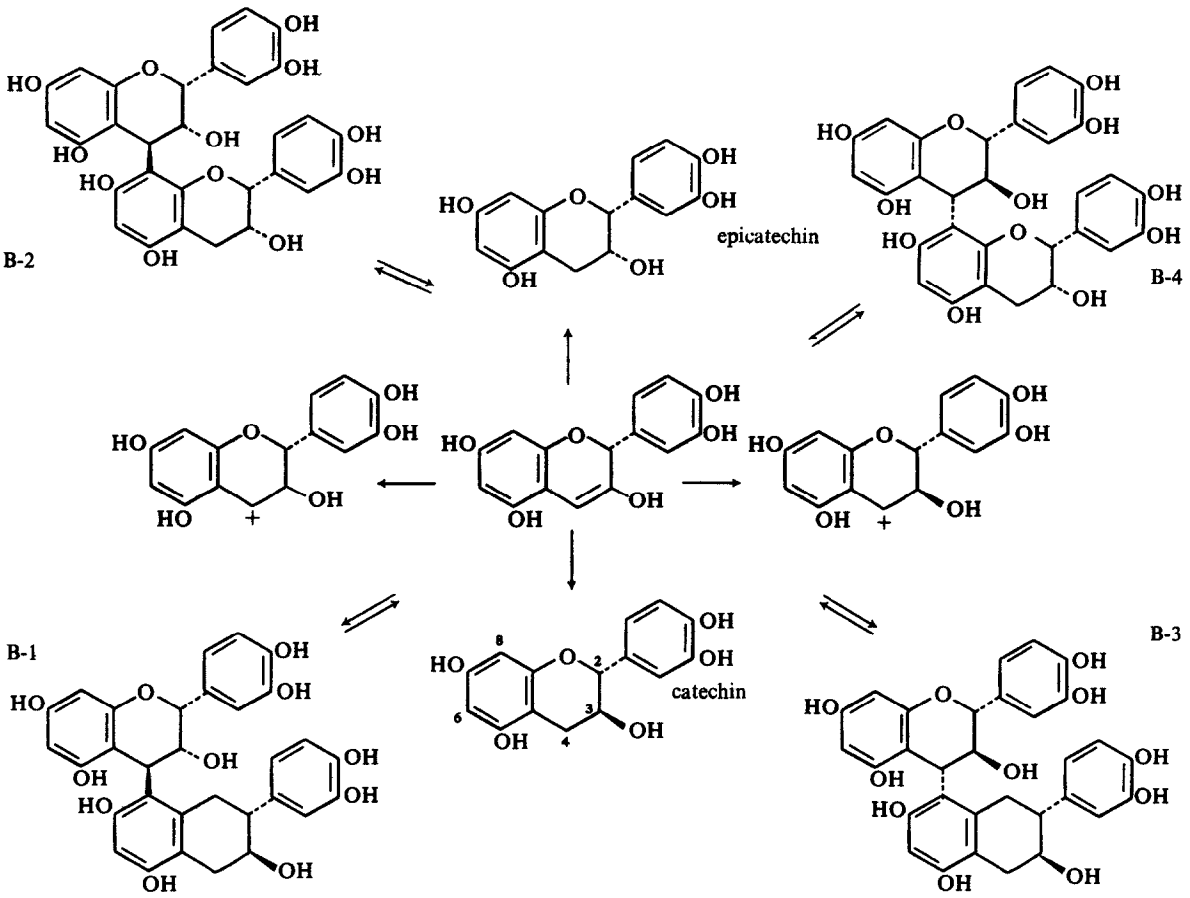


Fig. 7. Biogenetically patterned procyanidin synthesis.



Scheme 9. Procyanidins: structure, stereochemistry, degradation and biosynthesis.

be readily shown that the final array of products exactly matches both qualitatively and quantitatively that found in the several plants mentioned. Similar reactions can also be set up for the various other types of procyanidin patterns which have been found in plants (*vide supra*). The array of procyanidins obtained in the laboratory experiments in each case match the natural extract in the manner depicted for the procyanidins of *Crataegus*. The laboratory reactions are thermodynamically controlled and the close correspondence between the *in vitro* pattern of products produced by the entirely promiscuous encounter between the carbocation and flavan-3-ol and the pattern formed *in vivo* in the plant raises the very important question whether the procyanidin forming reactions observed in Nature are under enzymic control or not. The fact that certain products are formed predominantly in each reaction is determined solely by purely thermodynamic stability considerations. These reactions appear, therefore to be reactions taking place in the plant cell in the absence of enzymic control. Our picture of the procyanidins is now complete and full of awful symmetry (Scheme 9).

D. H. R. Barton, the Nobel laureate, once remarked ... "that it pays to speculate as widely and wildly as possible; people only remember when you are right". With this advice in mind may I conclude by tentatively drawing attention to possible relationships between procyanidin metabolism and certain forms of anthocyanidin biogenesis.

An interesting feature of many procyanidin producing plants is their ability to form distinctive and conspicuous anthocyanidin pigmentation in vegetative tissues at a late stage of growth. The autumnal colouration in leaves often contains anthocyanidin pigmentation as does the skin of ripening fruit. Linked to this latter observation we may also add the fact that many such fruit as they ripen lose their repellent astringent taste. We have considered these observations and briefly it would be pertinent to discuss studies made of the changes which occur amongst procyanidins and related molecules in fruit as they ripen (hawthorn and blackberry) and a cereal grain (sorghum) as it matures. In the case of the two fruits the analyses were based upon average values obtained from a fixed number of fruit at different stages of growth.

For both types of fruit, once formed from the green unripe to the point at which ripening commences a steady-state level of procyanidins is maintained over the whole of this period of maturation. The size and overall weight of the fruit increases over this period and thus the concentration of procyanidins *per fruit* decreases. For the hawthorn fruit little discernible change occurred in the pattern of procyanidins from October to January, the

level of (−)-epicatechin and procyanidin B-2 remained constant but that of the soluble oligomeric procyanidins slowly appeared to decrease. Significantly it was only in late January during a hard spell of weather that these fruit were taken by birds for food.

In the blackberry the range of polyphenols is more complex and amongst other polyphenols present are several ellagitannins which also therefore presumably contribute to the astringent taste of the unripe fruit. As the ripening proceeds the concentration of flavan-3-ol (predominantly (−)-epicatechin), procyanidin B-4 and the soluble oligomeric procyanidins falls substantially. It is clear that if these contribute to the unripe astringent taste of the blackberry then the increase in palatability may in part derive from the decrease in their concentration. However, at the same time there is little significant change during the ripening process in the concentration of ellagitannins which are present. This indicates that other factors, besides that of a reduction in quantity of complex polyphenols, are important in determining the loss of astringency in ripening fruit.

In the case of sorghum (*Sorghum vulgae* cv ensayo) the seed coat of the grain contains procyanidins. The grain develops initially in a sheath and at this etiolated stage the seed coat contains no flavan-3-ols or related molecules. As chlorophyll forms in the seed coat so also are (+)-catechin, procyanidin B-1 and its higher oligomers rapidly synthesised. Thereafter this level of non-hydrolysable vegetable tannins remains approximately constant until the appearance of the distinctive pink–red pigmentation of the ripening grain (luteolinidin is the major anthocyanidin present). At this stage a decrease in the level of the flavan-3-ol and its associated co-metabolites is observed and with the fully ripened sorghum the final oligomeric procyanidin which is obtained is usually of high molecular weight and is difficultly soluble.

In so far as generalisations can be made, the evidence obtained points to the fact that the soluble oligomeric procyanidins decline in concentration as the fruit or cereal grain matures. Taken in conjunction with the observations on sorghum this decrease in the concentration of soluble oligomeric procyanidins may be paralleled by an increase in concentration of the difficultly soluble and higher molecular weight procyanidins. In these cases therefore the loss of astringency may be due, as suggested earlier by Goldstein and Swain [12], to increased polymerisation of the flavan-based polyphenols (procyanidins). The evidence available is broadly consistent at present with the hypothesis that a steady state concentration of procyanidins is maintained in the vegetative tissues of the plant after an initial burst of procyanidin synthesis. As maturity approaches the photosynthetic activity of the tissue declines presumably and with it therefore the capacity to reduce the flav-3-en-3-ol to the flavan-3-ol Scheme 8. It seems probable—assuming that its synthesis is still in progress—that at least two fates may befall the flav-3-en-3-ol. It could be oxidised (by hydride transfer) to cyanidin and thus contribute in part to the characteristic anthocyanidin pigmentation of the ripened tissue. Alternatively it might react with the various procyanidins already present to produce higher oligomeric forms which would have decreased solubility and it is assumed, as far as the plant tissue is concerned decreased astringency.

CONCLUSION

It is clear that there is now a firm chemical and biochemical base from which the biological phenomena associated with the procyanidins (condensed tannins!) can be profitably exploited. Some of our initial rationalisations of biological events which we have advanced here must remain tentative explanations and perhaps at this stage we should content ourselves with the view that if Nature herself does not utilise these particular ideas then she has indeed missed an excellent opportunity!

Acknowledgements—Many have spoken and many more have undoubtedly written concerning the motivations which inspire a scientist in his work. For some an inherent curiosity about the workings of nature provides the inspiration and for others there is the desire to make significant and lasting contributions to human knowledge. It would nevertheless be idle to deny that, for a scientist, the recognition of his work by his peers is an event which engenders the deepest personal satisfaction. I should therefore like to express to the Phytochemical Society of Europe and the Tate and Lyle company my sincere thanks for the honour which they have bestowed upon me with this award. By honouring me, the Phytochemical Society also honours my colleagues who, over the years, have shared in this work. It is a pleasure to record my gratitude to them. My collaborators in this particular piece of research were Drs R. J. N. Tanner, R. S. Thompson, D. Jacques, L. J. Porter and R. K. Gupta, and Messrs. A. C. Fletcher and C. T. Opie.

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