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Review

Proanthocyanidin biosynthesis – still more questions than answers?

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Abstract

Proanthocyanidins, also known as condensed tannins, are oligomers or polymers of flavan-3-ol units. In spite of important breakthroughs in our understanding of the biosynthesis of the major building blocks of proanthocyanidins, (+)-catechin and (−)-epicatechin, important questions still remain to be answered as to the exact nature of the molecular species that undergo polymerization, and the mechanisms of assembly. We review the structures of proanthocyanidins reported over the past 12 years in the context of biosynthesis, and summarize the outstanding questions concerning synthesis of proanthocyanidins from the chemical, biochemical and molecular genetic perspectives.

Keywords: Proanthocyanidins; Tannins; Flavan-3-ols; Catechin; Epicatechin; Anthocyanidin reductase

Contents

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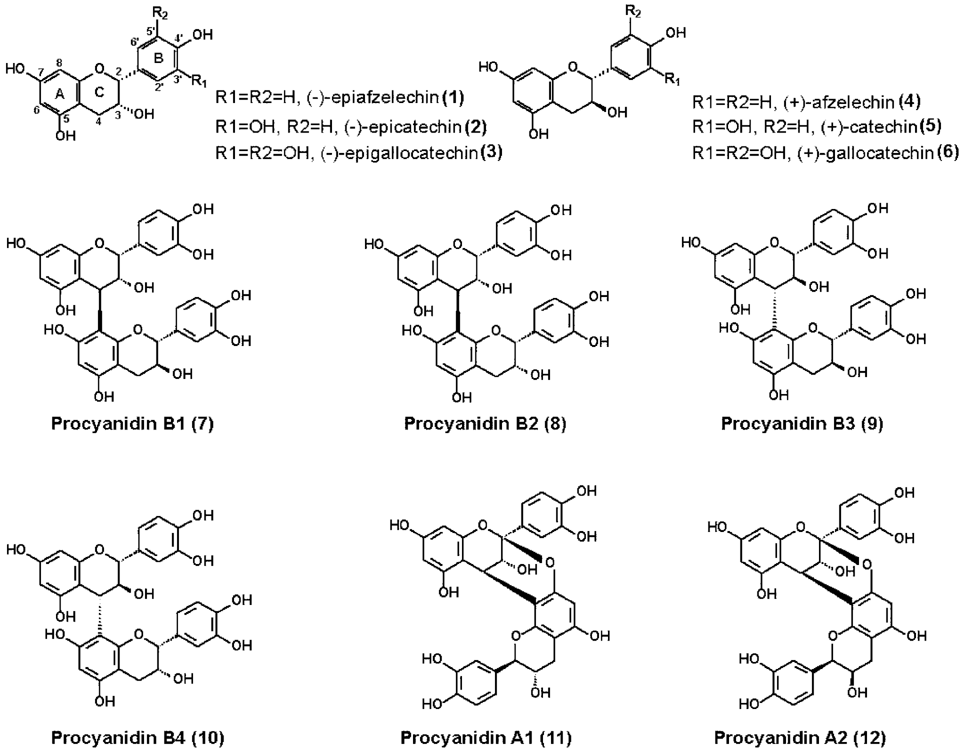
1. Introduction

Proanthocyanidins are widespread throughout the plant kingdom, where they accumulate in many different organs and tissues to provide protection against predation. At the same time, they impart astringency and flavor to beverages such as wines, fruit juices and teas, they are major quality factors for forage crops, and they are increasingly recognized as having health beneficial effects for humans (Dixon et al., 2005). The chemistry of proanthocyanidins has been studied for many decades. Their structures depend upon the nature (stereochemistry and hydroxylation pattern) of the flavan-3-ol starter and extension units, the position and stereochemistry of the linkage to the "lower" unit, the degree of polymerization, and the presence or absence of modifications such as esterification of the 3-hydroxyl group. On acid hydrolysis, the extension units are converted to colored anthocyanidins, this being the basis of a classical assay for these compounds (Porter, 1989).

Most proanthocyanidins are built from the flavan-3-ols (+)-catechin (2,3-*trans*-) (**5**) and (−)-epicatechin (2,3-*cis*) (**2**) (Fig. 1). However, all chiral intermediates in the flavonoid pathway up to and including leucoanthocyanidin are believed to be of the 2,3-*trans* stereochemistry. This raises the question of the origin of the 2,3-*cis* stereochemistry of the (−)-epicatechin extension units.

The B-ring hydroxylation pattern of the monomeric units in proanthocyanidins is determined by the presence or absence of the cytochrome P450 enzymes flavonoid 3′-hydroxylase and flavonoid 3′,5′-hydroxylase (Menting et al., 1994; Kaltenbach et al., 1999; Schoenbohm et al., 2000). Homo-oligomeric proanthocyanidins with two (3′,4′) B-ring hydroxyl groups [as (epi)catechin units] are termed procyanidins, whereas oligomers with at least one unit containing only a 4′-hydroxyl group [as (epi)afzelechin], or a 3′,4′,5′-tri-hydroxy pattern [as (epi)gallocatechin], are termed propelargonidins or prodelphinidins, respectively. This terminology, which reflects the nature of the monomer units in terms of the anthocyanidin product formed after acid hydrolysis, can be extended to cover the many other types of extension unit now known to be present in proanthocyanidin homo- and hetero-polymers. Thus, proanthocyanidins have been classified as procyanidins, propelargonidins, prodelphinidins, profisetinidins, prorobinetinidins, procassinidins, proapigeninidins, proluteolinidins, protricetinidins, prodistenidins, proguibourtinidins, proteracacinidins, propeltogynidins and promopanidins (Porter, 1989, 1993; Ferreira et al., 1999).

Linkage between successive monomeric units in proanthocyanidins is usually between the 4-position of the "upper" unit and the 8-position of the "lower" or "starter" unit (Fig. 1), and may be either α- or β-. Linkage can also occur between C_4 of the "upper" unit and C_6 of the "lower" unit (as in the mopane proanthocyanidins) (Ferreira et al., 2003). In A-type proanthocyanidins, linkages occur between both C_2 and C_4 of the



R1=R2=H, (-)-epiafzelechin (**1**)
R1=OH, R2=H, (-)-epicatechin (**2**)
R1=R2=OH, (-)-epigallocatechin (**3**)

R1=R2=H, (+)-afzelechin (**4**)
R1=OH, R2=H, (+)-catechin (**5**)
R1=R2=OH, (+)-gallocatechin (**6**)

Procyanidin B1 (7)

Procyanidin B2 (8)

Procyanidin B3 (9)

Procyanidin B4 (10)

Procyanidin A1 (11)

Procyanidin A2 (12)

Fig. 1. Structures of flavan-3-ol monomers and simple B-type and A-type dimeric proanthocyanidins.

upper unit and the oxygen at C_7 and positions 6 or 8, respectively, of the lower unit (Fig. 1).

Rarely, units other than flavan-3-ols are found in proanthocyanidins, one example being butiniflavan-epicatechin from *Cassia petersiana* (Coetzee et al., 1999). Quite commonly, the 3-hydroxyl group of the flavan-3-ol units is esterified, often with gallic acid. Free (−)-epigallocatechin gallate is a major phenolic compound in tea (Forrest and Bendall, 1969), and gallic acid substituted epicatechin, epigallocatechin and epiafzelechin units also occur in tea proanthocyanidins (Lakenbrink et al., 1999).

Several recent reviews have documented important concepts in proanthocyanidin research relating to stereochemistry, structure elucidation, taxonomic distribution, and condensation mechanism (Porter, 1989, 1993; Ferreira et al., 1999; Marles et al., 2003; Dixon et al., 2005). The purpose of the present article is to provide an update on recently identified proanthocyanidin structures, and to relate the structural information to still unanswered questions concerning proanthocyanidin biosynthesis and assembly.

2. Structural variation and biological activities among the B-type proanthocyanidins

B-type proanthocyanidins are oligomers or polymers of flavan-3-ols characterized by a single interflavan bond linkage usually between C_4 of the "upper" extension units and C_8 or C_6 of the "lower" or "starter" unit. Proanthocyanidins B1–B4 differ only in the arrangement of (+)-catechin (**5**) and (−)-epicatechin (**2**) starter and extension units (Fig. 1). The fact that different types of dimers are found in different plant species (procyanidin B1 (**7**) in grape, sorghum and cranberry, B2 (**8**) in apple, cocoa bean and cherry, B3 (**9**) in strawberry, hops and willow catkins, and B4 (**10**) in raspberry and blackberry (Haslam, 1977)) is consistent with, but does not prove, an enzymatic mechanism of dimerization.

B-type procyanidins represent the dominant class of natural proanthocyanidins. Among the dimers, procyanidins B1, B2, B3 and B4 (**7–10**) (Fig. 1) occur the most frequently in plant tissues. Procyanidin B5 (epicatechin-(4β → 6)-epicatechin), B6 (catechin-(4α → 6)-catechin), B7 (epicatechin-(4β → 6)-catechin) and B8 (catechin-(4α → 6)-epicatechin) are also widespread in plants (Porter, 1989, 1993; Ferreira et al., 1999). Analogues of procyanidin B1 (**7**) and B2 (**8**) with epicatechin chain extension units (2R, 3R-2,3-*cis* configuration) are very commonly represented in the plant kingdom (Porter, 1989, 1993; Ferreira et al., 1999), and many plants also produce analogues of procyanidin B3 to B8 (Porter, 1989, 1993; Ferreira et al., 1999).

Table 1 summarizes B-type proanthocyanidins identified from various plant species or food products since 1993. The list includes newly classified proanthocyanidins and also known structures, e.g., procyanidins B1–B7 which were, however, not previously isolated from the listed plants or food products. Many of the compounds listed in Table 1 represent novel structures, for example epioritin-(4β → 6)-oritin-(4α → 6)-epioritin-4α-ol, oritin-(4β → 6)-oritin-(4α → 6)-epioritin-4α-ol and epioritin-(4β → 6)-epioritin-(4β → 6)-epioritin-4α-ol isolated from *Acacia galpinii* and *A. caffra* stem bark (Bennie et al., 2004). New structures of proteracacinidins and promelacacinidins from *A. galpinii* and/or *A. caffra* stem bark (Bennie et al., 2000, 2002, 2004) and different stereochemical structures of prorobenitinidols from *Stryphnodendron adstringens* stem bark were also elucidated (de-Mello et al., 1996b). Usually, proanthocyanidins only form amorphous powders. However, one crystalline form of peracetylated prodelphinidin B3, dodecaacetylprodelphinidin B3, was isolated from dried leaf material of *Ziziphus spina-christi* (Weinges and Schick, 1995). Proanthocyanidins have been found in plant callus or cell suspension cultures, but little structure elucidation has been done. Procyanidin B2 (**8**) and higher oligomers of epicatechin were identified in *Vaccinium pahalae* cell cultures (Kandil et al., 2000).

An examination of Table 1 (and Table 2 reporting structures of A-type proanthocyanidins) indicates that several plant species contain a wide range of different linkage types. For example, almond fruit and faba beans collectively contain all the B1–B4 dimers, in addition to larger oligomers and variants with gallocatechin and epigallocatechin substituents. Does this imply that proanthocyanidin biosynthesis in such species is less tightly controlled than in other species where less broad subsets of oligomers are found? Is proanthocanidin composition in these and other species controlled primarily by monomer availability?

Elucidating the proanthocyanidin structures in food plants is important for understanding potential health beneficial effects of these dietary phytochemicals. Procyanidin B2 dimers from female hop (*Humulus lupus*) inflorescences showed inhibitory activity against nitric oxide synthase (nNOS) (Stevens et al., 2002). Two new proanthocyanidin structures were found in green tea, 3-*O*-galloyl-epiafzelechin-(4β → 8)-epicatechin-3-*O*-gallate and 3-*O*-galloyl-epiafzelechin-(4β → 6)-epicatechin-3-*O*-gallate (Lakenbrink et al., 1999). The procyanidin trimers [epicatechin-(4β → 8)]$_2$-catechin, [epicatechin-(4β → 6)]$_2$-epicatechin, [catechin-(4β → 8)]$_2$-catechin (procyanidin C2), epicatechin-(4β → 6)-epicatechin-(4β → 8)-catechin and epicatechin-(4β → 8)-epicatechin-(4β → 6)-catechin were isolated from unripe almond fruit (de Pascual et al., 1998). 3-*O*-Galloyl-epicatechin-(4β → 8)-epicatechin-3-*O*-gallate (procyanidin B2 3,3′-di-*O*-gallate) and 3-*O*-galloyl-epicatechin-(4β → 6)-epicatechin-3-*O*-gallate (procyanidin B5 3,3′-di-*O*-gallate) isolated from rhubarb (*Rhei rhizoma* and *Rheum palmatum* L.) were found to be potent inhibitors

Table 1
A survey of naturally occurring B-type proanthocyanidins characterized since 1993

Class and compound	Plant sources	References
1. Dimers		
(a) Procyanidins		
Epicatechin-(4β → 8)-catechin (procyandin B1) (**7**)	*Lotus corniculatus*	Foo et al. (1996)
	Lotus pedunculatus – leaf	Foo et al. (1997)
	Humulus lupulus (hop) – female inflorescence	Stevens et al. (2002)
	Pinus densiflora – bark	Sato et al. (1999)
	Prunus amygdalus (almond) – unripe fruits	de Pascual et al. (1998)
	Vicia faba (faba bean) – seed testa	Helsper et al. (1993)
Epicatechin-(4β → 8)-epicatechin (procyanidin B2) (**8**)	Cacao liquor, cocoa, and chocolate	Natsume et al. (2000)
	Crataegus monogyna	Rakotoarison et al. (1997)
	Crataegus sinaica	Shahat et al. (1996)
	Guazuma ulmifolia – bark	Hör et al. (1996)
	Humulus lupulus – female inflorescence	Stevens et al. (2002)
	Lotus corniculatus – leaf	Foo et al. (1996)
	Lotus pedunculatus – leaf	Foo et al. (1997)
	Prunus amygdalus – unripe fruits	de Pascual et al. (1998)
	Vaccinium pahalae – cell culture	Kandil et al. (2000)
Catechin-(4α → 8)-catechin (procyanidin B3) (**9**)	*Hamamelis virginiana* – bark	Hartisch and Kolodziej (1996)
	Humulus lupulus – female inflorescence	Stevens et al. (2002)
	Khaya senegalensis – bark	Kayser and Abreu (2001)
	Lotus pedunculatus – leaf	Foo et al. (1997)
	Pinus densiflora – bark	Sato et al. (1999)
	Prunus amygdalus – unripe fruits	de Pascual et al. (1998)
	Vicia faba – testa of faba beans	Helsper et al. (1993)
Catechin-(4α → 8)-epicatechin; (procyanidin B4) (**10**)	*Humulus lupulus* – female inflorescence	Stevens et al. (2002)
	Lotus pedunculatus – leaf	Foo et al. (1997)
	Prunus amygdalus – unripe fruits	de Pascual et al. (1998)
	Vicia faba – seed testa	Helsper et al. (1993)
Epicatechin-(4β → 6)-epicatechin (procyanidin B5)	*Crataegus sinaica*	Shahat et al. (1996)
	Guazuma ulmifolia – bark fruits	Hör et al. (1996)
Catechin – (4α → 6)-catechin (procyanidin B6)	*Khaya senegalensis* – bark	Kayser and Abreu (2001)
Epicatechin-(4β → 6)-catechin (procyanidin B7)	*Prunus amygolalus* – unripe fruits	de Pascual et al. (1998)
	Cistus incanus – aerial parts	Danne et al. (1993)
	Prunus amygdalus – unripe fruits	de Pascual et al. (1998)
ent-Epicatechin-(4α → 6)-*ent*-epicatechin	*Byrsonima crassifolia* – bark	Geiss et al. (1995)
ent-epicatechin-(4α → 8)-*ent*-epicatechin	*Byrsonima crassifolia* – bark	Geiss et al. (1995)
(b) Prodelphinidins		
Epigallocatechin-(4β → 8)-catechin	*Cistus incanus* – aerial parts	Danne et al. (1993)
	Hamamelis virginiana – bark	Hartisch and Kolodziej (1996)
	Lotus pedunculatus – leaf	Foo et al. (1997)
Gallocatechin-(4α → 8)-catechin	*Vicia faba* – seed testa	Helsper et al. (1993)
Epigallocatechin-(4β → 8)-epicatechin	*Lotus pedunculatus* – leaf	Foo et al. (1997)
Gallocatechin-(4α → 8)-epicatechin	*Vicia faba* – seed testa	Helsper et al. (1993)

Epigallocatechin-(4β → 8)-gallocatechin	*Cistus incanus* – aerial parts	Danne et al. (1993)
	Lotus pedunculatus – leaf	Foo et al. (1997)
	Stryphnodendron adstringens – stem bark	de-Mello et al. (1996a)
Gallocatechin-(4α → 8)-gallocatechin	*Ziziphus spina-christi* – leaf	Weinges and Schick (1995)
Epigallocatechin-(4β → 8)-epigallocatechin	*Lotus pedunculatus* – leaf	Foo et al. (1997)
	Stryphnodendron adstringens – stem bark	de-Mello et al. (1996a)
Epigallocatechin-(4β → 6)-epigallocatechin	*Stryphnodendron adstringens* – stem bark	de-Mello et al. (1996a)
Gallocatechin-(4α → 8)-epigallocatechin	*Lotus pedunculatus* – leaf	Foo et al. (1997)
	Vicia faba – seed testa	Helsper et al. (1993)
(c) Prorobinetinidins		
Robinetinidol-(4β → 8)-epigallocatechin	*Stryphnodendron adstringens* – stem bark	de-Mello et al. (1996b)
Robinetinidol-(4α → 8)-epigallocatechin		
Robinetinidol-(4α → 6)-epigallocatechin		
Robinetinidol-(4α → 6)-gallocatechin		
Robinetinidol-(4β → 6)-gallocatechin		
Robinetinidol-(4β → 8)-gallocatechin		
Robinetinidol-(4α → 8)-gallocatechin		
(d) Proteracacinidin		
Epioritin-(4β → *O* → 3)-epioritin-4β-ol	*Acacia caffra* – heartwood	Bennie et al. (2000)
Epioritin-(4β → *O* → 3)-epioritin-4α-ol	*Acacia caffra* – heartwood	Coetzee et al. (1998a)
Epioritin-(4β → *O* → 3)-oritin-4α-ol		
Epioritin-(4β → *O*→4)-epioritin-4α-ol		Coetzee et al. (1998b)
ent-Oritin-(4α → *O* → 4)-epioritin-4α-ol		
Epioritin-(4β → 6)-oritin-4α-ol	*Acacia galpinii* and *Acacia caffra* – heartwood	Bennie et al. (2002)
Epioritin-(4β → 6)-*ent*-oritin-4α-ol		
ent-Oritin-(4β → 6)-epioritin-4α-ol		
ent-Oritin-(4β → 6)-oritin-4α-ol		
ent-Oritin-(4α → 6)-epioritin-4α-ol		
ent-Oritin-(4α → 6)-oritin-4α-ol		
ent-Oritin-(4α → 6)-epioritin-4β-ol		
Proteracacinidins/-melacinidins		
Epioritin-(4β → 6)-epimesquitol-4α-ol	*Acacia galpinii* and *Acacia caffra* – heartwood	Bennie et al. (2002)
Epioritin-(4β → 6)-epimesquitol-4β-ol		
Epimesquitol-(4β → 6)-epioritin-4α-ol		
(e) Promelacacinidin		
Epimesquitol-(4β → *O* → 4)-epioritin-4β-ol	*Acacia caffra* – heartwood	Bennie et al. (2000)
Epimesquitol-(4β → 6)-epimesquitol-4β-ol	*Acacia galpinii and Acacia caffra* – heartwood	Bennie et al. (2002)
2. Trimers		
(a) Procyanidins		
[Epicatechin-(4β → 8)]$_2$-epicatechin (procyanidin Cl)	Cacao liquor, cocoa, and chocolate	Natsume et al. (2000)
	Crataegus sinaica	Shahat et al. (1996)
	Guazuma ulmifolia – bark	Hör et al. (1996)
	Prunus amygdalus – unripe fruit	de Pascual et al. (1998)

(*continued on next page*)

Table 1 (*continued*)

Class and compound	Plant sources	References
Epicatechin-(4β → 6)-epicatechin-(4β → 8)-epicatechin	*Guazuma ulmifolia* – bark	Hör et al. (1996)
	Prunus amygdalus – unripe fruit	de Pascual et al. (1998)
Epicatechin-(4β → 8)-epicatechin-(4β → 6)-epicatechin	*Guazuma ulmifolia* – bark	Hör et al. (1996)
[Epicatechin-(4β → 8)]$_2$-catechin	*Lotus corniculatus*	Foo et al. (1996)
	Prunus amygdalus – unripe fruit	de Pascual et al. (1998)
[Epicatechin-(4β → 6)]$_2$-epicatechin	*Prunus amygdalus* – unripe fruit	de Pascual et al. (1998)
[Catechin-(4α → 8)]$_2$-catechin (procyanidin C2)		
Epicatechin-(4β → 6)-epicatechin-(4β → 8)-catechin		
Epicatechin-(4β → -8)-epicatechin-(4β → 6)-catechin		
Epicatechin-(4β → 8)-catechin-(4α → 8)-catechin	*Humulus lupulus* – female inflorescences	Stevens et al. (2002)
(b) Prodelphinidins		
[Gallocatechin-(4α → 8)]$_2$-gallocatechin	*Cistus incanus* – aerial parts	Danne et al. (1993)
(c) Proteracacinidin		
Epioritin-(4β → 6)-oritin-(4α → 6)-epioritin-4α-ol	*Acacia galpinii* and *Acacia caffra* – heartwood	Bennie et al. (2004)
Oritin-(4β → 6)-oritin-(4α → 6)-epioritin-4α-ol		
Epioritin-(4β → 6)-epioritin-(4β → 6)-epioritin-4α-ol		
3. Tetramers		
(a) Procyanidins		
[Epicatechin-(4β → 8)]$_3$-epicatechin (cinnamtannin A2)	Cacao liquor, cocoa, and chocolate	Natsume et al. (2000)
	Guazuma ulmifolia – bark	Hör et al. (1996)
	Prunus amygdalus – unripe fruit	de Pascual et al. (1998)
[Epicatechin-(4β → 8)]$_3$-catechin	*Prunus amygdalus* – unripe fruit	de Pascual et al. (1998)
Gallate esters		
1. Dimers		
(a) Procyanidins		
3-*O*-Galloyl-epicatechin-(4β → 8)-catechin	*Hamamelis Virginiana* – bark	Hartisch and Kolodziej (1996)
3-*O*-Galloyl-*ent*-epicatechin-(4α → 8)-3-*O*-galloyl-*ent*-epicatechin	*Byrsonima crassifolia* – bark	Geiss et al. (1995)
3-*O*-Galloyl-*ent*-epicatechin-(4α → 8)-*ent*-epicatechin		
ent-Epicatechin-(4α → 8)-3-*O*-galloyl-*ent*-epicatechin		
3-*O*-Galloyl-epicatechin-(4β → 8)-epicatechin-3-*O*-gallate (procyanidin B2 3,3′-di-*O*-gallate)	Rhubarb *(Rhei rhizoma*, *Rheum palmatum)*	Abe et al. (2000)
3-*O*-Galloyl-epictechin-(4β → 6)-epicatechin-3-*O*-gallate (procyanidin B5 3,3′-di-*O*-gallate)		
(b) Prodelphinidins		
3-*O*-Galloyl-epigallocatechin-(4β → 8)-catechin	*Hamamelis virginiana* – bark	Hartisch and Kolodziej (1996)
3-*O*-Galloyl-epigallocatechin-(4β → 8)-gallocatechin	*Hamamelis virginiana* – bark	Hartisch and Kolodziej (1996)
	Cistus incanus – aerial parts	Danne et al. (1993)
3-*O*-Galloyl-epigallocatechin-(4β → 6)-gallocatechin	*Cistus incanus* – aerial parts	Danne et al. (1993)
3-*O*-Galloyl-epigallocatechin -(4β → 8)-epigallocatechin-3-*O*-gallate	*Stryphnodendron adstringens* – stem bark	de-Mello et al. (1996a)

Gallocatechin-(4α → 8)-epigallocatechin-3-*O*-gallate	*Stryphnodendron adstringens* – stem bark	de-Mello et al. (1996a)
Epigallocatechin-(4β → 8)-epigallocatechin 3-*O*-gallate	*Stryphnodendron adstringens* – stem bark	de-Mello et al. (1996a)
(c) Propelargonidins		
3-*O*-Galloyl-epiafzelechin-(4β → 8)-epicatechin-3-*O*-gallate	Green tea	Lakenbrink et al. (1999)
3-*O*-Galloyl-epiafzelechin-(4β → 6)-epicatechin-3-*O*-gallate		
(d) Prorobinetinidins		
Robinetinidol-(4β → 8)-epigallocatechin-3-*O*-gallate	*Stryphnodendron adstringens* – stem bark	de-Mello et al. (1996b)
Robinetinidol-(4α → 8)-epigallocatechin-3-*O*-gallate	*Stryphnodendron adstringens* – stem bark	
2. Trimers		
(a) Procyanidins		
3-*O*-Galloyl-*ent*-epicatechin-(4α → 8)-3-*O*-galloyl-*ent*-epicatechin-(4α → 8)-*ent*-epicatechin	*Byrsonima crassifolia* – bark	Geiss et al. (1995)
3-*O*-Galloyl-epicatechin-(4β → 8)-3-*O*-galloyl-*ent*-epicatechin-(4α → 8)-*ent*-epicatechin		
Other esters		
1. Dimers		
(a) Procyanidins		
Epicatechin-(4β → 8)-catechin-3-*O*-4-hydroxybenzoate	*Hamamelis virginiana* – bark	Hartisch and Kolodziej (1996)
(b) Prodelphinidins		
Epigallocatechin-(4β → 8)-epigallocatechin-3-*O*-4-hydroxybenzoate	*Stryphnodendron adstringens* – stem bark	de-Mello et al. (1996a)
Gallocatechin-(4α → 8)-epigallocatechin-3-*O*-4-hydroxybenzoate		
Glycosides		
(1) Propelargonidins		
3-*O*-(α-L-rhamnopyranosyl)-afzelechin-(4α → 8)-epiafzelechin-3-*O*-vanillate	*Joannesia princeps* – root and stem bark	Achenbach and Benirschke (1997)
3-*O*-(α-L-rhamnopyranosyl)-afzelechin-(4α → 8)-epicatechin-3-*O*-vanillate		
3-*O*-(α-L-rhamnopyranosyl)-afzelechin-(4α → 8)-epiafzelechin-3-*O*-syringate		
3-*O*-(α-L-rhamnopyranosyl)-afzelechin-(4α → 8)-epicatechin-3-*O*-syringate		
Miscellaneous proanthocyanidins		
Butiniflavan-(4α → 8)-epicatechin	*Cassia petersiana* – bark	Coetzee et al. (1999)
Butiniflavan-(4β → 8)-epicatechin		
Butiniflavan-(4β → 8)-epigallocatechin		
(2S)-7,8,4′-trihydroflavan-(4β → 6)-epioritin-4-ol	*Acacia caffra* – heartwood	Malan et al. (1997)
(2S)-3′,4′,7-trihydroxyflavan-(4β → 8)-catechin	*Cassia nomame* – fruit	Hatano et al. (1997)
(2S)-3′,4′,7-trihydroxyflavan-(4α → 8)-catechin		
Bis-leucoteracacinidin, epioritin-4α-ol-(6 → 6)-epioritin-4β-ol	*Acacia galpinii* and *Acacia caffra* – heartwood	Bennie et al. (2004)

Table 2
A survey of naturally occurring A-type proanthocyanidins characterized since 1991

Class and compound	Plant sources	References
Procyanidins		
1. Dimers		
Epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin (procyanidin A2) (**12**)	*Crataegus sinaica*	Shahat et al. (1996)
	Parameria laevigata – bark	Kamiya et al. (2001)
	Pavetta owariensis – stem-bark	Balde et al. (1991a)
	Arachis hypogaea – peanut skins	Lou et al. (1999)
	Vaccinium macrocarpon (cranberry) – fruit	Foo et al. (2000a)
Epicatechin-(4β → 8, 2β → *O* → 7)-catechin	peanut skins	Lou et al. (1999)
(procyanidin A1) (**11**)	*Lupinus angustifolius*-seeds	Stobiecki and Popenda (1994)
Epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-catechin (procyanidin A4)	*Pavetta owariensis* – stem bark	Balde et al. (1991a)
Epicatechin-(4β → 6, 2β → *O* → 7)-epicatechin		
Proanthocyanidin A6	*Parameria laevigata* – bark	Kamiya et al. (2001)
Epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-epicatechin	peanut skins	Lou et al. (1999)
Epicatechin-(4β → 6, 2β → *O* → 7)-catechin		
Epicatechin-(4β → 6, 2β → *O* → 7)-*ent*-catechin		
Epicatechin-(4β → 6, 2β → *O* → 7)-*ent*-epicatechin		
ent-Epicatechin-(4α → 8, 2α → *O* → 7)-*ent*-catechin (pavetannin A1)	*Pavetta owariensis* – stem bark	Balde et al. (1991a, 1995b)
ent-Epicatechin-(4α → 8, 2α → *O* → 7)-catechin (pavetannin A2)		Balde et al. (1991b)
3T-*O*-Arabinopyranosyl-*ent*-epicatechin-(4α → 8, 2α → *O* → 7)-catechin	*Theobroma cacao* – cacao liquor	Hatano et al. (2002)
3T-*O*-β-D-Gallactopyranosyl-*ent*-epicatechin-(4α → 8, 2α → *O* → 7)-epicatechin	*Theobroma cacao* – seeds	Natsume et al. (2000)
2. Trimers		
Epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4α → 8)-epicatechin (cinnamtannin B1)	*Parameria laevigata* – bark	Kamiya et al. (2001)
	Pavetta owariensis – stem bark	Balde et al. (1991b)
Epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin- (4β → 8)-epicatechin	*Vaccinium macrocarpon* – fruit	Foo et al. (2000b)
	Ecdysanthera utilis – stem	Lin et al. (2002)
Epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-catechin-(4β → 8)-epicatechin (aesculitannin B)	*Parameria laevigata* – bark	Kamiya et al. (2001)
Epicatechin-(4β → 6)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin	*Vaccinium macrocarpon* – fruit	Foo et al. (2000b)
Epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin		
Epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-*ent*-epicatechin (pavetannin B1)	*Pavetta owariensis* – stem bark	Balde et al. (1991b)
Epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin- (4β → 8)-epicatechin (pavetannin B2)		
Epicatechin-(4β → 6, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin (pavetannin B3)		
Epicatechin-(4β → 6, 2β → *O* → 7)-*ent*-epicatechin-(4β → 8)-epicatechin (pavetannin B4)		
Epicatechin-(4β → 6, 2β → *O* → 7)-catechin-(4β → 8)-epicatechin (pavetannin B5)		
Epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4α → 8)-catechin (pavetannin B6)		
Epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-epicatechin-(4α → 8, 2α → *O* → 7)-*ent*-catechin (pavetannin B7)		Balde et al. (1995b)
Epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-catechin (pavetannin B8)		
Epicatechin-(4β → 6, 2β → *O* → 7)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin	*Parameria laevigata* – bark	Kamiya et al. (2001)
3T-*O*-α-L-arabinopyranosyl-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4α → 8)-epicatechin (3T-*O*-α-L-arabinopyranosylcinnamtannin B1)	*Theobroma cacao* – cacao liquor	Hatano et al. (2002)
3T-*O*-β-D-galactopyranosyl-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4α → 8)-epicatechin (3T-*O*-β-D-galactopyranosylcinnamtannin B1)		
3. Tetramers		
Epicatechin-(4β → 8, 2β → *O* → 7)-[epicatechin-(4β → 6)]-epicatechin-(4β → 8)-epicatechin (parameritannin A1)	*Parameria laevigata* – bark	Kamiya et al. (2001)

Epicatechin-(4β → 8, 2β → *O* → 7)*ent*-epicatechin-(4α → 8)-*ent*-epicatechin-(4α → 8)-epicatechin (pavetannin C2)	*Pavetta owariensis* – stem bark	Balde et al. (1995a)
Epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-catechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin (pavetannin C6, aesculitannin F)		
Epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin	*Pavetta owariensis* – stem bark *Ecdysanthera utilis* – stem	Balde et al. (1995b) Lin et al. (2002)
Epicatechin-(4β → 6)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin (pavetannin C1)	*Pavetta owariensis* – stem bark	Balde et al. (1995b)
Epicatechin-(4β → 6, 2β → *O* → 5)-[epicatechin-(4β → 8, 2β → *O* → 7)]-epicatechin-(4β → 8)-epicatechin (parameritannin A2)	*Parameria laevigata* – bark	Kamiya et al. (2001)
4. Pentamers		
Epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin (pavetannin D1)	*Pavetta owariensis* – stem bark	Balde et al. (1995b)
Procyanidins/propelargonidins		
Epiafzelechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin (pavetannin C3)	*Pavetta owariensis* – stem bark	Balde et al. (1995a)
Epiafzelechin-(4β → 8, 2β → *O* → 7)-*ent*-afzelechin-(4α → 8)-*ent*-epicatechin-(4α → 8, 2β → *O* → 7)-*ent*-catechin (pavetannin C4)		
Epiafzelechin-(4β → 8, 2β → *O* → 7)-*ent*-catechin-(4α → 8)-*ent*-epicatechin-(4α → 8, 2β → *O* → 7)-*ent*-catechin (pavetannin C5)		
Propelargonidins		
1. Dimers		
Epiafzelechin-(4β → 8, 2β → *O* → 7)-afzelechin (geranin A)	*Geranium niveum* – root	Calzada et al. (1999, 2001)
Epicatechin-(4β → 8, 2β → *O* → 7)-afzelechin (geranin B)		
Epiafzelechin-(4β → 8, 2β → *O* → 7)-gallocatechin, (geranin C)		
Epiafzelechin-(4β → 8, 2β → *O* → 7)-epiafzelechin (mahuannin B)		
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-epicatechin	*Prunus armeniaca* – roots	Prasad (2000)
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-catechin.		
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-epiafzelechin (mahuannin A)	*Prunus armeniaca* – roots *Prunus spinosa* – branches	Rawat et al. (1999) Gonzalez et al. (1992)
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-epicatechin	*Prunus spinosa* – branches	Gonzalez et al. (1992)
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-afzelechin	*Prunus armeniaca* – roots	Rawat et al. (1999)
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-*ent*-afzelechin		
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-afzelechin	*Prunus prostrata* – aerial parts	Bilia et al. (1996)
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-*ent*-epicatechin		
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-*ent*-epiafzelechin		
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-catechin		
epiafzelechin-(4β → 8, 2β → *O* → 7)-epiafzelechin	*Cassipourea gummiflua* – stem bark	Drewes and Taylor (1994)
epiafzelechin-(4β → 8, 2β → *O* → 7)-*ent*-afzelechin	*Cassipourea gummiflua* – stem bark	Drewes and Taylor (1994)
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-quercetin	*Prunus prostrate* – aerial parts	Bilia et al. (1996)
ent-Epiafzelechin-(4α → 8, 2α → *O* → 7)-kaempferol		
3-*O*-*p*-Hydroxybenzoate-*ent*-epiafzelechin-(4α → 8, 2α → *O* → 7)-epiafzelechin	*Prunus armeniaca* – roots	Rawat et al. (1999)
7-OMe-epiafzelechin-(4β → 8, 2β → *O* → 7)-epiafzelechin	*Cassipourea gummiflua* – stem bark	Drewes and Taylor (1994)
7-OMe-Epiafzelechin-(4β → 8, 2β → *O* → 7)-*ent*-afzelechin		
2. Trimers		
Epiafzelechin-(4β → 8, 2β → *O* → 7)-afzelechin-(4β → 8, 2β → *O* → 7)-afzelechin (Geranins D)	*Geranium niveum* – root	Calzada et al. (2001)
Prodelphinidins		
1. Dimers		
Epigallocatechin-(4β → 8, 2β → *O* → 7)-epicatechin	*Dioclea lasio-hylla* – leaf	Barreiros et al. (2000)

of rat squalene epoxidase, a rate-limiting enzyme of cholesterol biogenesis and therefore a target for cholesterol-reducing drugs (Abe et al., 2000). Procyanidins B2 (**8**) and C1 from *Vicia faba* showed higher trypsin inhibitor activity than procyanidins B3 (**9**) and B4 (**10**) (which have fewer 2,3-*cis* units) (Helsper et al., 1993), and in a study of ten flavan dimers, including procyanidins B1 (**7**) and B2 (**8**), (2S)-3′,4′,7-trihydroxyflavan-(4α → 8)-catechin from *Cassia nomame* showed the highest lipase-inhibitory activity (Hatano et al., 1997). Together, these studies suggest that degree of polymerization, stereochemistry and the number of phenolic hydroxyl groups may all affect the biological activities of proanthocyanidins as potential dietary factors for human health.

The lack of proanthocyanidins in the forage legume alfalfa is an important factor in the occurrence of pasture bloat for ruminant animals (Aerts et al., 1999a). Mixtures of alfalfa fodder with proanthocyanidin producing legume plants, e.g. *Lotus* species, have therefore been used in ruminant feeds to help alleviate bloat (Wang et al., 1996). Procyanidins B1 (**7**) and B2 (**8**) were present in *L. corniculatus* leaf tissue in about equal proportions. The trimer [epicatechin-(4β → 8)]$_2$-catechin was also isolated (Foo et al., 1996). Compared with the proanthocyanidins in *L. corniculatus*, the structures of the proanthocyanidins in *L. pedunculatus* are more diverse, including procyanidins B1–B4 (**7–10**), epigallocatechin-(4β → 8)-catechin, epigallocatechin-(4β → 8)-epicatechin, epigallocatechin-(4β → 8)-gallocatechin, epigallocatechin-(4β → 8)-epigallocatechin and gallocatechin-(4α → 8)-epigallocatechin, and the trimer epicatechin-(4β → 8)]$_2$-catechin (Foo et al., 1997). While the extension units of proanthocyanidins in *L. corniculatus* mainly consist of epicatechin (67%) and epigallocatechin (30%), polymers of proanthocyanidins in *L. pedunculatus* are quite heterogeneous, consisting of catechin, epicatechin, gallocatechin and epigallocatechin units.

In addition to concentration, the exact structural properties of proanthocyanidins in forages may be important for the health of ruminant animals. For example, the proanthocyanidin extract from *L. pedunculatus* showed much stronger inhibitory effects on larval migration of the nematode *Trichostrongylus colubriformis* than the proanthocyanidin extract from *L. corniculatus* at the same feeding level (Molan et al., 2000). The *L. pedunculatus* extract was also more effective than that from *L. corniculatus* in preventing in vitro degradation of the large and small subunits of Rubisco by rumen microorganisms (Aerts et al., 1999b).

3. Structural variation and biological activities among the A-type proanthocyanidins

The A-type proanthocyanidins have a second ether linkage between an A-ring hydroxyl group of the lower unit and C-2 of the upper unit. Since they are not as frequently isolated from plants as the B-types, they have been considered as unusual structures (Porter, 1989, 1993; Ferreira et al., 1999). The first identified A-type proanthocyanidin was procyanidin A2 (**12**) isolated from the shells of fruit of *Aesculus hippocastanum* (Mayer et al., 1966) (Fig. 1). Since then, many more A-type proanthocyanidins have been found in plants, including dimers, trimers, tetramers, pentamers and ethers (Porter, 1989, 1993; Ferreira et al., 1999; Ferreira and Slade, 2002). Table 2 lists new A-type proanthocyanidins, or known compounds isolated from new tissue sources, reported in the past 13 years.

A-type proanthocyanidins were shown to be the major components with antibacterial and antiherpetic activity in extracts from *Pavetta owariensis*. Besides the two dimers *ent*-epicatechin-(4α → 8, 2α → *O* → 7)-*ent*-catechin (pavetannin A1) and epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin (procyanidin A2 (**12**)) (Balde et al., 1991a), structures of more A-type procyanidins were elucidated, including trimers (pavetannin B1 to B8), tetramers (pavetannin C1, C2 and C6, cinnamtannin B2) and pentamers (pavetannin D1), as well as tetramers of mixtures of procyanidin/propelargonidin (pavetannin C3 and C4) (Table 2) (Balde et al., 1991b, 1993, 1995a,b). A-type procyanidins in *P. owariensis* have either one or two double ether linkages, such as in epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-*ent*-epicatechin (pavetannin B1) (one double linkage) and epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-epicatechin-(4α → 8, 2α → *O* → 7)-*ent*-catechin (pavetannin B7) and epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-catechin (pavetannin B8) (two double linkages) (Balde et al., 1991b; Balde et al., 1995b).

Six dimers of A-type propelargonidins, including one new structure containing a quercetin moiety as the terminating unit, were isolated from *Prunus prostrata*, which has been used in folk medicine against gastrointestinal disturbances (Table 2) (Bilia et al., 1996). Four A-type propelargonidins were isolated from the stem bark of *Cassipourea gummiflua*, a local medicine in South Africa. These included two novel methylated derivatives, 7-*O*-Me-epiafzelechin-(4β → 8, 2β → *O* → 7)-epiafzelechin and 7-*O*-Me-epiafzelechin-(4β → 8, 2β → *O* → 7)-*ent*-afzelechin (Table 2) (Drewes and Taylor, 1994).

Activity-based fractionation has been used to assess the biological activities of certain A-type proanthocyanidins in relation to nutrition and pathology. The juice of cranberry (*Vaccinium macrocarpon*) is well known as a treatment for urinary tract infections. Three A-type procyanidins trimers, epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin, epicatechin-(4β → 6)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin and epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → *O* →

7)-epicatechin, isolated from ripe cranberry fruits, were shown to prevent adherence of P-fimbriated *Escherichia coli* (Foo et al., 2000a).

Cacao and its products (i.e., cacao liquor) show strong anti-oxidant activity due to their high level of polyphenols, of which proanthocyanidins are the major anti-oxidative components. Besides B-type procyanidins, i.e., procyanidin C1 and cinnamtannin A2, four new *O*-glycosides of A-type procyanidins, 3T-*O*-arabinopyranosyl-*ent*-epicatechin-(4α → 8, 2α → *O* → 7)-catechin, 3-*O*-galactopyranosyl-*ent*-epicatechin-(4α → 8, 2α → *O* → 7)-epicatechin, 3T-*O*-α-L-arabinopyranosyl-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4α → 8)-epicatechin (3T-*O*-α-L-arabinopyranosylcinnamtannin B1) and 3T-*O*-β-D-galactopyranosyl-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4α → 8)-epicatechin (3T-*O*-β-D-galactopyranosylcinnamtannin B1) where *T* = top unit), were isolated from cacao liquor (Natsume et al., 2000; Hatano et al., 2002).

4. Biosynthesis of (−)-epicatechin and (+)-catechin monomers

Flavan-3-ols are products of a branch pathway of anthocyanin biosynthesis. Several reviews have summarized our current understanding of the biosynthesis of anthocyanins and other flavonoids derived from earlier intermediates in the anthocyanin pathway (Shirley, 1996; Winkel-Shirley, 2001a,b; Saito and Yamazaki, 2002). Understanding the proanthocyanidin branch of the pathway has been more difficult because the potential substrates are not readily available, have multiple potential stereochemistries, and are readily oxidized in biological extracts.

Pioneering enzymological studies showed that crude extracts from cell or tissue cultures of *Ginko biloba* and Douglas fir (*Pseudotsuga menziesii*) converted dihydroflavonols ((+)-dihydromyricetin or (+)-dihydroquercetin) to the corresponding flavan-3,4-diols and catechin derivatives ((+)-gallocatechin (**6**) or (+)-catechin (**5**), respectively) (Stafford and Lester, 1984, 1985). Similarly, dihydroquercetin was converted to catechin in extracts from developing barley grains (Kristiansen, 1986). These early studies established the enzymatic basis for the formation of the 2,3-*trans*, catechin-derived series of flavan-3-ols, which was presumed to involve the consecutive action of a dihydroflavonol reductase (DFR), to yield leucoanthocyanidin, and a leucoanthocyanidin reductase (LAR). *DFR* genes have now been cloned from many plant species (Beld et al., 1989; Charrier et al., 1995; Tanaka et al., 1995). LAR activity was detected in extracts from leaves of tannin rich legumes (Tanner and Kristiansen, 1993; Skadhauge et al., 1997), and the first putative *LAR* gene was recently cloned from the forage legume *Desmodium uncinatum*. This gene encodes an enzyme that converts leucoanthocyanidin to a compound that co-elutes on HPLC with (+)-catechin (**5**), and has a molecular mass identical to that of a catechin (Tanner et al., 2003) (Fig. 2). LAR is a member of the isoflavone reductase-like group of the plant reductase-epimerase-dehydrogenase (RED) supergene family, and is not present in *Arabidopsis thaliana*, which apparently produces a seed coat proanthocyanidin consisting entirely of 2,3-*cis*-(−)-epicatechin units (Abrahams et al., 2003).

Most models of proanthocyanidin biosynthesis show the extension units arising by condensation of an electrophile, derived from leucoanthocyanidin, with the nucleophilic 8 or 6 position of the starter unit. However, this model fails to account for the fact that the stereochemistry of leucoanthocyanidin is most likely 2,3-*trans*, whereas, in many cases, the extension units are 2,3-*cis* (Fig. 1, Tables 1 and 2). One possible solution to this stereochemical paradox was discovered through a genetics-based approach. It was shown that the *BANYULS* genes from *Arabidopsis thaliana* and the model legume *Medicago truncatula* encode anthocyanidin reductases, that convert cyanidin to 2,3-*cis*-(−)-epicatechin (**2**) (Xie et al., 2003). *BANYULS* was named after the color of a French red wine to describe the anthocyanin accumulation (and lack of proanthocyanidins) in the seed testa of *ban* mutants (Devic et al., 1999). Although *BAN* expression in *M. truncatula* is primarily limited to young seed coats, ectopic expression of a *Medicago* BAN transgene in tobacco leads to accumulation of proanthocyanidins throughout the pigmented portions of the petals, with concomitant reduction in anthocyanin levels (Xie et al., 2003). The final stages in the biosynthesis of (+)-catechin (**5**) and (−)-epicatechin (**2**) are therefore as illustrated in Fig. 2. The alteration in stereochemistry at C_2-C_3 is brought about through the intermediacy of an achiral intermediate (the anthocyanidin), rather than through the action of an epimerase as proposed earlier (Stafford, 1983). Consistent with this new model, mutations in anthocyanidin synthase (ANS, also known as leucoanthocyanidin dioxygenase) result in a deficiency in proanthocyanidin accumulation in *Arabidopsis* (Abrahams et al., 2003; Xie et al., 2003).

MtANR exhibits a substrate preference of cyanidin > pelargonidin > delphinidin, with typical Michaelis–Menten kinetics for each, to yield (−)-epicatechin (**2**), (−)-epiafzelechin (**1**), and (−)-epigallocatechin (**3**), respectively (Xie et al., 2004). In contrast, AtANR exhibits the reverse preference, with substrate inhibition at high concentrations of cyanidin and pelargonidin (Xie et al., 2004). Nevertheless, the kinetic data are consistent with the hypothesis that a single ANR enzyme could be responsible for the formation of epicatechin, epigallocatechin or epiafzelechin, and ANR is encoded by a single gene in *M. truncatula*.

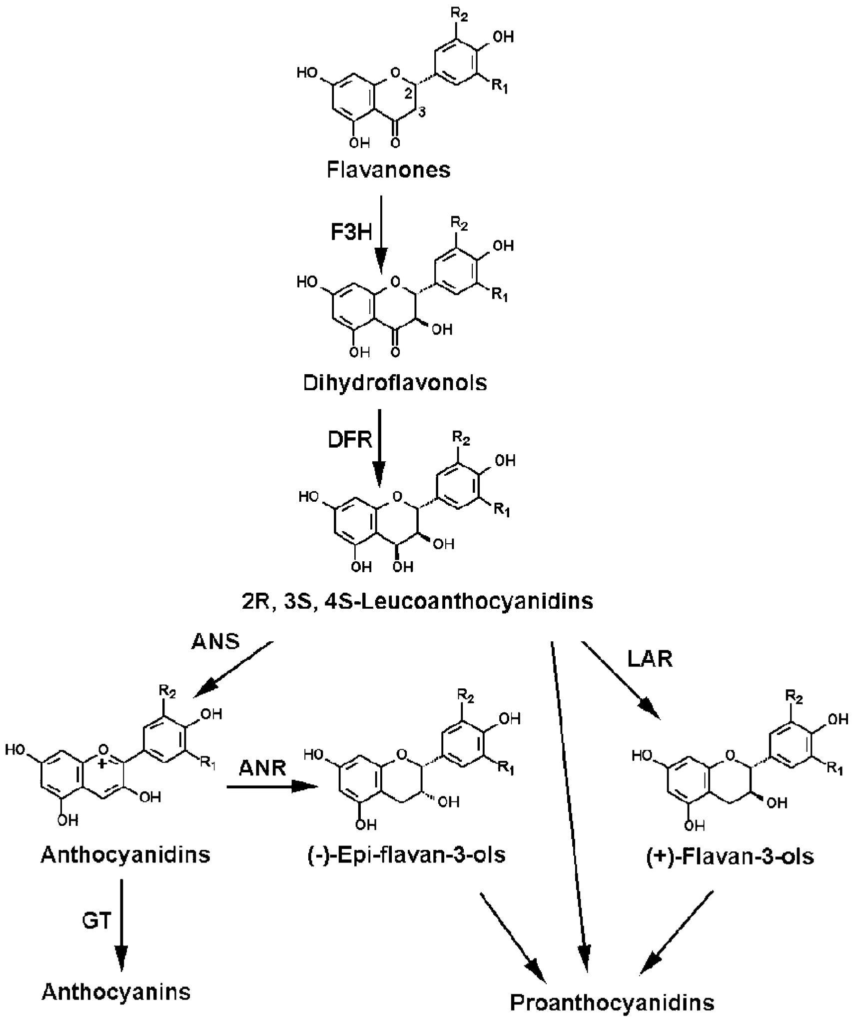


Fig. 2. Scheme of the enzymatic steps for biosynthesis of proanthocyanidins. F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol reductase; ANS, anthocyanidins synthase; ANR, anthocyanidin reductase; LAR, leucoanthocyanidin reductase; GT, anthocyanidin glycosyltransferase.

In addition to the clear genetic evidence of a role for ANR in proanthocyanidin bisoynthesis, biochemical correlations link both ANR and LAR enzymes to proanthocyanidin biosynthesis in vivo in several species. Thus, very high ANR activities are observed in extracts from *Desmodium* and *Lotus corniculatus* leaves, grape skins, and developing barley seeds, all sources rich in proanthocyanidins (Table 3). In contrast, alfalfa contains no proanthocyanidins in the leaves (Skadhauge et al., 1997), and no ANR or LAR activity is detectable (Table 3; (Skadhauge et al., 1997)). Likewise, LAR activity has been determined in barley, *Desmodium* leaves and the seeds of several legumes (Kristiansen, 1986; Skadhauge et al., 1997; Tanner et al., 2003). In a survey of developing tissues of five different legume species, there was an absolute correlation between the presence of extractable LAR activity and the accumulation of proanthocyanidins (Skadhauge et al., 1997).

Table 3
Anthocyanidin reductase extractable activity in various plant species

Species and tissue	ANR activity (pmol/min/mg protein)
Lotus corniculatus – leaf	721
Desmodium uncinatum – leaf	563
Flower	342
Pod	122
Grape – skin	143
Barley – grain	33
Alfalfa – leaf	0

The enzyme was assayed by measuring conversion of cyanidin to (−)-epicatechin.

5. Formation of proanthocyanidin oligomers and polymers

The debate concerning the operation of an enzymatic or non-enzymatic mechanism for proanthocyanidin condensation still continues. The problem is more fundamental than simply asking whether or not an enzyme is necessary, because, we contend, the nature of the substrate(s) for polymerization is still uncertain. Unambiguous resolution of this problem will require solid biochemical and genetic support for one or more enzymes involved in proanthocyanidin condensation.

(+)-Catechin (**5**) is often stated to be the most common starter unit in proanthocyanidins. However, in 58 structurally characterized procyanidin oligomers (including 44 trimers, 11 tetramers, 2 pentamers and 1 hexamer, consisting of only catechin and epicatechin), isolated from almond, *Areca catechu*, *Aesculus hippocastanum*, *Betula* spp., *Cinchona succirubra*, *Cinnamomum cassia*, *Crataegus oxyacantha*, *Craetaegus sinaica*, *Cryptomeria japonica*, *Davallia divaricata* rhizomes, *Disocorea cirrhosa*, *Fragaria* cv *annanasa*, *Guazuma ulmifolia*, *Hordeum vulgare*, *Humulus lupulus*, *Illicium anisatum*, *Kandelia candel*, *Lotus corniculatus*, *Nelia meyeri*, *Pinus radiate*, *Pinus taeda*, *Pseudotsuga menziesii*, *Rhaphiolepsis unbellata*, *Salix caprea*, *Salix* spp., *Thea sinensis* and *Theobroma cacao* (Porter, 1988, 1993), the ratio of proanthocyanidins with catechin as starter unit was only 45%. Among the 134 extension units in the above oligomers, epicatechin (**2**) accounted for 81%. Thus, 2,3-*cis*-(−)-epicatechin is the predominant extension unit among oligomeric proanthocyanidins, and also a common starter unit.

Quinone methides or carbocations derived from leucoanthocyanidin have been generally accepted as the precursors of the extension units of proanthocyanidins in vivo (Fig. 3), in spite of a lack of experimental proof. This model stems in large part from the demonstration of in vitro chemical condensation of either catechin (**5**) or epicatechin (**2**) with leucocyanidin derived from dihydroquercetin (Creasey and Swain, 1965). Leucocyanidin, leucopelargonidin or leucodelphinidin have not been isolated from plant tissues producing proanthocyanidins. Furthermore, this biogenetic sequence does not explain why the predominant extension units in proanthocyanidin oligomers or polymers are in the 2*R*,3*R*-2,3-*cis* configuration. The currently accepted pathway for leucoanthocyanidin formation results in a 2,3-*trans*-isomer (Fig. 2), but the stereochemistry of the in vivo "condensing" leucoanthocyanidin has not been demonstrated.

In spite of these problems, (−)-epicatechin (**2**) has not been viewed as a potential precursor of proanthocyanidins,

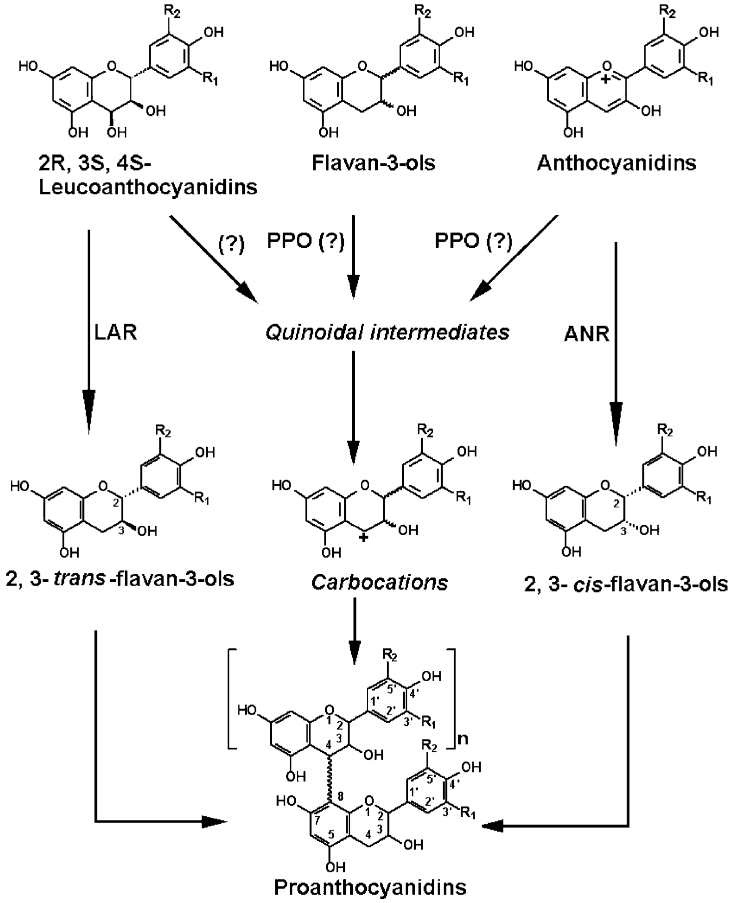


Fig. 3. Speculative scheme for the origin of carbocationic extension units for proanthocyanidin biosynthesis (see text for details). LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase, PPO, polyphenol oxidase. See Dixon et al. (2005) for further details and additional models.

other than as a starter unit. Crude enzyme preparations from barley, grape, *Desmodium uncinatum* and *Lotus corniculatus*, species that accumulate high levels of proanthocyanidins, all exhibit high ANR activity converting achiral anthocyanidins into their corresponding 2*R*,3*R*-2,3-*cis*-flavan-3-ols (Table 3). Ectopic expression of *Medicago* ANR in tobacco flowers results in accumulation of proanthocyanidin oligomers (Xie et al., 2003; Dixon et al., 2005). Epicatechin (**2**) and epigallocatechin (**3**) comprise 60% and 37%, respectively, of the extension units in *L. corniculatus* proanthocyanidins (Foo et al., 1996), and the major extension units of grape proanthocyanidins are epicatechin and epicatechin gallate (Souquet et al., 1996; Souquet et al., 2000). Although indirect, the above observations support a model in which ANR functions not only in provision of (−)-epicatechin as starter unit, but also provides the 2,3-*cis* monomers that become the extension units. By analogy, 2,3-*trans* units formed by LAR or an alternative enzyme could function as extension units in addition to being starter units, and a recent chemical study demonstrates the feasibility of producing dimeric and trimeric proanthocyanidins with controlled regio- and stereo-chemistry from (+)-catechin (Ohmori et al., 2004).

In one model of proanthocyanidin formation from a flavan-3-ol, epicatechin (**2**) or catechin (**5**) is converted to the corresponding quinone by polyphenol oxidase (PPO) (Fig. 3). The quinones can then be converted to carbocations via flav-3-en-ols, or reduced to carbocations through coupled non-enzymatic oxidation. Nucleophilic attack on the carbocations by epicatechin (**2**) or catechin (**5**) could produce dimers and then oligomeric proanthocyanidins linked through C_4–C_8 or C_4–C_6. In partial support of such a mechanism, formation of procyanidins B1 (**7**) and B2 (**8**) was observed when sorghum procyanidin polymers were added to white or red wines in the presence of catechin or epicatechin (Haslam, 1980). Epicatechin carbocations, released from polymeric procyanidins through the fission of interflavan bonds under mildly acidic conditions, were attacked by catechin (**5**) or epicatechin (**2**). Furthermore, tyrosinase has been shown to oxidize catechin to quinones accompanied by formation of procyanidin B3 (**9**) (Oszmianski and Lee, 1990). Further examples of PPO-catalyzed *o*-quinone formation from catechin (**5**) and epicatechin (**2**) have recently been reviewed (Dixon et al., 2005). PPO is generally believed to be localized to plastids. The PPO model for proanthocyanidin polymerization would therefore require the existence a novel form of PPO with alternative localization. Alternatively, plastidic PPO may be involved in proanthocyanidin polymerization following wounding or infection of plant tissues (Punyasiri et al., 2004), when cellular integrity may be destroyed.

Flav-3-en-3-ols have been suggested to be intermediates in the biogenesis of flavan-3-ol and anthocyanidin from leucoanthocyanidin (Haslam, 1977), and may also arise during the enzymatic conversion of anthocyanidins into flavan-3-ols by ANR (Xie et al., 2004). It is also possible to draw a scheme in which flav-3-en-3-ol is derived from catechin or epicatechin *o*-quinones by non-enzymatic reduction and then rearranged to form the corresponding carbocation (Dixon et al., 2005). Alternatively, the flav-3-en-3-ol involved in condensation might be the intermediate of the ANR reaction converting anthocyanidin into flavan-3-ol (Xie et al., 2004). Theoretically, flav-3-en-3-ol could be released from ANR, and then be converted to the flavan-4-yl carbocation, which can be attacked by epicatechin or catechin to form proanthocyanidins. However, no dimer or oligomer formation has been reported with ANR reactions in vitro. Direct involvement of ANR in proanthocyanidin condensation would suggest the need for localization of a significant proportion of the enzyme in the vacuole, or in (yet to be observed) vesicles involved in trafficking to the vacuole. The subcellular localization of ANR and LAR has yet to be determined. Both enzymes lack a targeting signal peptide.

Anthocyanins have also been considered as potential substrates for proanthocyanidin polymerization (Fig. 3). Condensation between anthocyanins and proanthocyanidin monomers (flavan-3-ols), oligomers or polymers is one of the main processes contributing to the changes in color and astringency as wines age (Haslam, 1980; Bishop and Charles, 1984). Under acidic conditions, anthocyanidins are mainly present as the corresponding flavylium ion, which may undergo PPO-catalyzed conversion to a quinone methide and then to a carbocation. For example, in the presence of the quinone of chlorogenic acid (CGA), PPO oxidizes anthocyanin into active quinoidal forms through a coupled oxidation with partial regeneration of CGA (Yokotsuka and Singleton, 1997; Kader et al., 1998). The nucleophilic epicatechin or catechin could theoretically attack the carbocation derivative to yield a proanthocyanidin (Fig. 3).

A-type dimeric or oligomeric proanthocyanidins can be formed in vitro by condensation between anthocyanin and epicatechin or catechin. For example, in aqueous 10 mM HCl, the bicyclic dimer of 3,5-*O*-diglucosyl-malvidin-catechin (3,5-*O*-diglucosyl-delphinidin-(4 → 8, 2 → *O*-7)-catechin) was formed through direct condensation of 3,5-*O*-diglucosyl-malvidin and catechin (**5**) (Bishop and Charles, 1984). The A-type prodelphinidin 3-*O*-glucosyl-delphinidin-(4 → 8, 2 → *O*-7)-epicatechin (bicyclic dimer of 3-*O*-glucosyl-malvidin-epicatechin) was identified from the incubation of malvidin 3-*O*-glucoside and epicatechin (**2**) in acidic ethanol (Remy-Tanneau et al., 2000; Remy-Tanneau et al., 2003). Similar types of condensation reactions have been proposed to account for the naturally occurring proanthocyanidins (Jacques et al., 1977). However, A-type proanthocyanidins can be formed from the B-proanthocyanidins in vitro. For example, treatment of proanthocyanidins B1 (**7**) or B2 (**8**) (Fig. 1) with 1,1-diphenyl-2-picrylhydrazyl radical resulted in formation of proanthocyanidins A1 (**11**) or A2 (**12**) (Kondo et al., 2000), suggesting that linkage to C_4 of the upper unit proceeds first as in the biosynthesis of B-type proanthocyanidins. Results of experiments on the oxidation of epicatechin in banana extracts suggest that A-type linkages may result from PPO mediated oxidation of the B-ring to a quinoidal structure, with subsequent addition of the C_7 hydroxyl to C_2 (Tanaka et al., 2000).

6. What we still do not know

In spite of the recent discovery of the enzymes and corresponding genes involved in conversion of leucoanthocyanidins into the 2,3-*cis* and 2,3-*trans* flavan-3-ols, many questions still remain concerning the biogenesis of proanthocyanidins. These include:

- What are the mechanisms for flux control at the interface between the anthocyanin and proanthocyanidin pathways? Do ANR and anthocyanidin glucosyltransferase compete in the cytosol for anthocyanidin substrate, or are the pathways of anthocyanin and proanthocyanidin regulated independently, e.g., by metabolic channeling through enzyme complexes, as suggested many years ago (Stafford, 1974).
- Is the product of the *LAR* genes described to date an enzyme whose activity is critical for proanthocyanidin biosynthesis? Although all the biochemical evidence to date points to this, genetic evidence is also required.
- Do LAR and ANR only produce monomeric flavan-3-ols as the "starter units" for proanthocyanidin biosynthesis, or are the extension units also synthesized via these enzymes? In other words, is the model in which the extension unit is formed from leucoanthocyanidin via a flav-3-en-3-ol or quinone methide intermediate still viable?
- If leucoanthocyanidin is the substrate for formation of the extension units, what is its stereochemistry? Can this intermediate be trapped in order to address this question?
- Does an ANR enzyme exist with the ability to generate 2,3-*trans*-flavan-3-ols from anthocyanidins?
- Where are LAR and ANR proteins localized within the cell? They do not appear to have targeting signal peptides and are presumable cytosolic, but this needs to be confirmed in view of the membrane localization of other operationally soluble plant enzymes (Liu and Dixon, 2001; Achnine et al., 2004)
- Are vesicles involved in proanthocyanidin transport and assembly?
- Is there a vacuolar-localized enzymatic system for proanthocyanidin polymerization? Does this involve a polyphenoloxidase-type enzyme?
- Is proanthocyanidin polymerization non-enzymatic, requiring simply a provision of substrate and correct physical conditions (e.g., vacuolar pH).
- Is proanthocyanidin composition in those species that accumulate a wide range of proanthocyanidins controlled primarily by monomer availability? This could be tested in the future by genetic modification of in vivo monomer availability.
- How "promiscuous" is the polymerization process? Is assembly of oligomers and polymers containing "non-traditional" units a strictly controlled process, or the result of a polymerization mechanism that is somewhat "forgiving" as to the nature of the monomer. This concept has been a matter of major controversy as regards lignin biosynthesis (Lewis, 1999; Sederoff et al., 1999). Interestingly, Stafford drew attention to several (different) analogies between lignin and proanthocyanidins in her 1988 review (Stafford, 1988).
- How is the stereochemistry of proanthocyanidin linkage controlled?

Several of these questions have been asked since the early days of proanthocyanidin research. Their solution will require the concerted application of chemical, biochemical, and genetic studies.

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References

Abe, I., Seki, T., Noguchi, H., Kashiwada, Y., 2000. Galloyl esters from rhubarb are potent inhibitors of squalene epoxidase, a key enzyme in cholesterol biosynthesis. Planta Medica 66, 753–756.

Abrahams, S., Lee, E., Walker, A.R., Tanner, G.J., Larkin, P., Ashton, A.R., 2003. The *Arabidopsis TDS4* gene encodes leucoanthocyanidin dioxygenase (LDOX) and is essential for proanthocyanidin synthesis and vacuole development. Plant Journal 35, 624–636.

Achenbach, H., Benirschke, G., 1997. Joannesialactone and other compounds from *Joannesia princeps*. Phytochemistry 45, 149–157.

Achnine, L., Blancaflor, E.B., Rasmussen, S., Dixon, R.A., 2004. Co-localization of L-phenylalanine ammonia-lyase and cinnamate 4-hydroxylase for metabolic channeling in phenylpropanoid biosynthesis. Plant Cell 16, 3098–3109.

Aerts, R.J., Barry, T.N., McNabb, W.C., 1999a. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agriculture Ecosystems and Environment 75, 1–12.

Aerts, R.J., McNabb, W.C., Molan, A., Brand, A., Barry, T.N., Peters, J.S., 1999b. Condensed tannins from *Lotus corniculatus* and *Lotus pedunculatus* exert different effects on the in vitro rumen degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein. Journal of the Science of Food and Agriculture 79, 79–85.

Balde, A.M., Pieters, L.A., Gergely, A., Kolodziej, H., Claeys, M., Vlietinck, A., 1991a. A-type proanthocyanidins from stem-bark of *Pavetta owariensis*. Phytochemistry 30, 337–342.

Balde, A.M., Pieters, L.A., Wary, V., Kolodziej, H., Vanden-Berghe, D., Claeys, M., Vlietinck, A., 1991b. Dimeric and trimeric proanthocyanidins possessing a doubly linked structure from *Pavetta owariensis*. Phytochemistry 30, 4129–4135.

Balde, A.M., de-Bruyne, T., Pieters, L., Kolodziej, H., Vanden-Berghe, D., Claeys, M., Vlietinck, A., 1995a. Oligomeric proanthocyanidins possessing a doubly linked structure from *Pavetta owariensis*. Phytochemistry 38, 719–723.

Balde, A.M., de-Bruyne, T., Pieters, L., Claeys, M., Vanden-Berghe, D., Vlietinck, A., Wary, V., Kolodziej, H., 1993. Proanthocyanidins from stem bark of *Pavetta owariensis*: 3. NMR study of acetylated trimeric proanthocyanidins possessing a doubly-linked structure. Journal of Natural Products 56, 1078–1088.

Balde, A.M., de-Bruyne, T., Pieters, L., Kolodziej, H., Vanden-Berghe, D., Claeys, M., Vlietinck, A., 1995b. Tetrameric proanthocyanidins containing a double interflavanoid (A-type) linkage from *Pavetta owariensis*. Phytochemistry 40, 933–938.

Barreiros, A.L., David, J.P., de Queiroz, L.P., David, J.M., 2000. A-type proanthocyanidin antioxidant from *Dioclea lasio-hylla*. Phytochemistry 55, 805–808.

Beld, M., Martin, C., Huits, H., Stuitje, A.R., Gerats, A.G.M., 1989. Flavonoid synthesis in *Petunia hybrida*: partial characterization of dihydroflavonol-4- reductase genes. Plant Molecular Biology 13, 491–502.

Bennie, L., Malan, E., Coetzee, J., Ferreira, D., 2000. Structure and synthesis of ether-linked proteracacinidin and promelacacinidin proanthocyanidins from *Acacia caffra*. Phytochemistry 53, 785–793.

Bennie, L., Coetzee, J., Malan, E., Ferreira, D., 2002. (4–6)-Coupled proteracacinidins and promelacacinidins from *Acacia galpinii* and *Acacia caffra*. Phytochemistry 60, 521–532.

Bennie, L., Coetzee, J., Malan, E., Slade, D., Marais, J.P.J., Ferreira, D., 2004. Trimeric proteracacinidin and a (6–6)-bis-leucoteracacinidin from *Acacia galpinii* and *Acacia caffra*. Phytochemistry 65, 215–220.

Bilia, A.R., Morelli, I., Hamburger, M., Hostettmann, K., 1996. Flavans and A-type proanthocyanidins from *Prunus prostrata*. Phytochemistry 43, 887–892.

Bishop, P.D., Charles, C.W., 1984. Characterization of condensation product of malvidin 3,5-diglucoside and catechin. Journal of Agricultural and Food Chemistry 32, 1022–1026.

Calzada, F., Cedillo-Rivera, R., Bye, R., Mata, R., 2001. Geranins C and D, additional new antiprotozoal A-type proanthocyanidins from *Geranium niveum*. Planta Medica 67, 677–680.

Calzada, F., Cerda-Garcia-Rojas, C.M., Meckes, M., Cedillo-Rivera, R., Bye, R., Mata, R., 1999. Geranins A and B, new antiprotozoal A-type proanthocyanidins from *Geranium niveum*. Journal of Natural Products 62, 705–709.

Charrier, B., Coronado, C., Kondorosi, A., Ratet, P., 1995. Molecular characterization and expression of alfalfa (*Medicago sativa* L.) flavanone-3-hydroxylase and dihydroflavonol-4-reductase encoding genes. Plant Molecular Biology 29, 773–786.

Coetzee, J., Malan, E., Ferreira, D., 1998a. Oligomeric flavanoids. Part XXVIII: Structure and synthesis of ether-linked [4-*O*-3]-bis-teracacinidins, a novel class of naturally occurring proanthocyanidins. Journal of Chemical Research (S) 526–527, (M) 2287–2296.

Coetzee, J., Malan, E., Ferreira, D., 1998b. Oligomeric flavanoids. Part XXIX: Structure and synthesis of novel ether-linked [4-*O*-4] bis-teracacinidins. Tetrahedron 54, 9153–9160.

Coetzee, J., Mciteka, L., Malan, E., Ferreira, D., 1999. Structure and synthesis of butiniflavan-epicatechin and -epigallocatechin probutinidins. Phytochemistry 52, 737–743.

Creasey, L.L., Swain, T., 1965. Structure of condensed tannins. Nature 208, 151–153.

Danne, A., Petereit, F., Nahrstedt, A., 1993. Proanthocyanidins from *Cistus incanus*. Phytochemistry 34, 1129–1133.

de Pascual, T.S., Gutierrez, F.Y., Rivas, G.J.C., Santos, B.C., 1998. Characterization of monomeric and oligomeric flavan-3-ols from unripe almond fruits. Phytochemical Analysis 9, 21–27.

de-Mello, J.P., Petereit, F., Nahrstedt, A., 1996a. Flavan-3-ols and prodelphinidins from *Stryphnodendron adstringens*. Phytochemistry 41, 807–813.

de-Mello, J.P., Petereit, F., Nahrstedt, A., 1996b. Prorobinetinidins from *Stryphnodendron adstringens*. Phytochemistry 42, 857–862.

Devic, M., Guilleminot, J., Debeaujon, I., Bechtold, N., Bensaude, E., Koornneef, M., Pelletier, G., Delseny, M., 1999. The *BANYULS* gene encodes a DFR-like protein and is a marker of early seed coat development. Plant Journal 19, 387–398.

Dixon, R.A., Sharma, S.B., Xie, D., 2005. Proanthocyanidins- a final frontier in flavonoid research?. New Phytologist 165, 9–28.

Drewes, S.E., Taylor, C.W., 1994. Methylated A-type proanthocyanidins and related metabolites from *Cassipourea gummiflua*. Phytochemistry 37, 551–555.

Ferreira, D., Slade, D., 2002. Oligomeric proanthocyanidins: naturally occuring *O* heterocycles. Natural Product Reports 19, 517–541.

Ferreira, D., Nel, R.J.J., Bekker, R., 1999. Condensed tannins. In: Barton, Sir D.H.R., Nakanishi, K., Meth-Cohn, O., Pinto, B.M. (Eds.), Comprehensive Natural Products Chemistry. Elsevier, Kidlington, Oxford, pp. 791–797.

Ferreira, D., Marais, J.P.J., Slade, D., 2003. Phytochemistry of the mopane, *Colophospermum mopane*. Phytochemistry 64, 31–51.

Foo, L.Y., Lu, Y., Howell, A.B., Vorsa, N., 2000a. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* in vitro. Phytochemistry 54, 173–181.

Foo, L.Y., Lu, Y., Howell, A.B., Vorsa, N., 2000b. A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. Journal of Natural Products 63, 1225–1228.

Foo, L.Y., Newman, R., Waghorn, G., McNabb, W.C., Ulyatt, M.J., 1996. Proanthocyanidins from *Lotus corniculatus*. Phytochemistry 41, 617–624.

Foo, L.Y., Lu, Y., McNabb, W.C., Waghorn, G., Ulyatt, M.J., 1997. Proanthocyanidins from *Lotus pedunculatus*. Phytochemistry 45, 1689–1696.

Forrest, G.I., Bendall, D.S., 1969. The distribution of polyphenols in the tea plant (*Camellia sinensis* L.). Biochemical Journal 113, 741–755.

Geiss, F., Heinrich, M., Hunkler, D., Rimpler, H., 1995. Proanthocyanidins with (+)-epicatechin units from *Byrsonima crassifolia* bark. Phytochemistry 39, 635–643.

Gonzalez, A.G., Irizar, A.C., Ravelo, A.G., Fernandez, M.F., 1992. Type A proanthocyanidins from *Prunus spinosa*. Phytochemistry 31, 1432–1434.

Hartisch, C., Kolodziej, H., 1996. Galloylhamameloses and proanthocyanidins from *Hamamelis virginiana*. Phytochemistry 42, 191–198.

Haslam, E., 1977. Symmetry and promiscuity in procyanidin biochemistry. Phytochemistry 16, 1625–1640.

Haslam, E., 1980. In vino veritas: oligomeric procyanidin and the aging of red wines. Phytochemistry 19, 2577–2582.

Hatano, T., Miyatake, H., Natsume, M., Osakabe, N., Takizawa, T., Ito, H., Yoshida, T., 2002. Proanthocyanidin glycosides and related polyphenols from cacao liquor and their antioxidant effects. Phytochemistry 59, 749–758.

Hatano, T., Yamashita, A., Hashimoto, T., Ito, H., Kubo, N., Yoshiyama, M., Shimura, S., Itoh, Y., Okuda, T., Yoshida, T., 1997. Flavan dimers with lipase inhibitory activity from *Cassia nomame*. Phytochemistry 46, 893–900.

Helsper, J.P.F.G., Kolodziej, H., Hoogendijk, J.M., Van-Norel, A., 1993. Characterization and trypsin inhibitor activity of proanthocyanidins from *Vicia faba*. Phytochemistry 34, 1255–1260.

Hör, M., Heinrich, M., Rimpler, H., 1996. Proanthocyanidin polymers with antisecretory activity and proanthocyanidin oligomers from *Guazuma ulmifolia* bark. Phytochemistry 42, 109–119.

Jacques, D., Opie, C.T., Porter, L.J., Haslam, E., 1977. Plant proanthocyanidins. Part 4. Biosynthesis of procyanidins and observations on the metabolism of cyanidin in plants. Journal of the Chemical Society, Perkin Transactions 1, 1637–1643.

Kader, F., Haluk, J.P., Nicolas, J.P., Metche, M., 1998. Degradation of cyanidin 3-glucoside by blueberry polyphenol oxidase: kinetic studied and mechanism. Journal of Agricultural and Food Chemistry 46, 3060–3065.

Kaltenbach, M., Schroder, G., Schmelzer, E., Lutz, V., Schroder, J., 1999. Flavonoid hydroxylase from *Catharanthus roseus*: cDNA, heterologous expression, enzyme properties and cell-type specific expression in plants. Plant Journal 19, 183–193.

Kamiya, K., Watanabe, C., Endang, H., Umar, M., Satake, T., 2001. Studies on the constituents of bark of *Parameria laevigata* Moldenke. Chemical and Pharmaceutical Bulletin 49, 551–557.

Kandil, F.E., Song, L., Pezzuto, J.M., Marley, K., Seigler, D.S., Smith, M.A.L., 2000. Isolation of oligomeric proanthocyanidins from flavonoid-producing cell cultures. In Vitro Cell and Developmental Biology – Plant 36, 492–500.

Kayser, O., Abreu, P.M., 2001. Antileishmania and immunostimulating activities of two dimeric proanthocyanidins from *Khaya senegalensis*. Pharmaceutical Biology 39, 284–288.

Kondo, K., Kurihawa, M., Fukuhara, K., Tanaka, T., Suzuki, T., Miyata, N., Toyoda, M., 2000. Conversion of procyanidin B-type (catechin dimer) to A-type: evidence for abstraction of C-2 hydrogen in catechin during radical oxidation. Tetrahedron Letters 41, 485–488.

Kristiansen, K.N., 1986. Conversion of (+)-dihydroquercetin to (+)-2,3-*trans*-3,4-*cis*-leucocyanidin and (+)-catechin with an enzyme extract from maturing grains of barley. Carlsberg Research Communications 51, 51–60.

Lakenbrink, C., Engelhardt, U.H., Wray, V., 1999. Identification of two novel proanthocyanidins in green tea. Journal of Agricultural and Food Chemistry 47, 4621–4624.

Lewis, N.G., 1999. A 20th century roller coaster ride: a short account of lignification. Current Opinion in Plant Biology 2, 153–162.

Liu, C.-J., Dixon, R.A., 2001. Elicitor-induced association of isoflavone *O*-methyltransferase with endomembranes prevents formation and 7-*O*-methylation of daidzein during isoflavonoid phytoalexin biosynthesis. Plant Cell 13, 2643–2658.

Lin, L.-C., Kuo, Y.-C., Chou, C.-J., 2002. Immunomodulatory proanthocyanidins from *Ecdysanthera utilis*. Journal of Natural Products 65, 505–508.

Lou, H.X., Yamazaki, Y., Sasaki, T., Uchida, M., Tanaka, H., Oka, S., 1999. A-type proanthocyanidins from peanut skins. Phytochemistry 51, 297–308.

Malan, E., Sireeparsad, A., Swinny, E., Ferreira, D., 1997. The structure and synthesis of a 7,8,4′-trihydroxyflavan–epioritin dimer from *Acacia caffra*. Phytochemistry 44, 529–531.

Marles, M.A.S., Ray, H., Gruber, M.Y., 2003. New perspectives on proanthocyanidin biochemistry and molecular regulation. Phytochemistry 64, 357–383.

Mayer, W., Goll, L., Arndt, E.M., Mannschreck, A., 1966. Procyanidino-(−)-epicatechol, a condensed proanthocyanidin of *Aesculus hippocastanum*. Tetrahedron Letters 4, 429–435.

Menting, J.G.T., Scopes, R.K., Stevenson, T.W., 1994. Characterization of flavonoid 3′,5′-hydroxylase in microsomal membrane fraction of *Petunia hybrida* flowers. Plant Physiology 106, 633–642.

Molan, A.L., Waghorn, G.C., Min, B.R., McNabb, W.C., 2000. The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration in vitro. Folia Parasitologica Ceske Budejovice 47, 39–44.

Natsume, M., Osakabe, N., Yamagishi, M., Takizawa, T., Nakamura, T., Miyatake, H., Hatano, T., Yoshida, T., 2000. Analyses of polyphenols in cacao liquor, cocoa, and chocolate by normal-phase and reversed-phase HPLC. Bioscience, Biotechnology and Biochemistry 64, 2581–2587.

Ohmori, K., Ushimaru, N., Suzuki, K., 2004. Oligomeric catechins: an enabling synthetic strategy by orthogonal activation and C(8) protection. Proceedings of the National Academy of Sciences USA 101, 12002–12007.

Oszmianski, J., Lee, C.Y., 1990. Enzymatic oxidation reaction of catechin and chlorogenic acid in a model system. Journal of Agricultural and Food Chemistry 38, 1202–1204.

Porter, L.J., 1988. Flavans and Proanthocyanidins. In: Harborne, J.B. (Ed.), The Flavonoids. Chapman & Hall, London, pp. 21–62.

Porter, L.J., 1989. Tannins. Methods in Plant Biochemistry 1, 389–419.

Porter, L.J., 1993. Flavans and proanthocyanidins. In: Harborne, J.B. (Ed.), The Flavonoids: Advances in Research Since 1986. Chapman & Hall, London, pp. 23–55.

Prasad, D., 2000. A-type proanthocyanidins from *Prunus armeniaca* roots. Fitoterapia 71, 245–253.

Punyasiri, P.A.N., Tanner, G., Abeysinghe, I.S.B., Kumar, V., Campbell, P.M., Pradeepa, N.H.L., 2004. *Exobasidium vexans* infection of leaves of *Camellia sinensis* increased 2,3-*cis* isomerisation and gallate esterification of proanthocyanidins. Phytochemistry 65, 2987–2994.

Rakotoarison, D.A., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., Vasseur, J., Cazin, M., Cazin, J.C., Pinkas, M., 1997. Antioxidant activities of polyphenolic extracts from flowers, in vitro callus and cell suspension cultures of *Crataegus monogyna*. Pharmazie 52, 60–64.

Rawat, M.S.M., Prasad, D., Joshi, R.K., Pant, G., 1999. Proanthocyanidins from *Prunus armeniaca* roots. Phytochemistry 50, 321–324.

Remy-Tanneau, S., le Guerneve, C., Meudec, E., Cheynier, V., 2003. Characterization of colorless anthocyanin-flavan-3-ol dimer containing both carbon–carbon and ether interflavanoid linkages by NMR and mass spectrometry. Journal of Agricultural and Food Chemistry 51, 3592–3597.

Remy-Tanneau, S., Fulcrand, H., Labarbe, B., Cheynier, V., Moutounet, M., 2000. First confirmation in red wine of products resulting from direct anthocyanintannin reactions. Journal of the Science of Food and Agriculture 80, 745–751.

Saito, K., Yamazaki, M., 2002. Tansley Review no. 138. Biochemistry and molecular biology of the late-stage of biosynthesis of anthocyanin: lessons from *Perilla frutescens* as a model plant. New Phytologist 155, 9–23.

Sato, M., Islam, S.Q., Awata, S., Yamasaki, T., 1999. Flavanonol glucoside and proanthocyanidins: oviposition stimulants for the cerambycid beetle, *Monochamus alternatus*. Journal of Pesticide Science 24, 123–129.

Schoenbohm, C., Martens, S., Eder, C., Forkmann, G., Weisshaar, B., 2000. Identification of the *Arabidopsis thaliana* flavonoid 3′-hydroxylase gene and functional expression of the encoded P450 enzyme. Biological Chemistry 381, 749–753.

Sederoff, R.R., MacKay, J.J., Ralph, J., Hatfield, R.D., 1999. Unexpected variation in lignin. Current Opinion in Plant Biology 2, 145–152.

Shahat, A.A., Hammouda, F., Ismail, S.I., Azzam, S.A., De-Bruyne, T., Lasure, A., Van-Poel, B., Pieters, L., Vlietinck, A.J., 1996. Anti-complementary activity of *Crataegus sinaica*. Planta 62, 10–13.

Shirley, B.W., 1996. Flavonoid biosynthesis: 'new' functions for an 'old' pathway. Trends in Plant Science 1, 377–382.

Skadhauge, B., Gruber, M.Y., Thomsen, K.K., von Wettstein, D., 1997. Leucocyanidin reductase activity and accumulation of proanthocyanidins in developing legume tissues. American Journal of Botany 84, 494–502.

Souquet, J.M., Cheynier, V., Brossaud, F., Moutounet, M., 1996. Polymeric proanthocyanidin from grape skins. Phytochemistry 43, 509–512.

Souquet, J.M., Labarbe, B., Le-Guerneve, C., Cheynier, V., Moutounet, M., 2000. Phenolic composition of grape stems. Journal of Agricultural and Food Chemistry 48, 1076–1080.

Stafford, H.A., 1974. Possible multienzyme complexes regulating the formation of C_6–C_3 phenolic compounds and lignins in higher plants. Recent Advances in Phytochemistry 8, 53–79.

Stafford, H.A., 1983. Enzymic regulation of procyanidin biosynthesis: lack of a flav-3-en-ol intermediate. Phytochemistry 22, 2643–2646.

Stafford, H.A., 1988. Proanthocyanidins and the lignin connection. Phytochemistry 27, 1–6.

Stafford, H.A., Lester, H.H., 1984. Flavan-3-ol biosynthesis. The conversion of (+)-dihydroquercetin and flavan-3,4-*cis*-diol (leucocyanidin) to (+)-catechin by reductases extracted from cell suspension cultures of Douglas fir. Plant Physiology 76, 184–186.

Stafford, H.A., Lester, H.H., 1985. Flavan-3-ol biosynthesis. The conversion of (+)-dihydromyrecetin to its flavan-3,4-diol (leucodelphinidin) and to (+)-gallocatechin by reductases extracted from tissue cultures of *Ginko biloba* and *Pseudotsuga menziesii*. Plant Physiology 78, 791–794.

Stevens, J.F., Miranda, C.L., Wolthers, K.R., Schimerlik, M., Deinzer, M.L., Buhler, D.R., 2002. Identification and in vitro biological activities of hop proanthocyanidins: inhibition of nNOS activity and scavenging of reactive nitrogen species. Journal of Agricultural and Food Chemistry 50, 3435–3443.

Stobiecki, M., Popenda, M., 1994. Flavan-3-ols from seeds of *Lupinus angustifolius*. Phytochemistry 37, 1707–1711.

Tanaka, T., Kondou, K., Kouno, I., 2000. Oxidation and epimerization of epigallocatechin in banana fruits. Phytochemistry 53, 311–316.

Tanaka, Y., Fukui, Y., Fukuchi-Mizutani, M., Holton, T.A., Higgins, E., Kusumi, T., 1995. Molecular cloning and characterization of *Rosa hybrida* dihydroflavonol 4-reductase gene. Plant and Cell Physiology 36, 1023–1031.

Tanner, G.J., Kristiansen, K.N., 1993. Synthesis of 3,4-*cis*-[3H]-leucocyanidin and enzymatic reduction to catechin. Analytical Biochemistry 209, 274–277.

Tanner, G.J., Francki, K.T., Abrahams, S., Watson, J.M., Larkin, P.J., Ashton, A.R., 2003. Proanthocyanidin biosynthesis in plants. Purification of legume leucoanthocyanidin reductase and molecular cloning of its cDNA. Journal of Biological Chemistry 278, 31647–31656.

Wang, Y., Douglas, G.B., Waghorn, G.C., Narry, T.N., Foote, A.G., Purchas, R.W., 1996. Effect of condensed tannins upon the performance of lambs grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). Journal of Agricultural Science 126, 87–98.

Weinges, K., Schick, H., 1995. Dodecaacetylprodelphinidin B3 from the dried leaves of *Ziziphus spina-christi*. Phytochemistry 38, 505–507.

Winkel-Shirley, B., 2001a. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiology 126, 485–493.

Winkel-Shirley, B., 2001b. It takes a garden. How work on diverse plant species has contributed to an understanding of flavonoid metabolism. Plant Physiology 127, 1399–1404.

Xie, D., Sharma, S.R., Paiva, N.L., Ferreira, D., Dixon, R.A., 2003. Role of anthocyanidin reductase, encoded by *BANYULS* in plant flavonoid biosynthesis. Science 299, 396–399.

Xie, D.-Y., Sharma, S.B., Dixon, R.A., 2004. Anthocyanidin reductases from *Medicago truncatula* and *Arabidopsis thaliana*. Archives of Biochemistry and Biophysics 422, 91–102.

Yokotsuka, K., Singleton, V.L., 1997. Disappearance of anthocyanins as grape juice is prepared and oxidized with PPO and PPO substrates. American Journal of Enology and Viticulture 48, 13–25.