Oligomeric proanthocyanidins: naturally occurring O-heterocycles

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This review covers the flavan-3-ols (catechins), flavan-4-ols/flavan-3,4-diols (leucoanthocyanidins), A-type proanthocyanidins, B-type proanthocyanidins including the procyanidins, prodelphinidins, propelargonidins, proteracacinidins, promelacacinidins, procassinidins, probutinidins, and non-proanthocyanidins with flavan-3-ol constituent units. Newly isolated proanthocyanidins, structure elucidation, syntheses, HPLC/MS analysis, NMR/conformational analysis, and the effects of proanthocyanidins on human nutrition and health are reported. The literature from January 1999 to December 2001 is reviewed, and 130 references are cited.

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Daneel Ferreira graduated from the University of Pretoria, South Africa in 1964. He completed the BSc (Hons.) and MSc programmes of the Chemistry Department, University of the Orange Free State, Bloemfontein, South Africa through part time studies. In 1969 he was appointed as Technical Assistant in the Chemistry Department at UOFS, obtained the DSc degree in Organic Chemistry in 1973 and progressed to the ranks of Professor of Organic Chemistry in 1985. He spent 1977 as a Visiting Lecturer at Imperial College, London where he worked under the supervision of the late Sir Derek Barton. His main area of research is in the study of the chemistry of flavonoids and proanthocyanidins where he focuses on structure elucidation (up to the tetraflavanoid level) via physical methods, especially NMR and CD, semi-synthesis of oligomers, stereoselective syntheses of monomeric precursors, and the development of general methodologies to manipulate the molecular backbone of the C6-C3-C6 unit. He was invited to establish a Research Unit for Polyphenol- and Synthetic Chemistry at UOFS by the Foundation for Research Development, Pretoria and was duly appointed as Director in 1990. He held this position until 1998 before joining the Thad Cochran National Center for Natural Products Research, University of Mississippi in 1999 as Visiting Scholar. He is currently a Principal Scientist in the Center where he continues with the endeavours into the Chemistry of Natural Products.

Desmond Slade graduated from the University of Stellenbosch, South Africa in 2000, where he obtained his PhD (Chemistry) on the chemical characterization of the interdigital secretion of the black wildebeest under the supervision of Professor Ben V. Burger. He started as a Postdoctoral Research Associate at the National Center for Natural Products Research, University of Mississippi at the end of 2000, working on the synthesis of antimalarial 8-aminoquinolines, under the supervision of Dr Daneel Ferreira.
1 Introduction

The oligomeric and polymeric proanthocyanidins (syn. condensed tannins) constitute one of the most ubiquitous groups of all plant phenolics.1,4 Leucoanthocyanidins are monomeric compounds which produce anthocyanidins 1 by cleavage of a C-O bond on heating with mineral acid. Proanthocyanidins are oligomers/polymer which give anthocyanidins by cleavage of a C-C bond under strongly acidic conditions in the presence of molecular oxygen. Together with the bi- and tri-flavonoids they represent the two major classes of complex C6C3C6 secondary metabolites. The bi- and tri-flavonoids are products of oxidative coupling of flavones, flavonols, dihydroflavonols, flavanones, isoflavones, aurones, chalcones, and 2-benzylbenzofuranones,5,6 and thus consistently possess a carbonyl group at C-4 or its equivalent in every constituent flavanyl unit. The proanthocyanidins, on the contrary, usually originate by coupling at C(4) (C-ring) of an electrophilic group at C-4 or its equivalent in every constituent flavanyl unit. The oligomeric and polymeric proanthocyanidins (syn. condensed tannins) constitute one of the most ubiquitous groups of all plant phenolics.1,4 Leucoanthocyanidins are monomeric compounds which produce anthocyanidins 1 by cleavage of a C-O bond on heating with mineral acid. Proanthocyanidins are oligomers/polymer which give anthocyanidins by cleavage of a C-C bond under strongly acidic conditions in the presence of molecular oxygen. Together with the bi- and tri-flavonoids they represent the two major classes of complex C6C3C6 secondary metabolites. The bi- and tri-flavonoids are products of oxidative coupling of flavones, flavonols, dihydroflavonols, flavanones, isoflavones, aurones, chalcones, and 2-benzylbenzofuranones,5,6 and thus consistently possess a carbonyl group at C-4 or its equivalent in every constituent flavanyl unit. The proanthocyanidins, on the contrary, usually originate by coupling at C(4) (C-ring) of an electrophilic flavanyl unit, presumably generated from a flavan-3,4-diol4 or a flavan-4-ol2 most commonly to C(8) or C(6) (A-ring) of a nucleophilic flavanyl unit, e.g. a flavan-3-ol. Compounds possessing at least one flavan or flavan-3-ol constituent unit constitute the subject of this report. The nomenclature system proposed by Hemingway8 and extended by Porter1 is applied consistently. 

The proanthocyanidins have recently attracted a considerable amount of attention in the fields of nutrition, health and medicine. This is the result of a rapidly growing body of evidence suggesting that the proanthocyanidins may act as potent antioxidants and/or modulate key biological pathways in vivo in mammals.11

2 Flavan-3-ols and flavan-3,4-diols/flavan-4-ols

Owing to the presumed key role of the flavan-3-ols (catechins) as nucleophilic chain-terminating units of flavan-3,4-diols/flavan-4-ols (leucoanthocyanidins) as electrophilic chain-extender units in proanthocyanidin biosynthesis,5 these three classes of compounds are also discussed.

2.1 Flavan-3-ols

One flavan-3-ol with a new hydroxylation pattern, (+)-3′,4′,5,6,7,8-hexahydroxyflavan-3-ol 2 (elephantorrhizhol) was identified in Elephantorrhiza goetzei.12 Its absolute configuration was assumed to be 2R,3S. A number of new flavan-3-ol derivatives were also reported. These are the fisetinidol-3-O-β-D-xylopyranoside, anadanthoside 3 from the bark of Anadenanthera macrocarpa,13 (2R,3S)-guibourtinidol 4, isolated for the first time from a natural source (Cassia abbreviata),14 its 7-O-methyl derivative 5 from Crinum bulbispermum Milne,15 epicatechin-5-O-β-D-glucosyl-3-benzoate 6 from Celastrols orbiculatus,16 and 3-acetyl-5-methoxy-7,3′,4′-trihydroxy-8-O-glucoside-flavan-3-ol, barbatoflavan 7 from Campanula barbata.17

It should be emphasized that the absolute configurations of the C(2) and C(3) stereocentres were not assessed for compounds 3, 5, and 7. This may conveniently be done by circular dichroism. The CD curves of flavan-3-ols exhibit two Cotton effects for the 1′La and 1′Lb transitions in the 240 and 280 nm regions, respectively. Analogue with 2R and 2S absolute configurations gave negative and positive Cotton effects, respectively in the 280 nm region. The sign of the Cotton effect of the 1′La transition at ca. 240 nm is consistently opposite to that at longer wavelength.

The group of naturally occurring flavan-3-ols with an additional C6C3 unit linked to the A-ring was extended by identification of four new analogues, apocynins A–D 8–11 from the leaves of Apocynum venetum.18 Their structures were determined by spectral analyses and the absolute configuration at C-7′ was established via the Cotton effects near 235 nm in their CD spectra. These compounds, which are based on galloatech (8, 9 and 11) and epigallocatechin 10, exhibited hepatoprotective activity against α-galactosamine (α-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes. A considerable number of papers dealing with the synthesis or chemical conversions of flavan-3-ols have been published. Among these are the development of a synthetic protocol towards the four diastereoisomers of flavan-3-ols with the typical hydroxylation patterns of naturally occurring analogues.14,19

This was achieved by selecting an acid-sensitive protecting
group for the phenolic functionalities in the previously developed protocol. The method is based on the asymmetric dihydroxylation of 1,3-diarylpropenes and subsequent acid-catalyzed cyclization to give the flavan-3-ol diastereoisomers in high yield and in essentially enantiopure form (see ref. 1 for a summary).

Two important papers focusing on the antioxidant chemistry of the green tea catechins (Epigallocatechin gallate (EGCG) and Epigallocatechin (EGC) were published. The identification of oxidation products formed by reactions of these flavan-3-ols with biologically relevant oxidants could provide information regarding the specific mechanisms of antioxidant reactions. Separate treatment of EGCG and EGC with peroxyl radicals generated by thermolysis of the initiator 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) in oxygenated acetonitrile gave the oxidation products 14–19 indicated in Scheme 1. The formation of products 14 and 15 was explained via the mechanism shown in Scheme 2. Thus, initial one-electron oxidation of EGCG/EGC by the peroxyl radical generates the phenoxyl radical of type 20 which is susceptible to reaction with a second peroxyl radical. The unstable AMVN adduct 21 is then susceptible to oxygen “insertion” leading to compounds 14 and 15 with their enlarged B-rings. For the formation of compounds 18 and 19 the phenoxyl radical 22 reacts with a second EGC/EGC molecule to form the dimeric radical 23 (Scheme 3). This is trapped by a second peroxyl radical to form the unstable adduct 24 which is susceptible to rearrangement via heterolytic cleavage of the peroxide bond. The formation of compound 17 was explained by a mechanism slightly different from the one depicted in Scheme 3 (see ref. 24). These results also settled the controversy regarding the oxidation site of EGCG since it unambiguously indicated that the principal oxidation site is the pyrogallol-type B-ring and not the same functionality of the 3-O-galloyl moiety. (However, it should be noted that the stereochemistry of the A,C-units in compounds 17 and 18/19 was incorrectly shown in the original papers.)

In order to study the formation of phenoxyl radicals on either the A- or B-ring by photo-oxidation or H-abstraction, catechin 25 was selectively protected at either its A- or B-ring phenolic functionalities (Scheme 4). Methylation with dichlorodiphenylmethane protected the B-ring catechol group to give 26 in 20% yield. This compound was partially methylated with dimethyl sulfate (1 mole eq.) to give a mixture of A-ring methylated analogues 27–29. Deprotection via hydrogenolysis over Pd(OH)₂/MeOH of the purified compounds gave the A-ring O-methyl ethers 30–32 which served as appropriate models for, respectively, A- and B-ring phenoxyl radical studies. The authors of this paper apparently overlooked a similar approach proceeding in better yields which was published more than 10 years ago.

Mushroom tyrosinase as polyphenol oxidase (PPO) source was recently utilized to construct the biaryl bond in the flavan-3-ols, catechin 25, fisetinidol 33 and mesquitol 34. The catechol-type B-ring in compounds 25 and 33 are readily susceptible to oxidation to an o-quinone moiety which is then susceptible to nucleophilic addition with phenolic nucleophiles like phloroglucinol. Mesquitol 34 with its pyrogallol-type A-ring is more susceptible to quinone formation at this ring hence leading to aryl–aryl bond formation at C(5). This method was successfully employed to synthesize the mesquitol-(5→8)-catechin atropisomers 35 and 36 which were previously isolated from Prosopis glandulosa.
Considerable effort has been focused on the reaction of catechin 25 and epicatechin [C(3) diastereoisomer of 25] with electrophilic reagents that may mimic the chemistry which is involved in the color changes produced during storage of red wine and grape-derived foods. The principles involved are demonstrated in Scheme 5 for condensation between catechin 25 and glyoxylic acid. Thus, treatment of catechin 25 with glyoxylic acid in aqueous ethanol afforded a mixture of the colorless bis-catechins, e.g. 37, bridged by a carboxymethine functionality via a process of two successive electrophilic aromatic substitution reactions. These compounds were gradually transformed via dehydration into yellowish pigments of type 38 which were susceptible to oxidation into the coloured xanthylum salts of type 39. It was later also demonstrated that the initially formed condensation products, e.g. the catechin analogue 40, were susceptible to acid-catalyzed loss of formic acid to give formyl derivatives of type 41. Similar principles also govern the reactions of the flavan-3-ols with other electrophilic reagents like acetaldehyde and furfural, and also in the acetaldehyde-induced condensation of epicatechin and malvidin 3-O-glucoside. An interesting new group of C-4 substituted flavan-3-ol derivatives were obtained from the acid-catalyzed degradation of the polymeric proanthocyanidin fraction of grape origin in the presence of cysteamine. The new derivatives, 4β-(2-aminoethylthio)epicatechin 42, 4β-(2-aminoethylthio)epicatechin 3-O-gallate 43 and 4β-(2-aminoethylthio)catechin 44 possess a
C-4 aminoethylsulfanyl functionality which facilitates their isolation from complex mixtures by cation-exchange gels or resins. This thus represents a method to efficiently obtain valuable antioxidant prototypes from otherwise wasted polymers from renewable sources.

A process for the preparation of 4-deuterio- or 4-tritio-(-)-epigallocatechin 3-O-gallate was patented. Treatment of the octa-O-acetyl derivative of (-)-epigallocatechin 3-O-gallate with NBS and AIBN afforded the 4-bromo derivative. This was treated with NaB\(_4\)H\(_4\) or NaB\(_3\)H\(_4\) which affected simultaneous reduction of the C–Br bond and deacetylation to form the 4-deuterio or 4-tritio analogue. However, it is not clear if and how the gallate ester moiety survived the de-esterification process.

The human intestinal bacterium, *Eubacterium* (E.) sp. strain SDG-2, cleaves the C-rings of (3S)- and (3R)-flavan-3-ols, e.g. catechin 25, *ent*-epicatechin 46, and *ent*-catechin 48, epicatechin 49 as well as *ent*-gallocatechin 52 and epigallocatechin 13 to give the corresponding 1,3-diphenylpropan-2-ol derivative.

Scheme 5  Proposed mechanism for the formation of xanthylum salt 39 from colorless dimer 37.

e.g. 47 and 50 (Scheme 6). The corresponding flavan-3-ol 3-O-gallate esters are not susceptible to similar cleavage of the etherocyclic bond. Furthermore, E. sp. strain SDG-2 effected 4′-dehydroxylation of the B-ring of (3R)-flavan-3-ols, e.g. ent-catechin 48, to give 1,3-diphenylpropan-2-ol derivatives of type 51. The C(4′)-OH bond in (3S)-flavan-3-ols, e.g. catechin 25 is stable under similar conditions. The sequence 48/49 ‒ 50 ‒ 51 was confirmed by incubation of the 1,3-diphenylpropan-2-ol 50 which gave the deoxygenated derivative 51. The gallatechins 52 and 13 were converted into the 4′-deoxy compound 54, though an intermediate of type 53 could not be detected.

Green tea polyphenols (catechins) are well known chemo-preventive agents with a variety of biological effects such as cholesterol lowering activity. It was recently demonstrated that epigallocatechin 3-O-gallate (EGCG) 12, epicatechin 3-O-gallate (ECG) 55, ent-gallocatechin 3-O-gallate (ent-EGC) 56 and theasinensin A 57 exhibited potent and selective inhibition of rat squalene epoxidase (SE), a rate-limiting enzyme of cholesterol biosynthesis. The 3′-O-methyl derivatives of compounds 12, 55 and 56, i.e. the major metabolites of orally administered 12, 55 and 56, showed as potent SE inhibition as EGCG 12. Flavan-3-ols without the 3-O-gallate functionality and with catechol-type B-rings did not show significant enzyme inhibition. Enzyme inhibition is postulated to involve specific binding of the flavan-3-ol to the enzyme, and by scavenging reactive oxygen species required for the mono-oxygenase reaction. It was also demonstrated that the pyrogallol-type functionality in flavan-3-ols, e.g. EGCG 12, was a prerequisite for inducing apoptosis in human histiocytic lymphoma U937 cells.
2.2 Flavan-3,4-diols/flavan-4-ols

One new flavan-3,4-diol derivative 58 (3β-methoxyxuulanin) and two flavan-4-ol derivatives 59 (xuulanin) and 60 (4β-demethoxyxuulanin-4β-ethyl ether) were identified from the stem bark of Lonchocarpus xuul.42 The indicated configurations are relative and the 4β-ethyl ether 60 presumably represents an artefact.

Flavan-3,4-diols are subject to facile conversion into flav-3-en-3-ols which are versatile precursors in flavonoid synthesis (Scheme 7).44 Treatment of 4',7,8-tri-O-methylxepioritin-4α-ol 61 with PBr3 in THF gave the 4β-bromo flavan-3-ol 62 which was susceptible to spontaneous dehydrobromination to give the flav-3-en-3-ol 63. This compound existed in solution as the keto tautomer 64 and was isolated in an 80% yield. Reduction of flavan-3-one 64 with NaBH4 afforded a diastereoisomeric mixture of 4',7,8-tri-O-methylxepioritin 66 and 4',7,8-tri-O-methyllepioritin 67 in ca. 70% overall yield. This represented the first synthetic access to the hitherto unknown oritin class of flavan-3-ols.

The flavan-3-one 65 was used to assess the feasibility of using its enolic tautomer as electrophile in flavonoid synthesis. Thus, treatment of 65 with benzylic mercaptaquin(tin(iv)chloride afforded the 2,4-cis-arylbenzylsulfanylflavan-3-one 71. The Lewis acid catalyzed α-sulfenylation of ketones involving a mercaptan, i.e. “nucleophilic” sulfur is unprecedented. The formation of the 4-benzylsulfanylflavan-3-one 71 is presumably triggered by initial formation of complex 68 which equilibrates with the tin(tin(iv)chloride enolate 69 under influence of the electron-rich A-ring. The tin(tin(iv)enolate then complexes with benzyl mercaptaquin leading to “umpolung” of the nucleophilic properties of sulfur in intermediate 70. The electrophilic sulfur in 70 is susceptible to intramolecular attack by the nucleophilic C(4) centre to give the 4-benzylsulfanylflavan-3-one 71.

7,8-Dihydroxy-2,3-cis-3,4-cis-flavan-3,4-diols, e.g. the teraacidin 72, and some of their all-cis (C-ring) oligomers are conspicuously stable.44,45 The electronic, stereochemical and conformational effects contributing to such stability were highlighted in a paper describing the synthesis and chemistry of the all-cis 4α-benzylsulfanyllepioritin 73.45

A series of flavan-4-ols, e.g. 74, was conveniently prepared by metal hydride reduction of the corresponding flavanone.46 The flavan-4-ols were converted into the 4-methoxyflavans, e.g. 75,

Scheme 6 Metabolism of flavan-3-ols by Eubacterium sp. strain SDG-2.

by acid-catalyzed solvolysis in methanol. Both these classes of compounds are currently being evaluated as anticancer drugs.

3 A-Type proanthocyanidins

The A-type proanthocyanidins, with their unusual second ether linkage between an A-ring hydroxyl function of the bottom unit to C(2) of the T-unit, continued to receive considerable attention. Three new analogues with substantial activity against hyaluronidase were isolated from the water-soluble fraction of peanut skins. These compounds are epicatechin-(2β → 7,4β → 6)-catechin 76, epicatechin-(2β → 7,4β → 6)-ent-catechin 77 and epicatechin-(2β → 7,4β → 6)-ent-epicatechin 78. Their structures were properly elucidated by NMR and chiroptical methods as well as by controlled chemical degradation using sodium cyanoborohydride in acidic medium. ¹³C NMR chemical shift rules to differentiate between (2 → 7,4 → 8) and (2 → 7,4 → 6) doubly linked hepta-O-methyl ethers of A-type proanthocyanidins were also proposed.
ent-Epiafzelechin-(2α → 7,4α → 8)-afzelechin 79 and ent-epiafzelechin-(2α → 7,4α → 8)-ent-afzelechin 80 were obtained from the root of Prunus armeniaca. This paper, however, did not show structures for 79 and 80 and is also confusing as far as proper nomenclature, e.g. use of (-)-afzelechin instead of ent-afzelechin, is concerned. A separate investigation of the same natural source also indicated the presence of ent-epiafzelechin-(2α → 7,4α → 8)-epicatechin 81 and ent-epiafzelechin-(2α → 7,4α → 8)-catechin 82.

For compound 81 the indicated structure again did not correspond to the name given for the DEF constituent unit. Epigallocatechin-(2β → 7,4β → 8)-epicatechin, which exhibited potent antioxidant properties, was obtained from the leaves of Dioclea lasiophylla. This compound was independently also isolated from the wood of Xanthoceras sorbifolia.

Geranins A–D, i.e. epiafzelechin-(2β → 7,4β → 8)-afzelechin 84, epicatechin-(2β → 7,4β → 8)-afzelechin 85, epiafzelechin-(2β → 7,4β → 8)-gallocatechin 86 and epiafzelechin-(2β → 7,4β → 8)-afzelechin 87 were identified from the roots of Geranium niveum. This plant is highly valued by the Tarahumara Indians for the treatment of gastrointestinal conditions. The geranins showed antiprotozoal activity when tested against axenically grown trophozoites of Giardia lamblia and Entamoeba histolytica. Geranin D 87 complements the rare series of trimeric A-type proanthocyanidins with two double linkages between constituent flavanyl moieties (see ref. 3). Valuable information regarding the conformation of the F-ring in geranin A 84 was also reported.

The trimeric epicatechin-(2β → 7,4β → 6)-epicatechin-(2β → 7,4β → 8)-epicatechin 88, tetrameric epicatechin-(2β → 7,4β → 8)-(epicatechin-(4β → 6)-epicatechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin 89 (parameritannin A-1) and epicatechin-(2β → 5,4β → 6)-(epicatechin-(2β → 7,4β → 8)-epicatechin-(4β → 8)-epicatechin 90 (parameritannin A-2) were isolated from the bark of Parameria laevigata Moldenke. Analogues 89 and 90 are the first tetrameric A-type proanthocyanidins possessing a “branched” chain of constituent flavanyl units.

Cranberry (Vaccinium macrocarpon Ait.) fruit juice has been used traditionally for the treatment and prevention of urinary tract infections. Its effectiveness was scientifically demonstrated by a randomized, double-blind placebo-controlled trial. The attachment of Escherichia coli, the principal bacterial species responsible for urinary tract infection, is facilitated by fimbriae, which are proteinaceous fibers on the bacterial cell wall. Fimbriae produce specific adhesins that attach to specific oligosaccharide receptors on uroepithelial cells. It
was recently demonstrated that trimeric A-type proanthocyanidins from Cranberry prevented adherence of P-fimbriated E. coli isolates from the urinary tract to cellular surfaces containing α-Gal (1→4)β-Gal receptor sequences similar to those on uroepithelial cells. The compounds that inhibited adherence were shown to be the known epicatechin-(2β7,4β8)-epicatechin-(4β8)-epicatechin, epicatechin-(4β8)-epicatechin-(2β7,4β8)-epicatechin and the new epicatechin-(4β6)-epicatechin-(2β7,4β8)-epicatechin. An interesting paper reported the conversion of B- into A-type proanthocyanidins via oxidation using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals under neutral conditions. Procyanidins B1 and B2 were converted into procyanidins A1 and A2, respectively, by oxidation with DPPH in ethanol (Scheme 8). The formation of 96 and 97 indicates that H(2) (C-ring) in the 4β-substituted epicatechin ABC moiety is probably abstracted as a hydrogen radical following proton loss and one-electron oxidation at the C(4) (B-ring) phenolic functionality. The resulting p-quinomethanes 94 and 95 are then susceptible to ring closure via the 1,6-Michael addition indicated in Scheme 8. Indirect evidence for the intermediacy of a p-quinomethane of type 94 in the oxidative conversion of B- into A-type proanthocyanidins came from the oxidation of epigallocatechin with the homogenate of banana fruit flesh polyphenol oxidase. Besides racemization at C(2), the oxidative conversion also gave the retro-α-hydroxydihydrochalcone (Scheme 9), presumably via initial oxidation of EGC to the p-quinomethane. Hydration then gave the unstable hemiacetal which would equilibrate with the 1,3-diarylketone.

4 B-Type proanthocyanidins

Proanthocyanidins of the B-type are characterized by singly linked flavanyl units, usually between C(4) of the flavan-3-ol chain-extender unit and C(6) or C(8) of the chain-terminating moiety. They are classified according to the hydroxylation pattern(s) of the chain-extender unit(s) and several of the known classes were supplemented during the review period. A considerable number of papers also reported synthetic efforts which are leading to an increased level of understanding the intricate principles that govern the physico-chemical properties of these compounds.

4.1 Procyanidins (3,5,7,3',4'-pentahydroxylation)

The procyanidins represent a dominant and widespread class of naturally occurring proanthocyanidins. New analogues that were added during the review period included procyanidin B5 3'-O-gallate from the seeds of Vitis amurensis. The same source also afforded vitisinol and amurenisin with relative configurations as indicated. Although both and were classified as procyanidins, per definition they do not belong to this class of compounds. Vitisinol is, rather, a member of the non-proanthocyanidin class with flavan or flavan-3-ol constituent units (see ref. 1 and Section 4.8), while amurenisin is simply a gallic acid derivative of epicatechin. A number of “mixed” procyanidins/prodelphinidins with exceptionally complex structures have been identified from the roots of Clementsia semenovii and Rhodiola panirotuloida. Owing to the space requirements for the structures of these macroolecules, only the names of compounds given by the authors are reported. In addition the authors stated that...
indicated configurations were relative. Thus, the analogues from *C. semenovii* are CS-3, 7-O-(6-O-galloyl-β-d-Glcp) → 6-O-β-d-Glcp → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-(−)-epigallocatechin(4β → 8)-(−)-catechin(4α → 8)-(−)-epigallocatechin(4β → 8)-(−)-epigallocatechin(4β → 8)-epigallocatechin, and CS-4, 3-O-galloyl-7-O-[6-O-galloyl-β-d-Glcp] → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-gallo catechin(4α → 8)-(−)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-1, 3-O-galloyl-7-O-(6-O-galloyl-β-d-Glcp) → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-2, 3-O-galloyl-7-O-(6-O-galloyl-β-d-Glcp) → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-4, 3-O-galloyl-7-O-[6-O-galloyl-β-d-Glcp] → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-gallocatechin(4α → 8)-(−)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-1, 3-O-galloyl-7-O-(6-O-galloyl-β-d-Glcp) → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-2, 3-O-galloyl-7-O-(6-O-galloyl-β-d-Glcp) → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-4, 3-O-galloyl-7-O-[6-O-galloyl-β-d-Glcp] → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-1, 3-O-galloyl-7-O-(6-O-galloyl-β-d-Glcp) → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-2, 3-O-galloyl-7-O-(6-O-galloyl-β-d-Glcp) → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin, and CS-4, 3-O-galloyl-7-O-[6-O-galloyl-β-d-Glcp] → 6-O-β-d-Glcp → 6-O-β-d-Glcp→(+)-catechin(4α → 8)-3-O-galloyl-(−)-epigallocatechin.

**Scheme 8** Oxidative conversion of B-type procyanidins B₈ and B₉ into A-type compounds 9₆ and 9₇.

An approach utilizing phenolic O-protected flavanyl precursors to synthesize proanthocyanidins found in cocoa was recently described (Scheme 10). Tetra-O-benzylepicatechin **10₄** was obtained via oxidation of tetra-O-benzylecatechin to the 3-keto derivative of type 6₄ by the Dess–Martin periodinane.

followed by reduction with lithium tri-sec-butyldiborohydride in THF. Derivative 104 also served as precursor to the flavan-3,4-diol derivative 105 [C(4) stereochemistry not defined] via DDQ oxidation in CH₂Cl₂ containing ethylene glycol. Lewis acid (TiCl₄) catalyzed condensation of the nucleophilic flavan-3-ol derivative 104 and the electrophilic flavan-3,4-diol analogue 105 afforded epicatechin-(4β → 8)-epicatechin perbenzyl aryl ether 106. Galloylation using 3,4,5-tri-O-benzyl galloyl chloride in pyridine containing DMAP afforded the diester which was debenzylated by hydrogenation over Pd(OH)₂/C to give the bis-gallate 107 of procyanidin B2. Compound 107 possesses notable protein kinase C inhibiting and anticancer activity.

Unequivocal proof of the 4β-stereochemistry in procyanidin B2 [epicatechin-(4β → 8)-epicatechin] was obtained by oxidative degradation of the O-alkylated derivative 109 to (R)-(−)-2,4-diphenylbutyric acid 117 (Scheme 11).⁶ Condensation of tetra-O-methylcatechin 108 (prepared via a similar procedure as for 104) with the flavan-3,4-diol derivative 105 mediated by TiCl₄ afforded the procyanidin B2 derivative 109 bearing differential protecting groups in its extender and terminating units. Thioacetylation of 109 with PhO(C=S)Cl/DMAP in 1,2-dichloroethane gave the bis[(phenoxyl)thiocarbonyl] derivative 110 which was deoxygenated by means of the Barton protocol with H₃PO₂/Et₃AlNIBN⁶⁶ to give the bis-flavan 111. Debenzylation afforded 112 which was triflated with N,N-bis(trifluoromethanesulfonyl)aniline in DMF containing DBU. Hydrogenolysis of the tetratriolate 113 in the presence of Et₃AlN proceeded efficiently over Pearlman’s catalyst to give the 1-flavanyl-1,3-diarylpropane 114 via phenol deoxygenation and scission of the benzyl etherocyclic bond of the C-ring.† Deoxygenation as above then gave the trisubstituted propane derivative 116 via triolate 115. Oxidative degradation of 116 with NaIO₄/RuCl₃ afforded the (−)-2,4-diphenylbutyric acid 117. Its 2R absolute configuration was established by X-ray crystal structure analysis of the (R)-(+)α-methylbenzylamine salt.

A highly stereoselective synthesis of the hitherto inaccessible, unnatural procyanidin diastereoisomer epicatechin-(4α → 8)-epicatechin 123 has been reported (Scheme 12).⁷ The 8-lithio derivative 118 of the selective protected epicatechin derivative was prepared from the 8-bromocatechin-3α,4α-diol 119 by halogen-metal exchange using t-BuLi in THF.‡ Treatment of

† See ref. 67 for the formation of small amounts of artifacts.
‡ The sequence in Scheme 12 was also done with 3-O-Bn protection in stead of O-TBDMS.

Scheme 9 Oxidative conversion of epigallocatechin 13.

Scheme 10 Synthesis of procyanidins using phenolic O-protected flavanol precursors.

Scheme 12  Synthesis of epicatechin-(4α → 8)-epicatechin 123 and its mono-O-gallate 124.
the lithio derivative 118 with the protected 2,3-cis-dihydroflavonol derivative 119 afforded the biflavonoid tertiary alcohol 120 as a single isomer. This was smoothly reduced with n-Bu3SnH/CF3CO2H to give the protected epicatechin-(4α → 8)-catechin 121 which was desilylated with HF in acetonitrile to afford 122. Hydrogenolysis with 20% Pd(OH)2/C then gave the C(4) (C-ring) diastereoisomer 123 of procyanidin B2. Owing to severe steric constraints at C(3)(OH) (C-ring), derivative 124 was susceptible to regioselective galloylation at C(3)(OH) (F) leading to useful synthetic access to the mono-O-galloyl ester of, especially, 125.

A process to synthesize (6 → 6), (6 → 8)- and (8 → 8)-linked catechin and epicatechin dimers as well as their 3,3-di-O-gallate esters was patented. The protocol is based on the oxidative (FeCl3) or reductive [Ni(0) reagents] coupling of protected monomers and is demonstrated in Scheme 13 for the (8 → 8)-linked catechin 128. Halogen–metal exchange of the 8-bromo-catechin derivative 125 gave the 8-lithio analogue 126 which was susceptible to oxidative coupling using FeCl3 to give the (8 → 8)-bis-catechin 127. The appropriate sequence of deprotection/galloylation provided access to the free phenol 128 or the 3,3-digallate ester 129.

A considerable effort has been devoted to the production of, especially, 77,78 13-C and, to a lesser extent, also 14-C-labelled catechins and procyanidins. Administration of [U-13C]-phenylalanine or [1-13C]acetate to willow tree shoots, led to the isolation of procyanidin B3 [catechin-(4α → 8)-catechin], procyanidin B6 [catechin-(4α → 6)-catechin], procyanidin C2 [catechin-(4α → 8)-catechin-(4α → 6)-catechin] and a related polymer of high radiopurity. The 13-C-labelled anthocyanins, cyanidin-3-O-β-D-glucoside, peonidin-3-O-β-D-glucoside and malvidin-3-O-β-D-glucoside were similarly produced by incorporation of [1-13C]phenylalanine into Vitis vinifera cell suspension cultures.

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Rac 4-[13C]catechin 137 was synthesized by the sequence outlined in Scheme 14.14 (E)-1-[13C]-di-O-benzylcaffeic acid 131 was synthesized from CH3Cl–CN and 3,4-di-O-benzylbenzaldehyde. Friedel-Crafts acylation of tri-O-benzylphloroglucinol 130 with 131 in TFAA afforded the labelled chalcone 132. This was selectively deprotected with TiCl4 and the resulting chalcone 133 was transformed into the racemic flav-3-ene 134 via successive reduction (NaBH4) and Lewis acid (BF3·OEt2) cyclization. Osmium-catalyzed dihydroxylation gave the flavan-3,4-diol derivative 135 with high diastereoselectivity. Subsequent reduction with Na(CN)BH3/HOAc gave the protected rac-catechin 136, which was then hydrogenolized to afford rac 4-[13C]catechin 137 (99% enrichment). The same synthetic sequence but without isotopic labelling was also used to provide access to both enantiomers of catechin. The unlabelled racemic mixture 136 was resolved via esterification with the monomethyl ester of dibenzoyl-l-tartaric acid. This latter protocol in turn was then utilized to produce optically pure 4-[13C]catechin 137 and 4-[13C]epicatechin.76 The same theme was further exploited to synthesise gram quantities of 4-[13C]procyanidin B3 [catechin-(4α → 8)-catechin] 138.77,78

The structure of procyanidin B1 92 was unequivocally confirmed by X-ray analysis of its deca-O-acetyl derivative 139.79

4.2 Prodelphinidins (3,5,7,3’,4’,5’-hexahydroxylation)

Besides the “mixed” procyanidin–prodelphinidin oligomers from C. semenovii84 and R. panirolaxa85 indicated in Section 4.1, the roots of the former plant also afforded a complex series of prodelphinidin oligomers.80,81 These are compounds CS-1, 7-
Scheme 14  Synthesis of $^{13}$C-labelled rac-catechin 137.

$O$-$[6$-$O$-galloyl$]$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc-$6 → $O$-$β$-$D$-$Glc;$(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin; $(4β → 8)$-$(-)$-epigallocatechin.

The structures were deduced by chemical and enzymatic degradation, as well as spectral data. Rhodichimoside and rhodichin possess hypocholesterinemic, hypolipidemic and anti-inflammatory activities.

$4'$-$O$-Methylgallocatechin $(4α → 8')$-$4'$-$O$-methylgallocatechin 140 was obtained from the stem bark of Stryphnodendron adstringens. Prodelphinidin polymers of undefined structure were also obtained from white clover (Trifolium repens L.) flowers and Onobrychis viciifolia.

**4.3 Propelargonidins (3,5,7,4'-$O$-tetrahydroxylation)**

In addition to the propelargonidin-type proanthocyanidins in the A-series (Section 3), e.g. 79, only two new entries into the B-class were made. 3-$O$-Galloylepigallocatechin $(4β → 8)$-$3$-$O$-Galloylepicatechin and 3-$O$-Galloylepigallocatechin $(4β → 8)$-$3$-$O$-Galloylepicatechin were isolated from green tea, yet again demonstrating the remarkable diversity of the polyphenolic pool of this natural source.

**4.4 Profisetinidins (3,7,3',4'-$O$-tetrahydroxylation) and proробinetinidins (3,7,3',4',5'-$O$-pentahydroxylation)**

No new analogues or new information relevant to the chemistry of these industrially important classes of proanthocyanidins were reported during the review period.

**4.5 Proteracacinidins (3,7,8,4'-$O$-tetrahydroxylation) and promelacacinidins (3,7,8,3',4'-$O$-pentahydroxylation)**

Since the oritin- and mesquitol-type structural moieties that constitute the proteracacinidin and promelacacinidin classes of proanthocyanidins, respectively, are often found in the same molecule, these compounds are grouped together.

The proanthocyanidin pool in plants usually involves the presence of carbon–carbon bonds linking predominantly flavan-3-ol constituent moieties. Such an ensemble of flavan-3-ol units originates via electrophilic aromatic substitution of flavan-4-yl carbocations (or their equivalents) presumably derived from flavan-3,4-diols and the nucleophilic centres of the m-oxygenated A-rings of flavan-3-ols. In the absence of these potent flavan-3-ol nucleophiles with their aptitude for the formation of C–C bonds, alternative centres emerge as participants in interflavanyl bond formation. This is especially prevalent in natural sources containing precursors with a 7,8-dihydroxy functionality of their A-rings where C–C linked proanthocyanidins are often accompanied by ether-linked analogues.

Epioritin-(4β → 3)-epioritin-4β-ol 143 and epimesquitol-(4β → 4)-epioritin-4β-ol 144, the first biflavonoids containing both leucomeracacinidin and leucoteracacinidin units, were isolated from the heartwood of *Acacia caffra*. In designing a synthetic sequence towards ether-linked proanthocyanidins, cognizance had to be taken of the acid-lability of the C(4) benzylic ether functionality. Analogue 143 was formed in low yield when epioritin-4β-ol 145 was activated with AgBF₄ in order to induce self-condensation (Scheme 15). The C–C coupled analogue, epioritin-(4β → 6)-epioritin-4β-ol 146 was also formed. The stereochemical course of the reaction is explicable in terms of a neighbouring group mechanism triggered by interaction of the Lewis acid and the near-axial C(4) hydroxyl group of the flavan-3,4-diol 145. The epimesquitol-(4β → 4)-epioritin-4β-ol 144 and compound 146 were similarly formed when a mixture of 4β-benzylsulfonylepimesquitol 147 and epioritin-4β-ol was treated with AgBF₄ in THF. The same Lewis acid also catalyzed the condensation of epioritin-4α-ol 148 and epioritin-4β-ol 145 to afford the epioritin-(4β → 4)-epioritin-4α-ol 149, epioritin-(4β → 3)-epioritin-4α-ol 150 and epioritin-(4β → 6)-epioritin-4α-ol 151 (Scheme 16). Although the yields of the ether-linked analogues were consistently below 10%, the semisynthesis provided invaluable chiroptical data facilitating establishment of the absolute configuration of this class of naturally occurring proanthocyanidins.
epioritin-4β-ol 152, epioritin-(4β → 3)-epioritin-(4β → 6)-epioritin-(4β-ol 154, and epioritin-(4β → 3)-epioritin-(4β → 6)-epimesquitol-4α-ol 156, as well as the symmetrical dimeric epiortinin-(4β → 4)-epioritin-4β-ol 158 were also identified in the heartwood of A. caffra.

The absence of a second aromatic chromophore in close proximity of the C-4 stereocentre in ether-linked leucoanthocyanidins at both the di- and tri-meric levels precludes assessment of their absolute configuration by chiroptical methods. Trimeric derivatives 153, 155 and 157 exhibited negative and positive Cotton effects in the ca. 280 and 220–250 nm regions, respectively, in their CD spectra (please note that each compound exhibits Cotton effects in the two wavelength regions). The negative Cotton effects near 280 nm probably indicated the cumulative effects of the 1Lb transitions of all the constituent units exhibiting 2R absolute configuration. Positive Cotton effects in the 220–250 nm region were then reminiscent of 1La transitions as well as contributions resulting from the biphenylmethylidene chromophore at C(4)(C). However, the ethereal interflavanyl bond in both the di- and tri-meric analogues are readily susceptible to reductive cleavage with Na(CN)BH$_3$ in TFA/DCM which permitted the unequivocal assignment of the absolute configuration of constituent flavanyl units. Such a protocol is demonstrated in Scheme 17 for cleavage of the permethyl aryl ether diacetate 160 of epioritin-(4β → 4)-epioritin-4α-ol 149. Treatment of 160 with Na(CN)BH$_3$ in TFA/DCM for 45 min at 0 °C afforded epioritin-tri-O-methylether acetate 163 (65%). Under these conditions the protonated C(4)β hydroxyl bond of the C-ring is presumably preferentially cleaved due to the anchimeric effect of the axial C(3)-OH bond. The resulting flavan-3,4-diol derivative 162 that accompanies the epioritin derivative 163 is eventually also reduced to afford the latter compound as sole product of the reductive cleavage process. A similar protocol was also used to reductively cleave the benzylic interflavanyl ether linkages of derivatives 153, 155, 157 and 159.
absolute configuration of the flavan-3-ols could then be determined by comparison of their CD spectra with those of authentic samples. The rare series of doubly-linked proteracacinidin-type oligoflavanoids was extended by identification of four new analogues, oritin-(4α → 7,5→6)-epioritin-4α-ol 164, oritin-(4β → 7,5→6)-epioritin-4α-ol 165, epioritin-(4β → 7,5→6)-epioritin-4α-ol 166, epioritin-(4β → 7,5→6)-epioritin-4α-ol 167 and epioritin-(4β → 5,3→4)-oritin-4α-ol 168. The UV spectra of the permethylaryl acetate derivatives of compounds 164–167 showed three major absorption bands in the 225–235, 275–285 and 315–325 (inflexion) nm regions. Their CD spectra all exhibited high amplitude Cotton effects near 225 nm (negative for 164 and positive for 165, 166 and 167) while 168 showed a positive high amplitude Cotton effect at 240 nm. The Cotton effects in analogues 164–167 result from the helicity imposed by the twisted biaryl chromophore (π→π* transition) similar to observations in the aporphine class of benzyltetrahydroisoquinolines. Thus, the negative Cotton effect near 255 nm for 164 reflects M-helicity of the biaryl bond and hence (R) absolute configuration at C(4) (C). The positive Cotton effects in the same region for the derivatives of 165, 166 and 167 are accordingly indicative of P-helicity of the biaryl bond and hence (S) absolute configuration at C(4) (C). The high amplitude Cotton effect at 240 nm in the CD spectrum of the derivative of compound 168 indicated a C(4) (C) stereocentre carrying a β-substituent and hence (S) absolute configuration by application of the aromatic quadrant rule. The combined (4β → 5) C–C bond and the (3 → 4)-ether linkage in 168 is a unique structural feature in naturally occurring proanthocyanidins.

4.6 Procassinidins (7,4′-dihydroxylation)

In the previous review, two procassinidin analogues with catechin bottom units were listed under "Proanthocyanidins with flavan chain extender units". The procassinidins represent a rare group of naturally occurring proanthocyanidins and only four compounds have thus far been reported. Seven new analogues were identified in the bark of Cassia petersiana, an aqueous extract which is used in traditional African medicine to treat fevers, gonorrhoea and skin infections.

4.7 Probutinidins (7,3′,4′-trihydroxylation)

The bark of A. petersiana also afforded three dimers with a
Scheme 17  Reductive cleavage of the C–O–C bond in leucoteracacinidin derivative 160.

7,3',4'-trihydroxylation chain extender unit.\textsuperscript{39} Owing to the close structural relationship of the ABC moiety, \textit{i.e.} the chain extender unit, in compounds 176–178 with the (2S)-7,3',4'-trihydroxyflavanone, butin, the trivial name butinflavan, was proposed for the (2S)-7,3',4'-trihydroxyflavan ABC unit. \textit{Ent}-butinflavan is a (2R)-7,3',4'-trihydroxyflavan ABC moiety.
and analogues with these 7,3',4'-trihydroxyflavan chain extender units then belong to the probutinidin class of proanthocyanidins. Thus, the new compounds are butinflavan-(4α → 8)- and (4β → 8)-epicatechins 176 and 177, and butinflavan-(4β → 8)-epigallocatechin 178. Their structures and absolute configurations were again confirmed by synthesis via reduction of racemic tetra-O-methylbutin to the diastereoisomeric flavan-4-ols and condensation with the relevant permethylether flavan-3-ols using TiCl₄ as Lewis acid.

4.8 Non-proanthocyanidins with flavan-3-ol constituent units

Owing to their importance for the colour and flavour of “black tea”, the theaflavins continued to be the focus of much attention during the review period. Treatment of a mixture of epicatechin 49 and epigallocatechin 13 with banana fruit homogenate afforded theanaphthoquinone 181, bistheaflavins A and B, 180 and 182, respectively, and the known compound theaflavin 179 (Scheme 18).[^19][^20] The genesis of these compounds was explained as follows. Oxidation of theaflavin 179 (Scheme 19) affords the α-quinone 183, a tautomer 184 of which undergoes hydration to give the gem-diol 185. Rearrangement affords the carboxylic acid 186 which is decarboxylated to the catechol derivative 187. This compound is susceptible to oxidative conversion into theanaphthoquinone 181. The 1,3-diene type functionality of the B-ring of 187 and the dienophlic type functionality of the E-ring in 183 furthermore permit an intermolecular Diels–Alder type cyclization to afford bistheaflavin B 182. The authors also related the sequence of reactions in Scheme 18 to the formation of thearubigins during tea fermentation. Theanaphthoquinone 181 was also formed when a mixture of epicatechin 49 and epigallocatechin 13 was treated with fresh tea leaf extract.[^24]

In *vitro* oxidation experiments using polyphenol oxidase (PPO) from fresh tea leaf resulted in higher content of theaflavins at pH 4.5 in comparison with pH 5.5, the normal pH of macerated tea leaf.[^25] Such an increase of theaflavins is probably due to lower turnover of formed theaflavins into thearubigins. It was also demonstrated that the theaflavins in black tea and the catechins in green tea are equally effective antioxidants,[^26] while epigallocatechin gallate 12, a main constituent of green tea, was demonstrated to possess potent tumor necrosis factor-α (TNF-α) release inhibiting activity.[^27]

Complexation of the theaflavin group of polyphenols with caffeine is thought to be largely responsible for the formation of tea cream, the precipitate that forms as tea cools. The self-association of theaflavin 179 with caffeine was thus studied using ¹H NMR techniques.[^28] These studies indicated that caffeine forms stacks of molecules, while theaflavin forms stable dimers. Theaflavin 179 consists of a planar benzotropolone ring system, with the two 3,4-dihydro-2H-1-benzopyran-3,5,7-triols approximately orthogonal to this plane, and stacked against each other. In the dimer, two benzotropolone rings align with an antiparallel geometry. Two molecules of caffeine bind to one molecule of theaflavin in a strictly sequential manner. It was proposed that the first caffeine unit inserts between the two flavan rings, and the second then binds to the newly liberated benzopyranyl surface. A simple but very descriptive model to explain these associations/interactions was proposed (see ref. 100).

Similar studies focusing on the interactions of procyanidins with salivary proteins and other polypeptides were also published.[^101][^102]

5 Miscellaneous

The colour of a large number of berries that play an important role in human nutrition, is usually associated with the presence of anthocyanin analogues. Investigation of the compounds contributing to this natural colouration thus continued during the review period.

Four new pyranoanthocyanins, pyranocyanins A and B, 188 and 190, and pyranodelphinidins A and B, 189 and 191, were isolated from the seed of *Ribes nigrum* (black currant).[^103] It was subsequently demonstrated that compounds 188–191 were indeed artefacts resulting from oxidative addition of acetone, the solvent used for extraction, to the parent anthocyanins present in black currant seed.[^104] The formation of pyrananthocyanins was explained *via* the sequence outlined in Scheme 20. Thus, the cycloaddition between acetone and a flavilium compound 192 gives adduct 193 which is susceptible to dehydration to form intermediate 194. This is oxidized to the pyranoanthocyanins 195/196. These results clearly indicate that acetone should be avoided as solvent of choice for the quantitative extraction of plant anthocyanins.[^105] Similar adducts resulting from the reaction between the anthocyanins and pyruvic acid excreted by yeast were also reported in 1-year-old bottled Port wines from the Duoro region.[^106] Adduct formation apparently converts anthocyanins into stable pigments with structural features that improve their food colour properties. Evidence for the presence of products resulting from direct anthocyanin–proanthocyanidin adduct formation was also disclosed.[^107]
Numerous methods have been developed for routine qualitative analysis of the catechins (flavan-3-ols) and proanthocyanidins up to the tetrameric level. These methods include paper chromatography, thin-layer chromatography, countercurrent chromatography, centrifugal partition chromatography, several gel separation techniques and high-performance liquid chromatography (HPLC). Most of these techniques are capable of adequately separating monomers, dimers and trimers, but...
are unable to resolve the more structurally diverse higher oligomers. The analytical method usually employed to estimate the amount of catechins and proanthocyanidins is the colorimetric assessment of their total content after reaction with aromatic aldehydes. However, use of a spectroscopic method typically gives estimations of total flavan-3-ol content instead of the quantitative contribution of each compound within its class. Recently, this trend has changed to incorporate the use of HPLC for the quantification of individual proanthocyanidins in various food products. The most effective HPLC method for the separation of proanthocyanidin oligomers into their different molecular mass classes employs the use of normal-phase techniques. Various detection techniques have been explored. UV detection is the most common but specificity for proanthocyanidins is difficult due to the narrow range of UV absorption of many phenolics. To circumvent this problem a method was developed for the postcolumn reaction of proanthocyanidins with 4-dimethylaminocinnamaldehyde to produce an adduct with absorbance at 640 nm. The various MS methods to determine the molecular composition of the constituent monomeric units in proanthocyanidin oligomers are summarized in ref. 112. Contributions focusing on proanthocyanidin analysis via the HPLC/MS protocol included a wide range of plant-derived foods and beverages, cocoa (Theobroma cacao), quantification of procyanidins in cocoa and correlation to total antioxidant capacity, identification of procyanidins and anthocyanins in blueberries and cranberries (Vaccinium spp.), and analysis of polymeric proanthocyanidins from grape (Vitis vinifera) seeds. HPLC analysis of the acid-catalyzed thiolysis products provided information regarding a large range of polymerization states of the procyanidins of two cider apple varieties. The utility of the HPLC/MS protocol is demonstrated for analysis of the procyanidins from grape seeds. The HPLC profile for the grape seed extract is shown in Fig. 1(a), which demonstrates the presence of thirteen distinct UV-absorbing peaks with good baseline separation. Ultrafiltration through a 3000 Da NMWCO (nominal molecular weight cutoff) filter led to separation of the “polymeric” fraction (peak 13, Fig. 1(b) and the lower molecular weight analogues (filtrate, Fig. 1(c)).

Scheme 20  Proposed mechanism for the formation of pyrananthocyanins of type 195/196.

Fig. 1  Reversed-phase HPLC of grape seed extract before ultrafiltration (a), after ultrafiltration through a 3000 Da NMWCO membrane (b), the filtrate after ultrafiltration (c).
1(c). Peaks 1–12 in Fig. 1(a) were identified by LC-ESI/MS as gallic acid (1), procyanidin trimer (P3) (2), procyanidin tetramer (P4) (3), procyanidin dimer (P2) (4), procyanidin dimer (P1) (5), procyanidin trimer (P3) (6), catechin (7), pro-
cyanidin dimer (P2) (8), procyanidin dimer (P2) (9), epicatechin (10), monogallate procyanidin dimer (P2G1) (11), and epi-
catechin gallate (12). The major molecular ions distributed in
the LC-ESI/MS spectrum m/z range of 250–3000 for peak 13
are given in Table 1 (the ESI/MS data is given in Fig. 2).
Although the major ions appeared to be singly charged with
some evidence of multiple charged species, Hammerstone et al. listed several multiple charged ions in their pioneering
work on identification of procyanidins in cocoa. The HPLC/
MS protocol is also a useful indicator of the presence of
A-type proanthocyanidins which show a molecular mass of
2 units lower than that of the B-type analogues. Although
this method is useful to define the degree of polymerization
and the level of derivatization, e.g. galloylation, it does not
provide information regarding stereochemistry and mode of
interflavanyl linkage(s).

7 NMR/conformational analysis of proanthocyanidins

The utilization of the full array of modern 1H and 13C NMR
methodology, as well as the implementation of molecular
mechanics and molecular orbital calculations that were dis-
cussed in the previous review, are now being used routinely
in the analysis of the NMR and conformational properties
of the proanthocyanidins. A useful summary of NMR
studies relevant to the conformation of polyphenols and their
association with proteins was also published.

8 Effects of proanthocyanidins on human nutrition and health

The proanthocyanidins are widely distributed in nature and are
often the active compounds of the medicinal plants in which they occur. Reports of several in vitro assays demonstrate
potentially significant interactions with biological systems such as antiviral, antibacterial, molluscidal, enzyme-inhibiting,
anti-
oxidant and radical-scavenging properties. Their tendency to interfere with biological systems results, at least in part, from
the characteristic ability to form complexes with other bio-
molecules. The increasing interest in the use of “natural remedies” and of appropriate diets to control disease and ill-
ness has been paralleled, over the past twenty years, by studies whose aim has been to pin-point the origins of the particular
biological activities observed. Notable studies in this area have
been made by several groups worldwide and were summarized
by Haslam. A number of other useful contributions pertaining
to the nutritional and health promoting properties of the
proanthocyanidins and related polyphenols are listed as refs.
Insofar as the possible modes of action of natural polyphenols present in foodstuffs and beverages, and as con-
stituents of herbal medicines, is concerned there is circum-
stantial in vitro evidence that they act in at least three general
areas, i.e. transition metal ion complexation, as antioxidants
and by complexation with macromolecules such as peptides,
proteins and polysaccharides.

In summary, the proanthocyanidins have continued to
receive a considerable amount of attention, both from the
chemistry and biological research spheres. There is now a firm
foundation from which journeys aimed at comprehending the
mode(s) of action of these important biomolecules may be
launched.

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Table 1 Major m/z signals identified from peak 13 by LC-ESI/MS analysis

| m/z | Compound* | a | b | c | d | e | f | g | h | i | j |
|-----|-----------|---|---|---|---|---|---|---|---|---|---|---|
| 291 | P1        |   |   |   |   |   |   |   |   |   |   |   |
| 443 | P1G1      |   |   |   |   |   |   |   |   |   |   |   |
| 579 | P2        |   |   |   |   |   |   |   |   |   |   |   |
| 731 | P2G1      |   |   |   |   |   |   |   |   |   |   |   |
| 867 | P2G2      |   |   |   |   |   |   |   |   |   |   |   |
| 1019| P3        |   |   |   |   |   |   |   |   |   |   |   |
| 1155| P3G1      |   |   |   |   |   |   |   |   |   |   |   |
| 1171| P3G2      |   |   |   |   |   |   |   |   |   |   |   |
| 1307| P4        |   |   |   |   |   |   |   |   |   |   |   |
| 1443| P4G1      |   |   |   |   |   |   |   |   |   |   |   |
| 1459| P4G2      |   |   |   |   |   |   |   |   |   |   |   |
| 1595| P5        |   |   |   |   |   |   |   |   |   |   |   |
| 1731| P5G1      |   |   |   |   |   |   |   |   |   |   |   |
| 1748| P5G2      |   |   |   |   |   |   |   |   |   |   |   |
| 1884| P6        |   |   |   |   |   |   |   |   |   |   |   |
| 2021| P6G1      |   |   |   |   |   |   |   |   |   |   |   |
| 2036| P6G2      |   |   |   |   |   |   |   |   |   |   |   |
| 2172| P6G3      |   |   |   |   |   |   |   |   |   |   |   |
| 2188| P6G4      |   |   |   |   |   |   |   |   |   |   |   |
| 2310| P7        |   |   |   |   |   |   |   |   |   |   |   |
| 2324| P7G1      |   |   |   |   |   |   |   |   |   |   |   |
| 2460| P7G2      |   |   |   |   |   |   |   |   |   |   |   |
| 2477| P7G3      |   |   |   |   |   |   |   |   |   |   |   |
| 2612| P8        |   |   |   |   |   |   |   |   |   |   |   |
| 2740| P8G1      |   |   |   |   |   |   |   |   |   |   |   |
| 2749| P8G2      |   |   |   |   |   |   |   |   |   |   |   |
| 2802| P8G3      |   |   |   |   |   |   |   |   |   |   |   |

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AAbbreviations: P, procyanidin; P2, procyanidin dimer, etc.; G, gallate; G1, monogallate, etc.

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Fig. 2 ESI/MS data for the procyanidin polymer peak of grape seed extracts. Data is represented in three m/z ranges: 250–1000, 1000–2000 and 2000–3000.