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.

Daneel Ferreira

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 focusses 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 C_6·C_3·C_6unit. 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

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.

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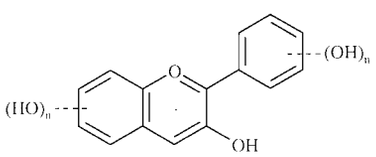
1 Introduction

The oligomeric and polymeric proanthocyanidins (syn. condensed tannins) constitute one of the most ubiquitous groups of all plant phenolics.[1–5] Leucoanthocyanidins are monomeric compounds which produce anthocyanidins **1** by cleavage of a C–O bond on heating with mineral acid. Proanthocyanidins are oligomers/polymers 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 $C_6.C_3.C_6$ secondary metabolites. The bi- and tri-flavonoids[6] are products of oxidative coupling of flavones, flavonols, dihydroflavonols, flavanones, isoflavones, aurones, chalcones, and 2-benzylbenzofuranones[7–9] 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-diol[4] or a flavan-4-ol[2] 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 Hemingway[10] and extended by Porter[2] is applied consistently.



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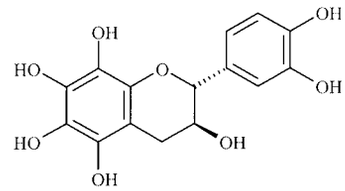
The proanthocyanidins have recently attracted a considerable amount of attention in the fields of nutrition, health and medicine. This is the result of a rapidly growing body of evidence suggesting that the proanthocyanidins may act as potent antioxidants and/or modulate key biological pathways *in vivo* 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 and of flavan-3,4-diol/flavan-4-ols (leucoanthocyanidins) as electrophilic chain-extender units in proanthocyanidin biosynthesis,[4] 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** (elephantorrhizol) was identified in *Elephantorrhiza goetzei*.[12] Its absolute configuration was assumed to be 2*R*,3*S*. 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 *Anadenathera macrocarpa*,[13] (2*R*,3*S*)-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 *Celastrus orbiculatus*,[16] and 3-acetyl-5-methoxy-7,3′,4′-trihydroxy-8-*O*-glucoside-flavan-3-ol, barbatoflavan **7** from *Campanula barbata*.[17]



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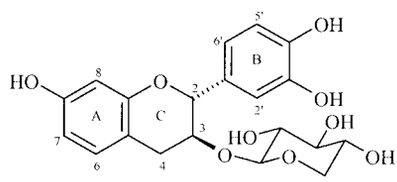

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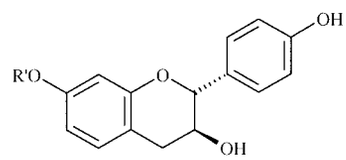
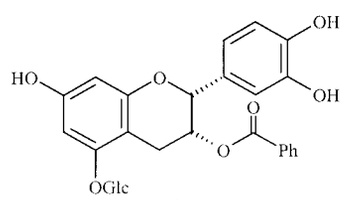


4 R′ = H
5 R′ = Me



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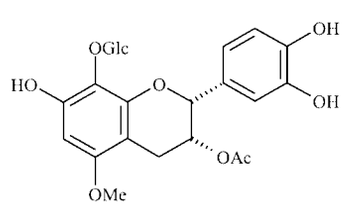

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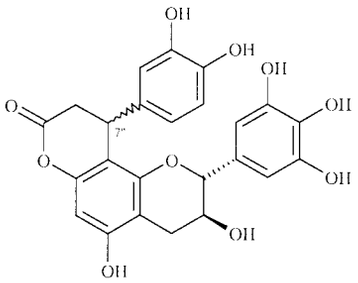
It should be emphasized that the absolute configurations of the C(2) and C(3) stereocentres were not assessed for compounds **3**, **5**, and **7**. This may conveniently be done by circular dichroism. The CD curves of flavan-3-ols exhibit two Cotton effects for the ^{1}La and ^{1}Lb transitions in the 240 and 280 nm regions, respectively.[18–20] Analogues with 2*R* and 2*S* 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 $C_6.C_3$ 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*.[21] 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 gallocatechin (**8**, **9** and **11**) and epigallocatechin **10**, exhibited hepatoprotectitive activity against D-galactosamine (D-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.[14,20] This was achieved by selecting an acid-sensitive protecting



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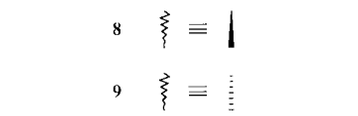

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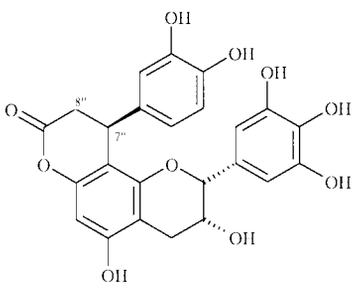

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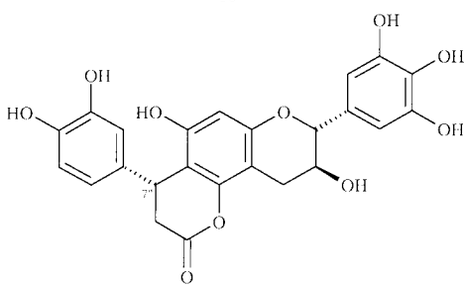

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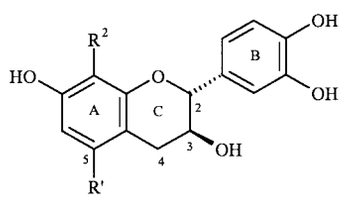
group for the phenolic functionalities in the previously developed protocol.[22] The method is based on the asymmetric dihydroxylation of 1,3-diarylpropenes and subsequent acid-catalyzed cyclization to give the flavan-3-ol diastereoisomers in high yield and in essentially enantiopure form (see ref. 1 for a summary).

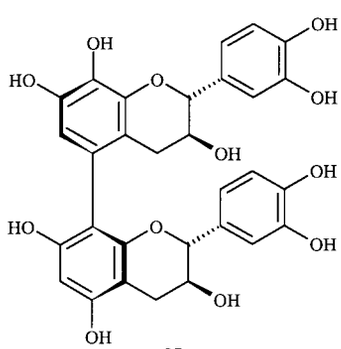
Two important papers focusing on the antioxidant chemistry of the green tea catechins (−)-epigallocatechin gallate (EGCG) **12** and (−)-epigallocatechin (EGC) **13** were published.[23,24] The identification of oxidation products formed by reactions of these flavan-3-ols with biologically relevant oxidants could provide information regarding the specific mechanisms of antioxidant reactions. Separate treatment of EGCG **12** and EGC **13** 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 EGCG/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_2C_2-units in compounds **17** and **18/19** was incorrectly shown in the original papers.[23,24])

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).[25] Methylenation 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.[26]

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**.[27] 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*.[28]



25 R′ = OH, R^2 = H
33 R′ = R^2 = H
34 R′ = H, R^2 = OH



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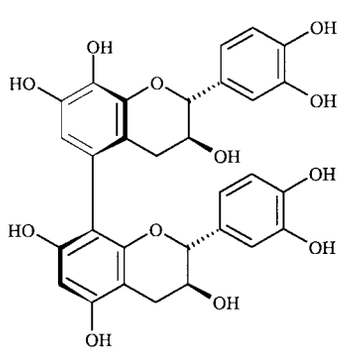

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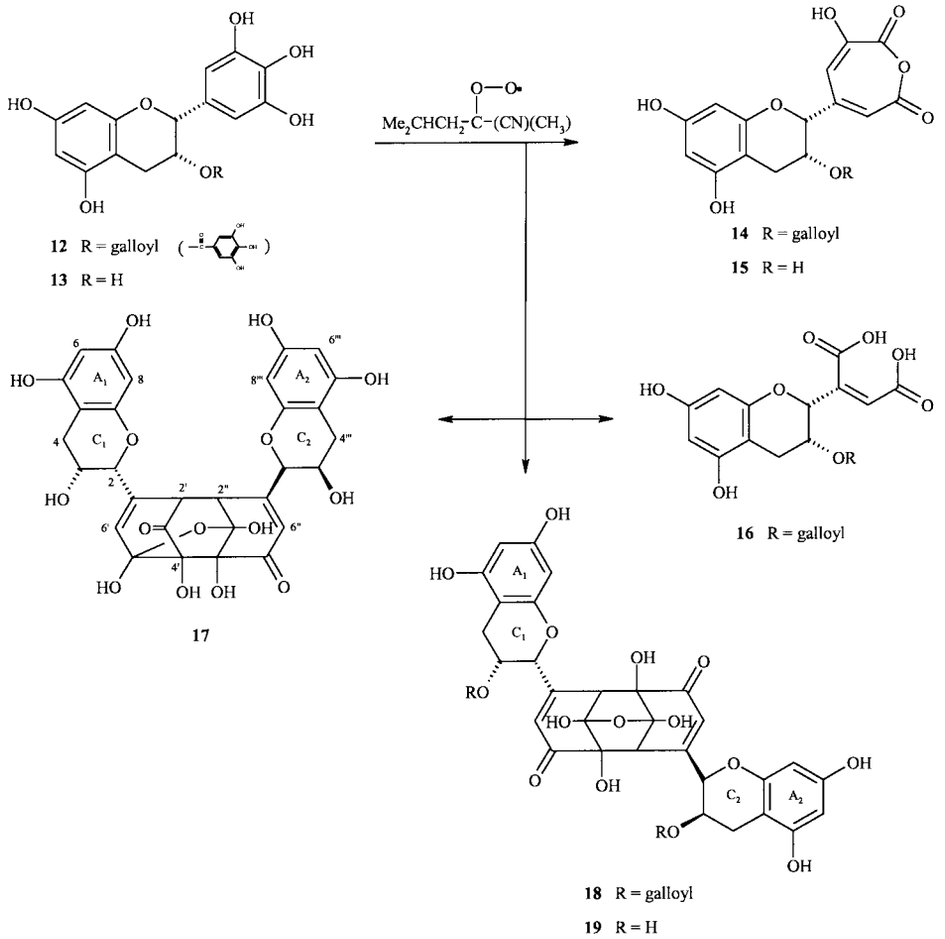
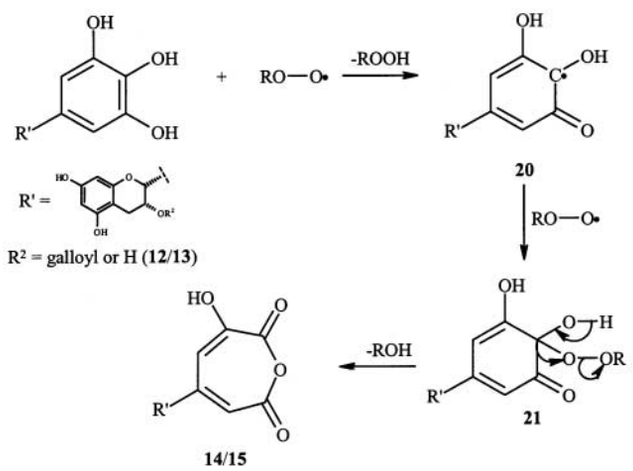
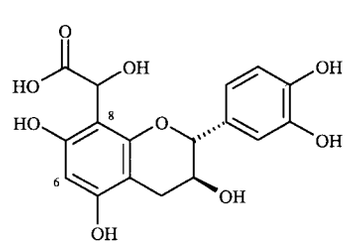


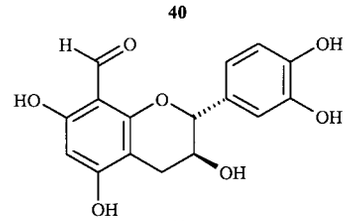
Scheme 1 Oxidation products of EGCG **12** and EGC **13**.



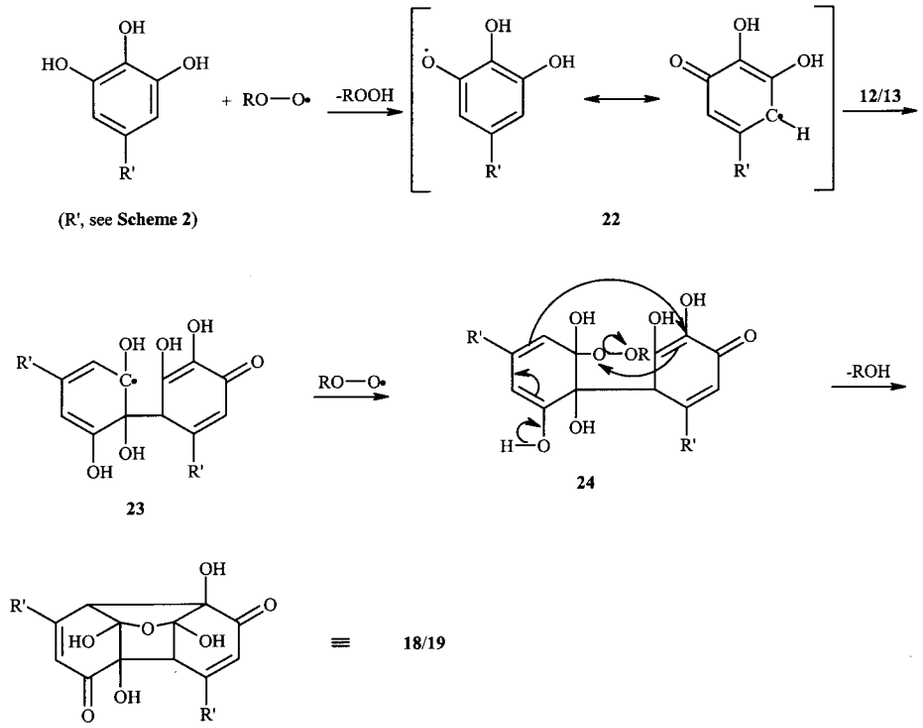
Scheme 2 Proposed mechanism for the formation of compounds **14** and **15**.

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.[29–35] The principles involved are demonstrated in Scheme 5 for condensation between catechin **25** and glyoxylic acid.[30,31,33] 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 xanthylium salts of type **39**. It was later also demonstrated[35] 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[29] and furfural,[33] and also in the acetaldehyde-induced condensation of epicatechin and malvidin 3-*O*-glucoside.[30]

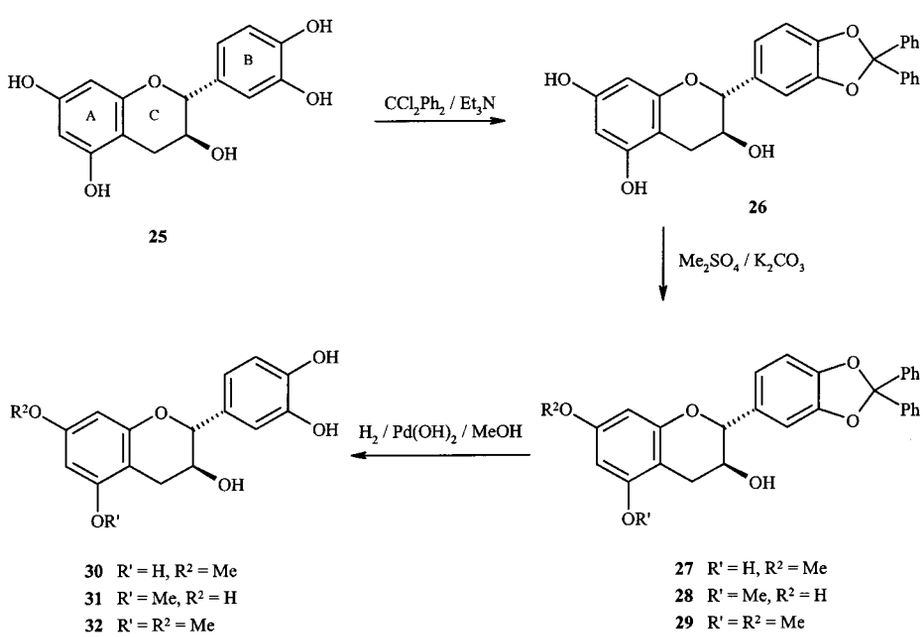




An interesting new group of C-4 substituted flavan-3-ol derivatives were obtained from the acid-catalyzed degradation of the polymeric proanthocyanidin fraction of grape origin in the presence of cysteamine.[36] The new derivatives, 4β-(2-aminoethylthio)epicatechin **42**, 4β-(2-aminoethylthio)epicatechin 3-*O*-gallate **43** and 4β-(2-aminoethylthio)catechin **44** possess a



Scheme 3 Proposed mechanism for the formation of compounds **18** and **19**.

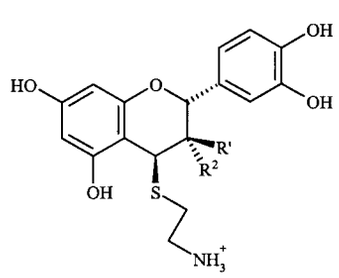


Scheme 4 Synthesis of selectively methylated catechin.

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.

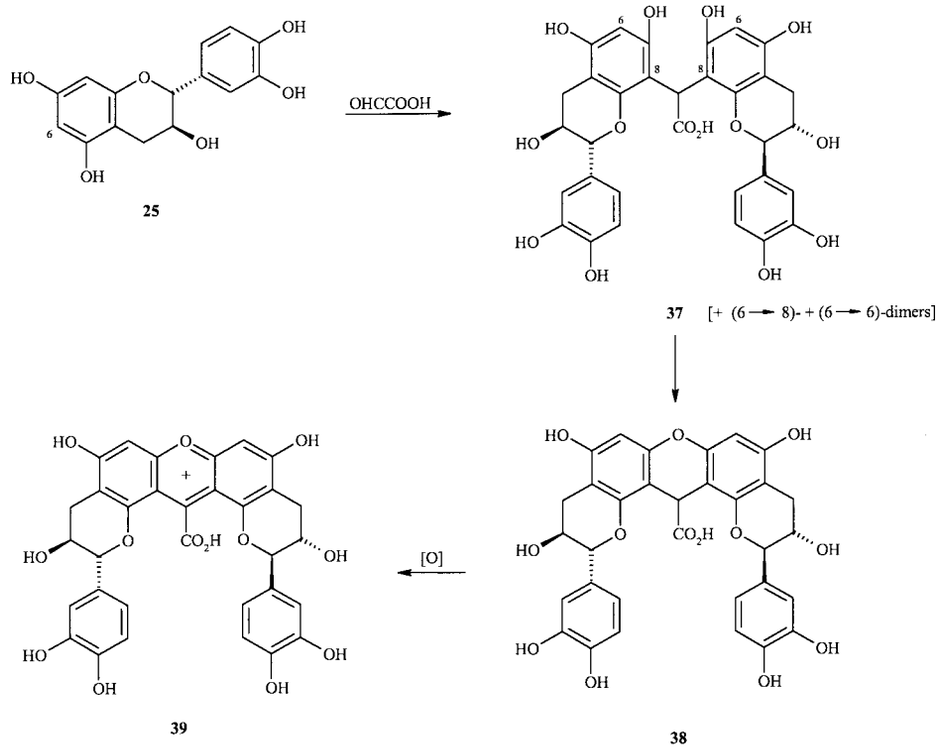
A process for the preparation of 4-deuterio- or 4-tritio-(−)-epigallocatechin 3-*O*-gallate was patented.[37] Treatment of the octa-*O*-acetyl derivative of (−)-epigallocatechin 3-*O*-gallate **12** with NBS and AIBN afforded the 4-bromo derivative. This was treated with NaB^2H_4 or NaB^3H_4 which affected simultaneous reduction of the C–Br bond and deacetylation to form the 4-deuterio or 4-tritio analogue **45**. 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 (3*S*)- and (3*R*)-flavan-3-ols, *e.g.*

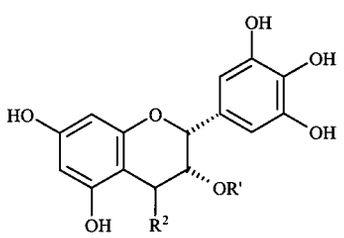


42 R' = H, R² = OH
43 R' = H, R² = galloyl
44 R' = OH, R² = H

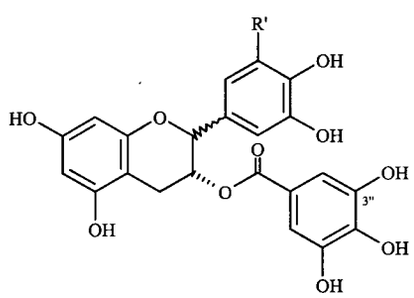
catechin **25**, *ent*-epicatechin **46**, and *ent*-catechin **48**, epicatechin **49** as well as *ent*-gallocatechin **52** and epigallocatechin **13** to give the corresponding 1,3-diphenylpropan-2-ol derivative,



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



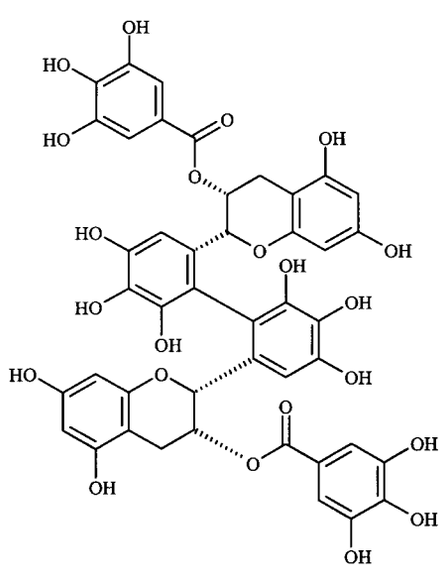
45 R' = galloyl, R^2 = ^2H or ^3H



12 ≡ , R' = OH
55 ≡ , R' = H
56 ≡ , R' = OH

e.g. **47** and **50** (Scheme 6).[38] 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 (3*R*)-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 (3*S*)-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 gallocatechins **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 chemopreventive agents with a variety of biological effects such as cholesterol lowering activity.[39] It was recently demonstrated[40] 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 biogenesis. 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 hystiocytic lymphoma U937 cells.[41]



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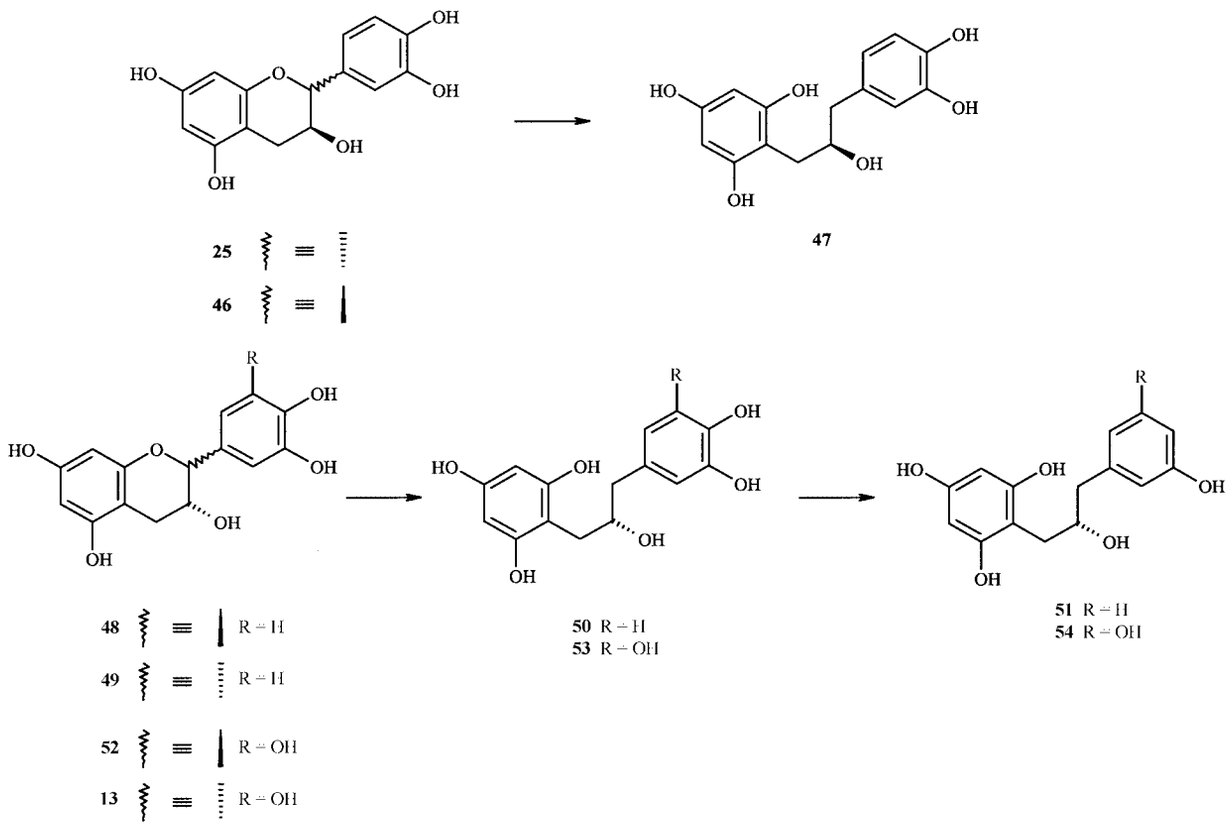
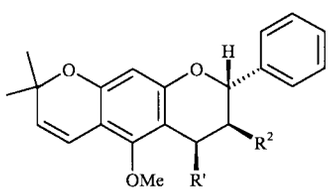


Scheme 6 Metabolism of flavan-3-ols by *Eubacterium* sp. strain SDG-2.

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-demethylxuulanin-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.

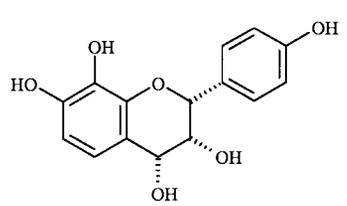


58 R' = R^2 = OMe
59 R' = OMe, R^2 = H
60 R' = OEt, R^2 = H

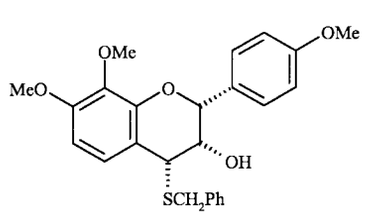
Flavan-3,4-diols are subject to facile conversion into flav-3-en-3-ols which are versatile precursors in flavonoid synthesis (Scheme 7).[43] Treatment of 4′,7,8-tri-*O*-methylepioritin-4α-ol **61** with PBr_3 in THF gave the 4β-bromoflavan-3-ol **62** which was susceptible to spontaneous dehydrobromination 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 $NaBH_4$ afforded a diastereoisomeric mixture of 4′,7,8-tri-*O*-methyloritin **66** and 4′,7,8-tri-*O*-methylepioritin **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 benzyl mercaptan/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(IV)chloride enolate **69** under influence of the electron-rich A-ring. The tin(IV)enolate then complexes with benzyl mercaptan 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 teracacidin **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-benzylsulfanylepioritin **73**.[45]

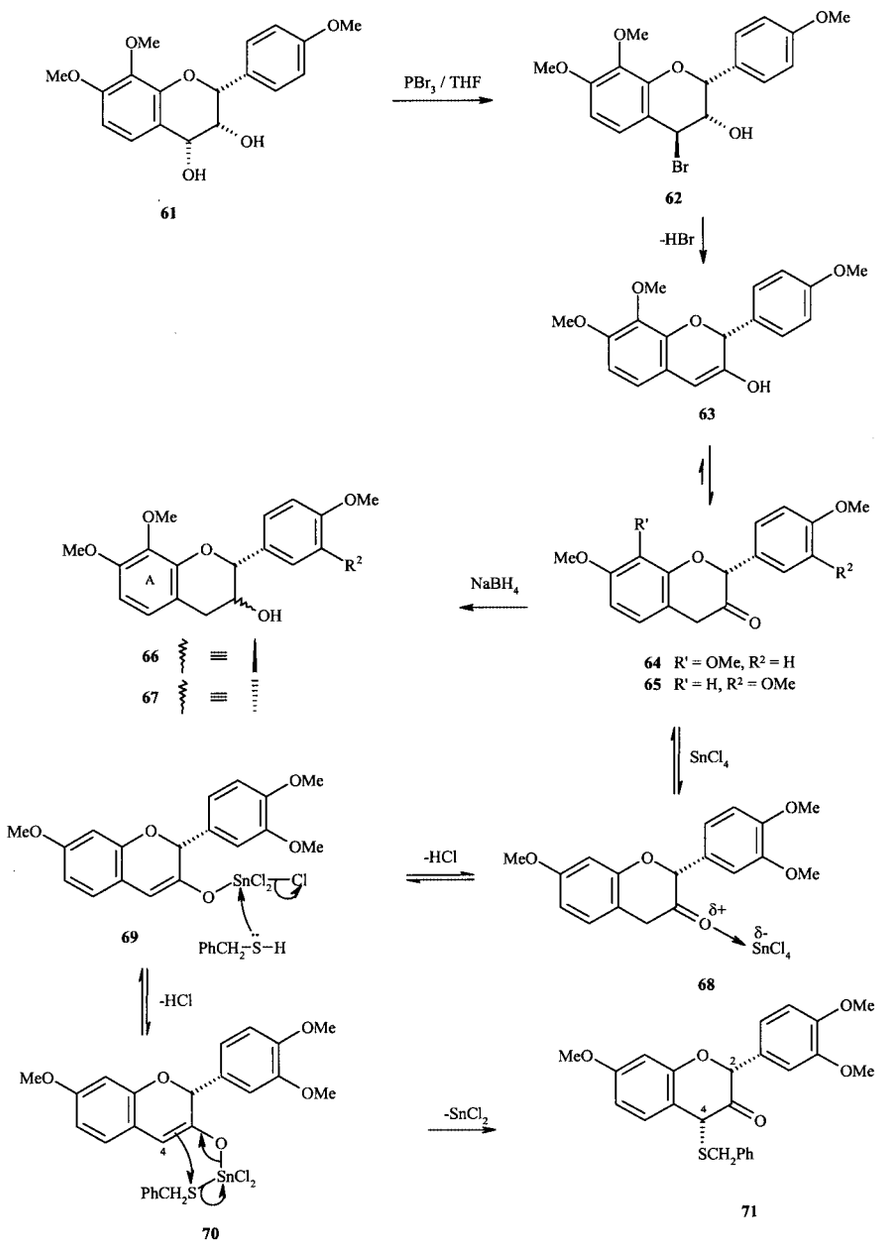


72



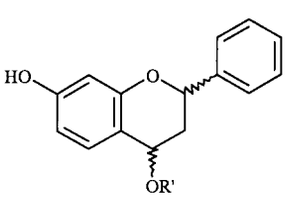
73

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 7 Synthesis and conversion reactions of flavan-3-en-3-ols.

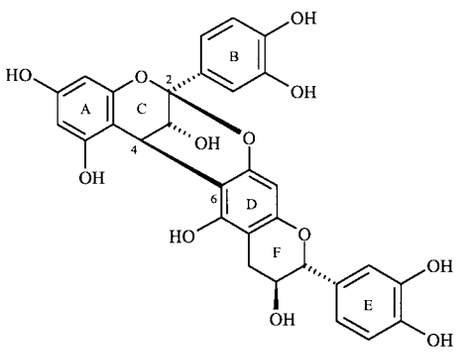
by acid-catalyzed solvolysis in methanol. Both these classes of compounds are currently being evaluated as anticancer drugs.



74 R' = H
75 R' = Me

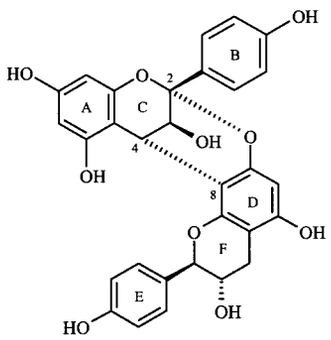
3 A-Type proanthocyanidins

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.[47] 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.[48] ^{13}C 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.



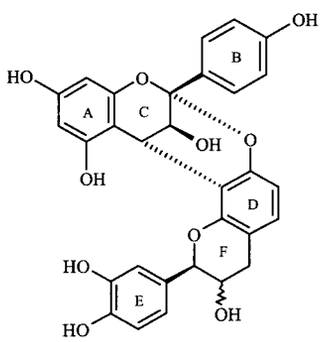
76
77 F-ring enantiomer of **76**
78 C(2)(F) diastereoisomer of **76**

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*.[49] This paper, however, did not show structures for **79** and **80** and is also confusing as far as proper nomenclature,[10] *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**.[50] 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 **83**, which exhibited potent antioxidant properties, was obtained from the leaves of *Dioclea lasiophylla*.[51] This compound was independently also isolated from the wood of *Xanthoceras sorbifolia*.[52]



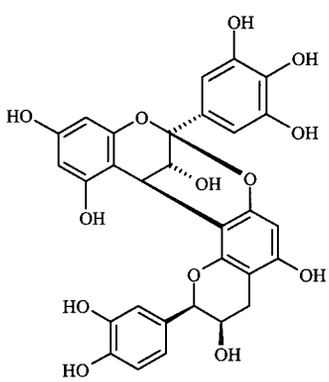
79

80 F-ring enantiomer of **79**



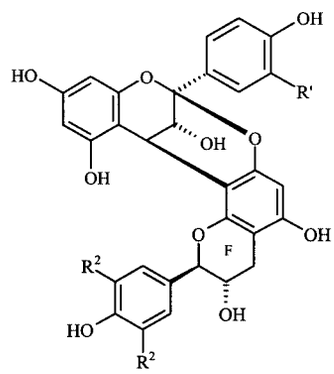
81 ≡ |

82 ≡ ⋮



83

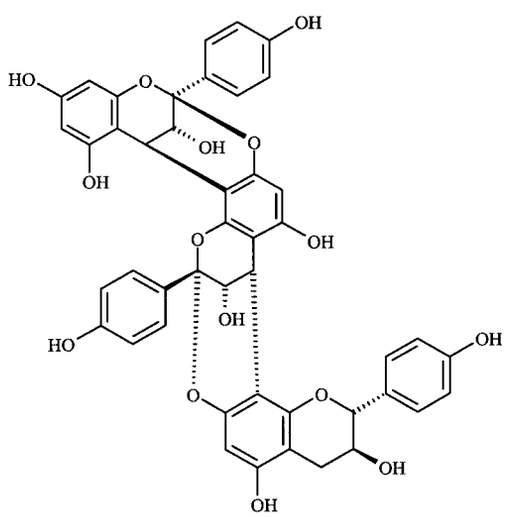
Geranins A–D, *i.e.* epiafzelechin-(2β → 7,4β → 8)-afzelechin **84**, epicatechin-(2β → 7,4β → 8)-afzelechin **85**, epiafzelechin-(2β → 7,4β → 8)-gallocatechin **86** and epiafzelechin-(2β → 7,4β → 8)-afzelechin-(2β → 7,4β → 8)-afzelechin **87** were identified from the roots of *Geranium niveum*.[53,54] 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 *Girardia 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.[53]



84 R' = R² = H

85 R' = OH, R² = H

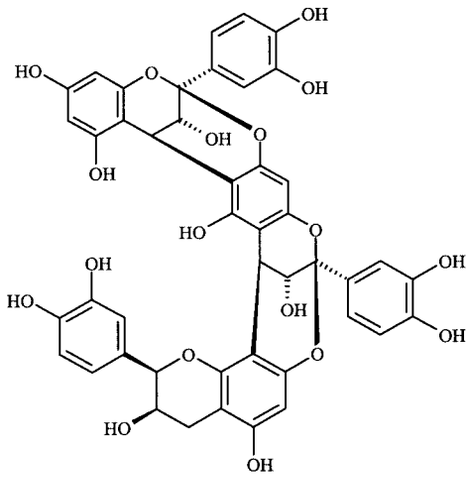
86 R' = H, R² = OH



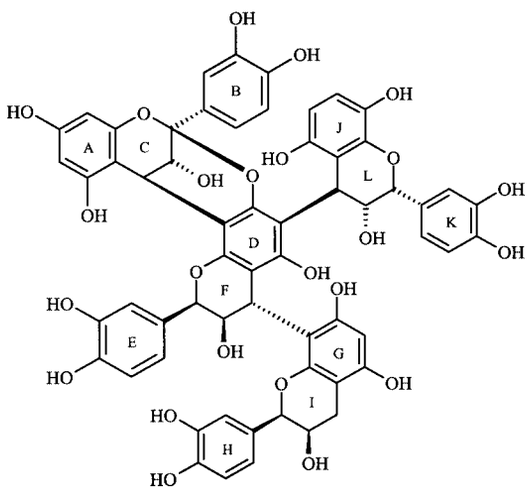
87

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 **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.[55] Analogues **89** and **90** are the first tetrameric A-type proanthocyanidins possessing a "branched" chain of constituent flavanyl units.

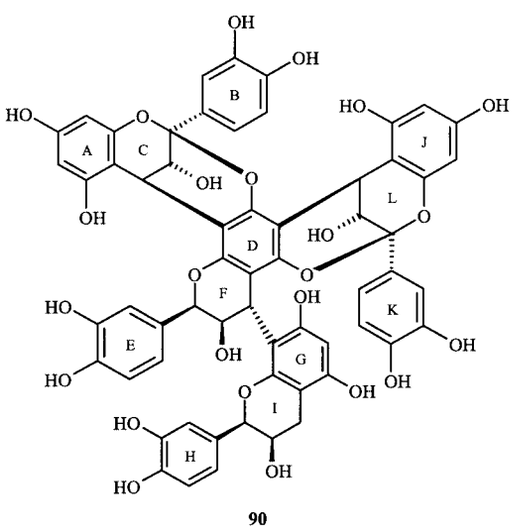
Cranberry (*Vaccinium macrocarpon* Ait.) fruit juice has been used traditionally for the treatment and prevention of urinary tract infections.[56] Its effectiveness was scientifically demonstrated by a randomized, double-blind placebo-controlled trial.[57] The attachment of *Escherichia coli*, the principal 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.[58] It



88



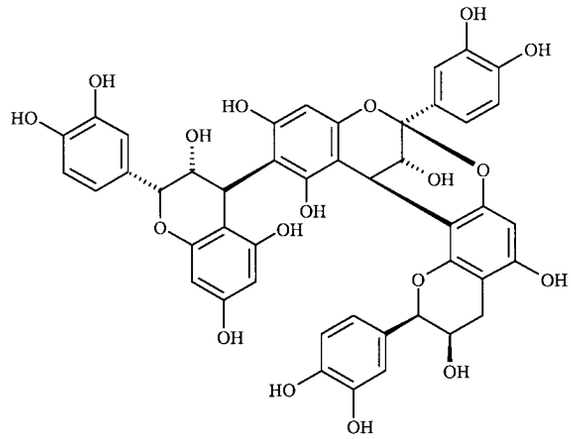
89



90

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.[56,59,60] The compounds that inhibited adherence were shown to be the known epicatechin-(2β → 7,4β → 8)-epicatechin-(4β → 8)-epicatechin, epicatechin-(4β → 8)-epicatechin-(2β → 7,4β → 8)-epicatechin and the new epicatechin-(4β → 6)-epicatechin-(2β → 7,4β → 8)-epicatechin **91**.[60]

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.[61] Procyanidins B1 **92** and B2 **93** were converted into procyanidins A1 **96** and A2 **97**, 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 **13** with the homogenate of banana fruit flesh polyphenol oxidase.[62] Besides racemization at C(2), the oxidative conversion also gave the *retro*-α-hydroxydihydrochalcone **100** (Scheme 9), presumably *via* initial oxidation of EGC **13** to the *p*-quinomethane **98**. Hydration then gave the unstable hemiacetal **99** which would equilibrate with the 1,3-diarylketone **100**.



91

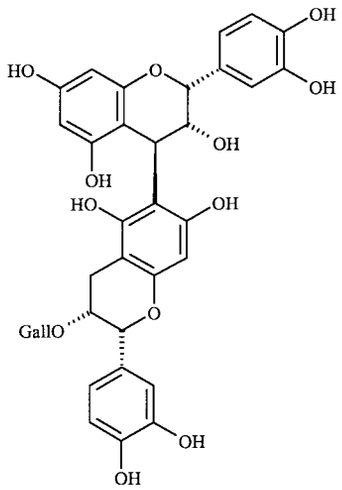
4 B-Type proanthocyanidins

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 **101** from the seeds of *Vitis amurensis*.[63] The same source also afforded vitisinol **102** and amurensisin **103** with relative configurations as indicated. Although both **102** and **103** were classified as procyanidins, per definition they do not belong to this class of compounds. Vitisinol **102** 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 amurensisin **103** 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*[64] and *Rhodiola pamiroalaica*.[65,66] Owing to the space requirements for the structures of these macromolecules, only the names of compounds given by the authors are reported. In addition the authors stated that



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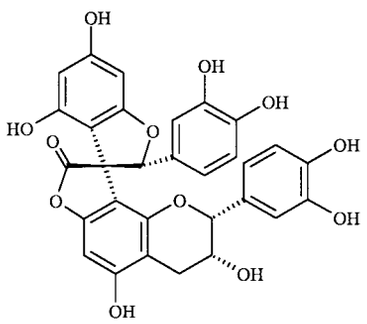

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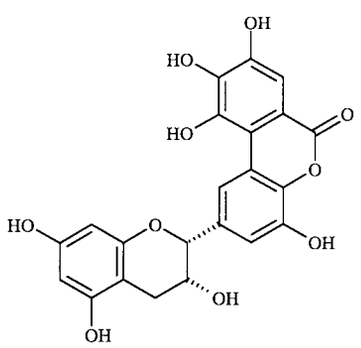
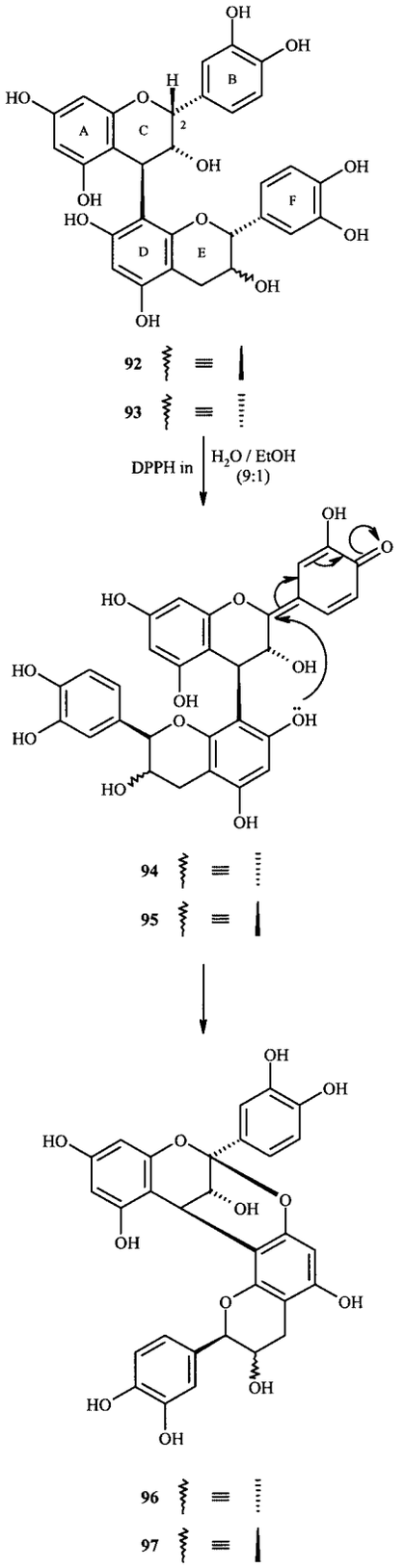


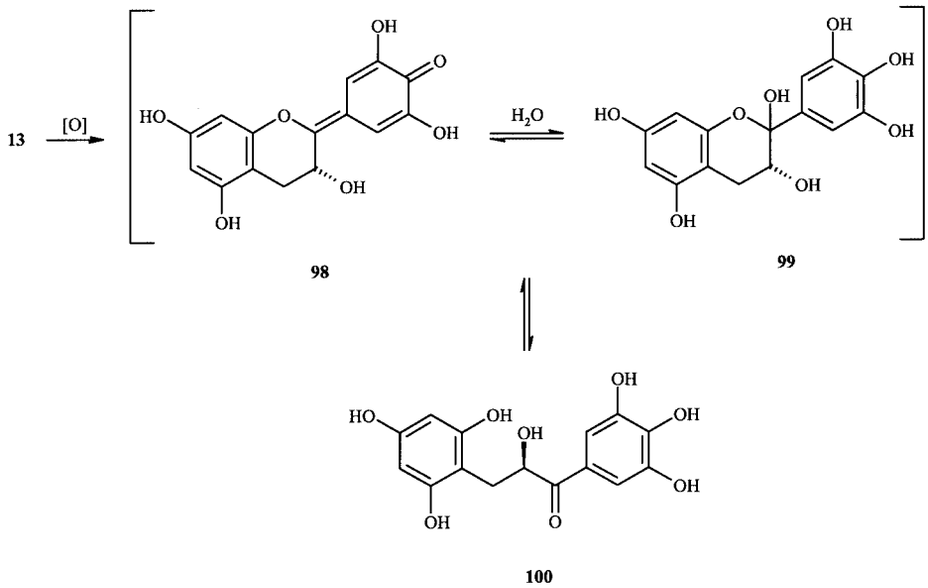
103

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 → 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]-(+)-gallocatechin-(4α → 8)-[(+)-catechin-(4α → 8)-3-*O*-galloyl-(-)-epigallocatechin]$_2$-(4β → 8)-epigallocatechin. The compounds from *R. pamiroalaica* are RP-1, 7-*O*-[6-*O*-galloyl-β-D-Glcp → *O*-β-D-Glcp → *O*-β-D-Glcp]-(+)-gallocatechin-(4α → 8)-(−)-epicatechin-(4β → 8)-epicatechin-(4β → 8)-(+)-catechin-(4α → 8)-5-*O*-[6-*O*-galloyl-β-D-Glcp → *O*-β-D-Glcp → *O*-β-D-Glcp]-(+)-catechin, RP-2, 7-*O*-[*O*-β-D-Glcp → *O*-β-D-Glcp]-(−)-epicatechin-(4β → 6)-7-*O*-β-D-Glcp-(−)-epicatechin-(4β → 6)-3-*O*-galloyl-(−)-epigallocatechin-(4β → 6)-3-*O*-galloyl-(−)-epigallocatechin-(4β → 6)-3-*O*-galloyl-5-*O*-β-D-Glcp-(−)-epicatechin, RP-3,7-*O*-(6-*O*-galloyl-β-D-Glcp)-3-*O*-galloyl-(−)-epigallocatechin-(4β → 8)-[(−)-epicatechin-(4β → 8)-(3-*O*-galloyl-(−)-epigallocatechin)]$_2$-(4β → 8)-[5-*O*-(β-D-Glcp → 6-*O*-β-D-Glcp)-(+)-catechin, and RP-4, 7-*O*-(6-*O*-galloyl-β-D-Glcp)-3-*O*-galloyl-(−)-epigallocatechin-(4β → 8)-[3-*O*-galloyl-(−)-epicatechin-(4β → 8)-[3-*O*-galloyl-(−)-epigallocatechin]-(4β → 8)-[3-*O*-galloyl-(−)-epicatechin]-(4β → 8)-[3-*O*-galloyl-5-(β-D-Glcp → 6-*O*-β-D-Glcp)]-(−)-epigallocatechin.

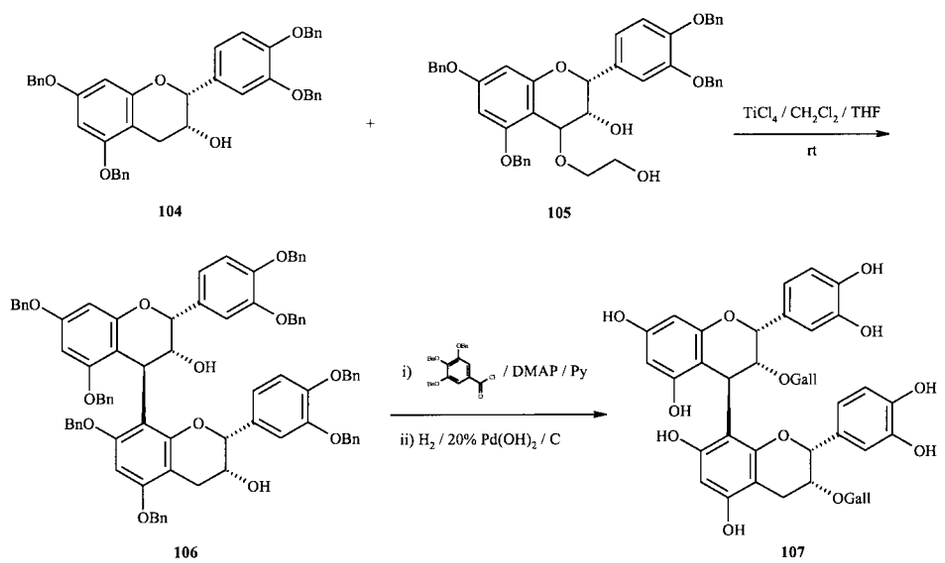


Scheme 8 Oxidative conversion of B-type procyanidins B_1 **92** and B_2 **93** into A-type compounds **96** and **97**.

An approach utilizing phenolic O-protected flavanyl precursors to synthesize proanthocyanidins found in cocao was recently described (Scheme 10).[67] Tetra-*O*-benzylepicatechin **104** was obtained *via* oxidation of tetra-*O*-benzylcatechin to the 3-keto derivative of type **64** by the Dess–Martin periodinane



Scheme 9 Oxidative conversion of epigallocatechin **13**.



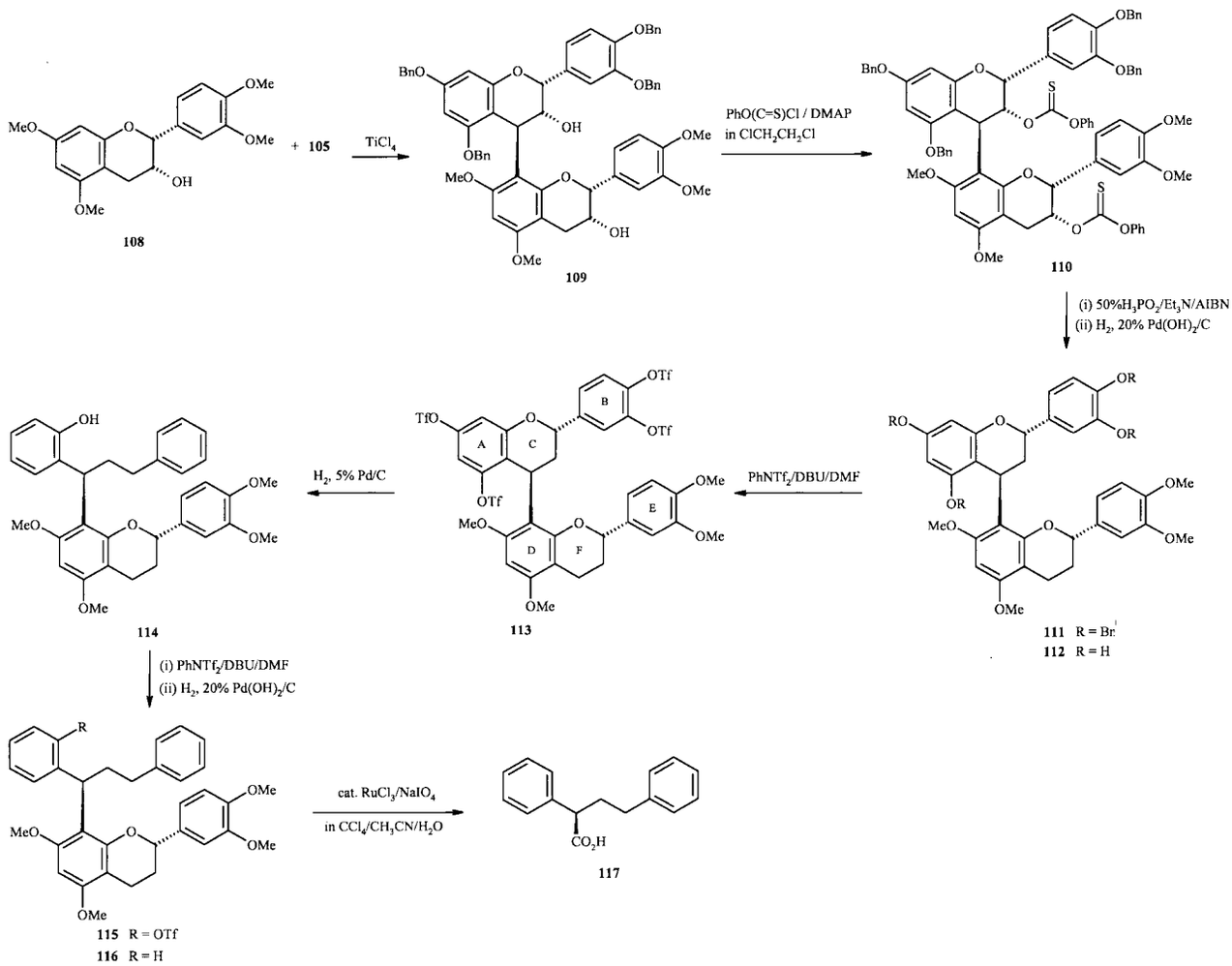
Scheme 10 Synthesis of procyanidins using phenolic *O*-protected flavanyl precursors.

followed by reduction with lithium tri-*sec*-butylborohydride 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 CH_2Cl_2 containing ethylene glycol. Lewis acid ($TiCl_4$) 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.

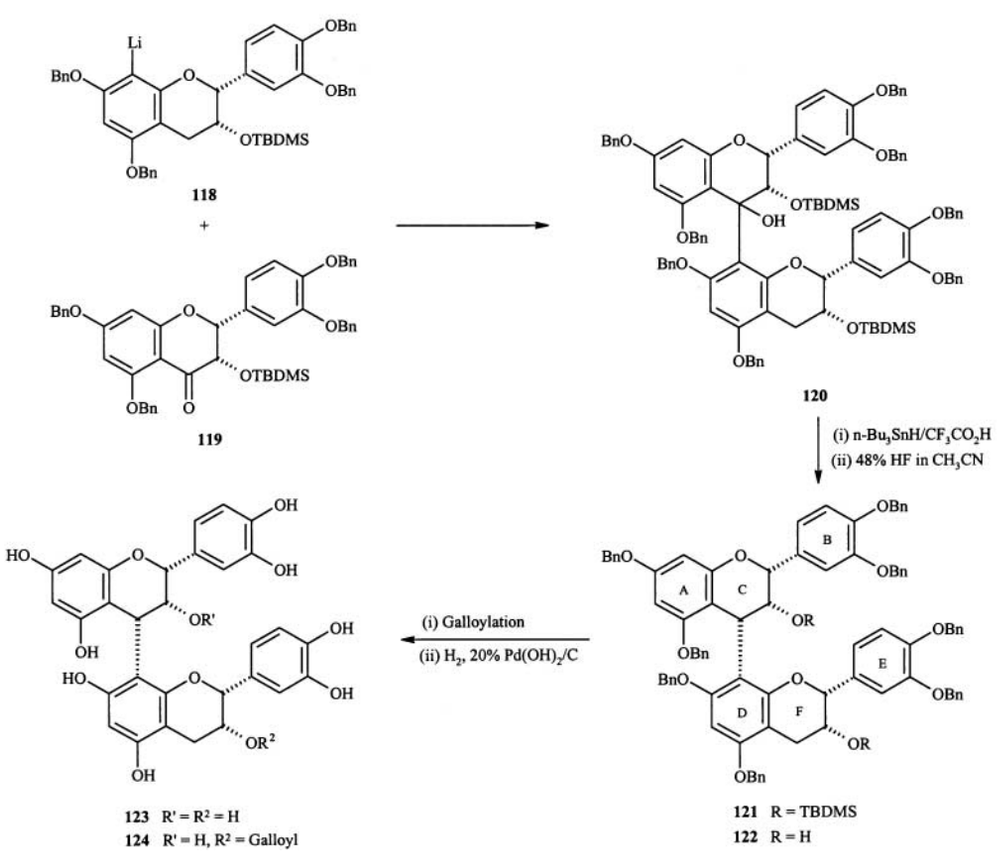
Unequivocal proof of the 4β-stereochemistry in procyanidin B2 **93** [epicatechin-(4β ⟶ 8)-epicatechin] was obtained by oxidative degradation of the *O*-alkylated derivative **109** to (*R*)-(−)-2,4-diphenylbutyric acid **117** (Scheme 11).[68] Condensation of tetra-*O*-methylepicatechin **108** (prepared *via* a similar procedure as for **104**) with the flavan-3,4-diol derivative **105** mediated by $TiCl_4$, afforded the procyanidin B2 derivative **109** bearing differential protecting groups in its extender and terminating units. Thioacylation of **109** using PhO(C=S)Cl/DMAP in 1,2-dichloroethane gave the bis[(phenoxy)thiocarbonyl] derivative **110** which was deoxygenated by means of the Barton protocol with H_3PO_2/Et_3N/AIBN[69] to give the bis-flavan **111**. Debenzylation afforded **112** which was triflated with *N*,*N*-bis(trifluoromethylmethanesulfonyl)analine in DMF containing DBU. Hydrogenolysis of the tetratriflate **113** in the presence of Et_3N proceeded efficiently over Pearlman's catalyst to give the 1-flavanyl-1,3-diarylpropane **114** *via* phenol deoxygenation and scission of the benzylic etherocyclic bond of the C-ring.† Deoxygenation as above then gave the trisubstituted propane derivative **116** *via* triflate **115**. Oxidative degradation of **116** with $NaIO_4/RuCl_3$ afforded the (−)-2,4-diphenylbutyric acid **117**. Its 2*R* 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).[70] The 8-lithio derivative **118** of the selective protected epicatechin derivative was prepared from the 8-bromoepicatechin derivative 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.



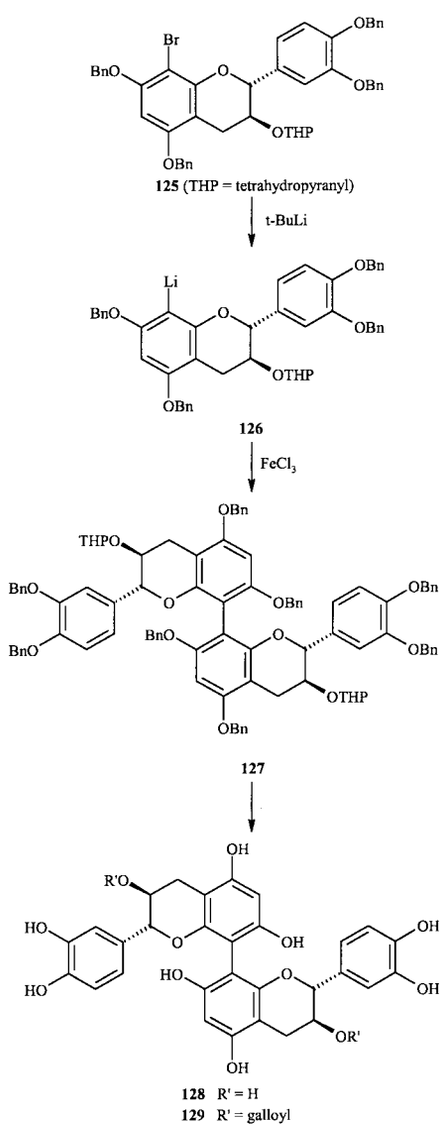
Scheme 11 Proof of 4β-stereochemistry of procyanidin B2 *via* oxidative degradation of derivative **109**.



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 biflavanoid tertiary alcohol **120** as a single isomer. This was smoothly reduced with n-Bu_3SnH/CF_3CO_2H 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 **122** was susceptible to regioselective galloylation at C(3)(OH) (F) leading to useful synthetic access to the mono-*O*-gallate ester **124**.

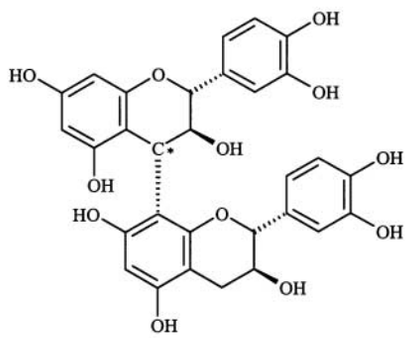
A process to synthesize (6 → 6)-, (6 → 8)- and (8 → 8)-linked catechin and epicatechin dimers as well as their 3,3-di-*O*-gallate esters was patented.[71] The protocol is based on the oxidative ($FeCl_3$) or reductive [Ni(0) reagents] coupling of protected monomers and is demonstrated in Scheme 13 for the (8 → 8)-bis-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 $FeCl_3$ 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**.



Scheme 13 Synthesis of (8 → 8)-bis-catechin **128** and its digallate **129**.

A considerable effort has been devoted to the production of, especially, ^{13}C- and, to a lesser extent, also ^{14}C-labelled catechins and proanthocyanidins. Administration of [U-^{14}C]-phenylalanine or [1-^{14}C]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 → 8)-catechin] and a related polymer of high radiopurity.[72] 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-^{13}C]phenylalanine into *Vitis vinifera* cell suspension cultures.[73]

Rac 4-[^{13}C]catechin **137** was synthesized by the sequence outlined in Scheme 14.[74] (*E*)-1-[^{13}C]-di-*O*-benzylcaffeic acid **131** was synthesized from CH_3–^{13}CN and 3,4-di-*O*-benzyl-benzaldehyde. Friedel–Crafts acylation of tri-*O*-benzyl-phloroglucinol **130** with **131** in TFAA afforded the labelled chalcone **132**. This was selectively deprotected with $TiCl_4$ and the resulting chalcone **133** was transformed into the racemic flav-3-ene **134** *via* successive reduction ($NaBH_4$) and Lewis acid ($BF_3{\cdot}OEt_2$) cyclization. Osmium-catalyzed dihydroxylation gave the flavan-3,4-diol derivative **135** with high diastereo-selectivity. Subsequent reduction with $Na(CN)BH_3$/HOAc gave the protected *rac*-catechin **136**, which was then hydrogenolized to afford *rac* 4-[^{13}C]catechin **137** (99% enrichment). The same synthetic sequence but without isotopic labelling was also used to provide access to both enantiomers of catechin.[75] 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-[^{13}C]catechin **137** and 4-[^{13}C]epicatechin.[76] The same theme was further exploited to synthesize gram quantities of 4-[^{13}C]procyanidin B3 [catechin-(4α → 8)-catechin] **138**.[77,78]



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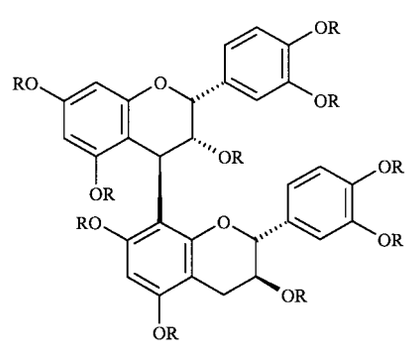
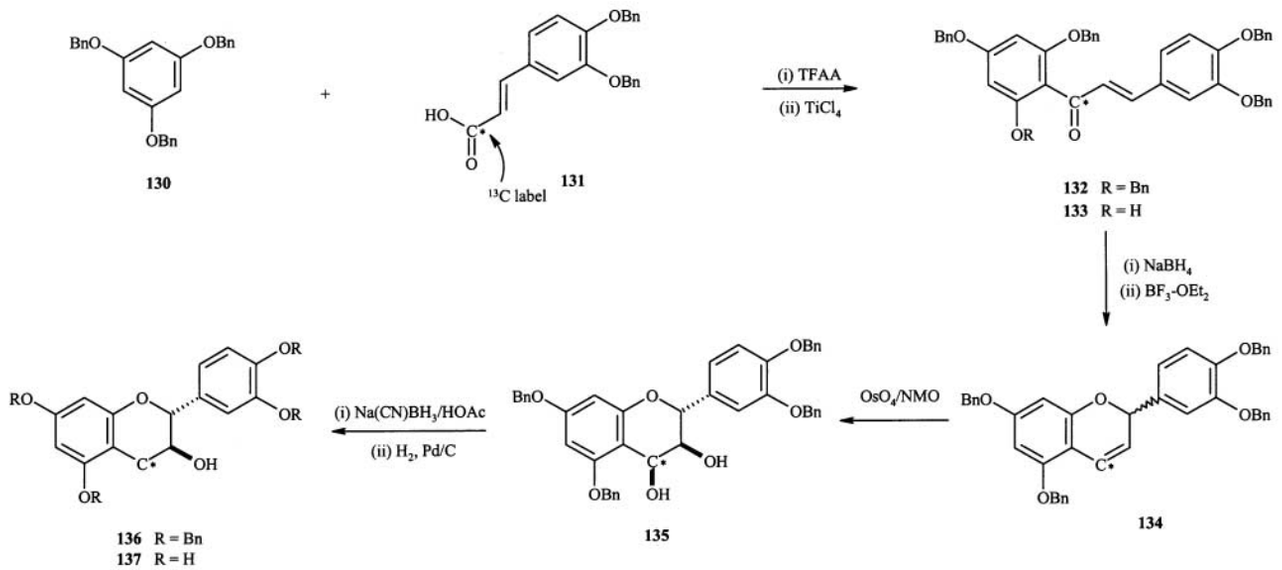
The structure of procyanidin B1 **92** was unequivocally confirmed by X-ray analysis of its deca-*O*-acetyl derivative **139**.[79]



92 R = H
139 R = Ac

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

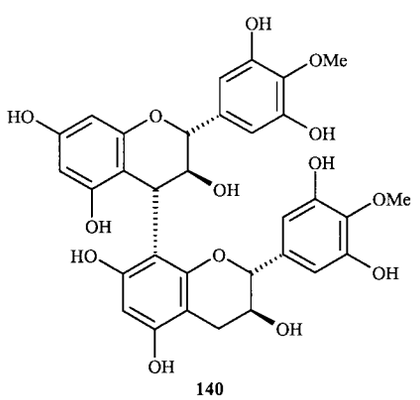
Besides the "mixed" procyanidin–prodelphinidin oligomers from *C. semenovii*[64] and *R. pamirolaica*[65,66] indicated in Section 4.1, the roots of the former plant also afforded a complex series of prodelphinidin oligomers.[80,81] These are compounds CS-1, 7-



Scheme 14 Synthesis of ^{13}C-labelled *rac*-catechin **137**.

O-[6-*O*-galloyl-β-D-Glcp-6 ⟶ *O*-β-D-Glcp-6 ⟶ *O*-β-D-Glcp-6 ⟶ *O*-β-D-Glcp]-(+)-gallocatechin-(4α ⟶ 8)-(+)-gallocatechin-(4α ⟶ 8)-(−)-epigallocatechin-(4β ⟶ 8)-(−)-epigallocatechin-(4β ⟶ 8)-(−)-epigallocatechin-(4β ⟶ 8)-(+)-catechin, CS-2,3-*O*-galloyl-7-*O*-(β-D-Glcp-6 ⟶ *O*-β-D-Glcp-(−)-epigallocatechin-(4β ⟶ 8)-[3-*O*-galloyl-(−)-epicatechin-(4β ⟶ 8)-[3-*O*-galloyl-(−)-epigallocatechin]-(4β ⟶ 8)-[3-*O*-galloyl-5-*O*-(6-*O*-galloyl-*O*-β-D-Glcp)]-(−)-epicatechin, rhodichimoside, 7-*O*-[β-D-Glcp-*O*-β-D-Glcp]$_2$-3-*O*-galloyl-(−)-epigallocatechin-(4β ⟶ 8)-[3-*O*-galloyl-(−)-epigallocatechin]-(4β ⟶ 8)-3-*O*-galloyl-(−)-epigallocatechin and rhodichin, 7-*O*-β-D-Glcp-3-*O*-galloyl-(−)-epigallocatechin-(4β ⟶ 8)-[(−)-epigallocatechin]$_2$-(4β ⟶ 8)-epigallocatechin-(4β ⟶ 6)-3-*O*-galloyl-(−)-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*.[82] Prodelphinidin polymers of undefined structure were also obtained from white clover (*Trifolium repens L.*) flowers[83] and *Onobrychis viciifolia* (*sainfoin*).[84]



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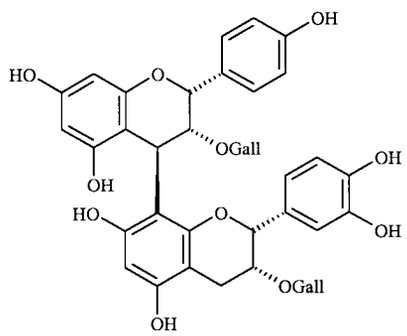
4.3 Propelargonidins (3,5,7,4′-tetrahydroxylation)

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*-Galloylepiafzelechin-(4β ⟶ 8)-3-*O*-galloylepicatechin **141** and 3-*O*-galloylepiafzelechin-(4β ⟶ 6)-3-*O*-galloylepicatechin **142** were isolated from green tea,[85] yet again demonstrating the remarkable diversity of the polyphenolic pool of this natural source.



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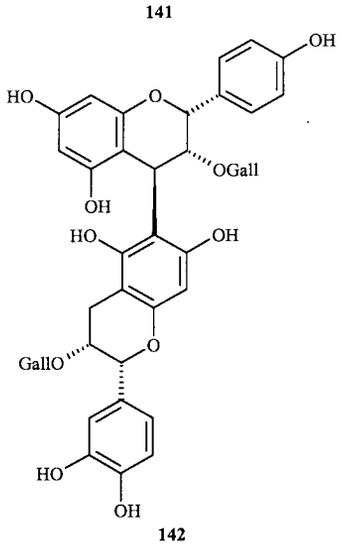

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4.4 Profisetinidins (3,7,3′,4′-tetrahydroxylation) and prorobinetinidins (3,7,3′,4′,5′-pentahydroxylation)

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

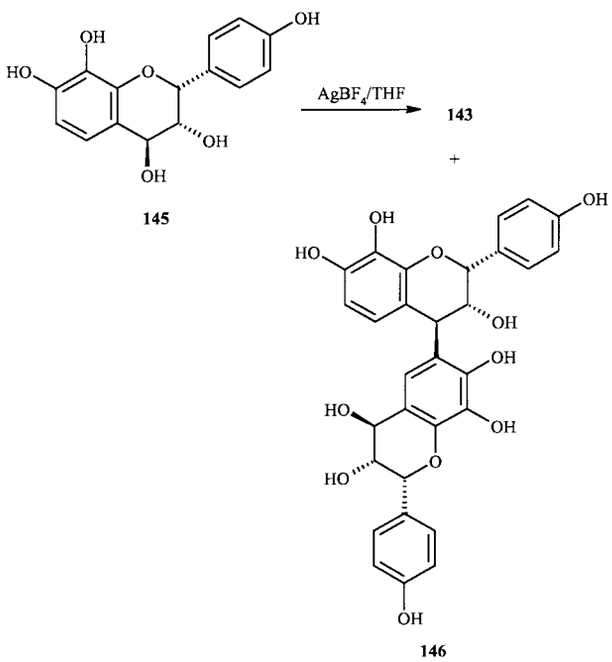
4.5 Proteracacinidins (3,7,8,4′-tetrahydroxylation) and promelacacinidins (3,7,8,3′,4′-pentahydroxylation)

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.

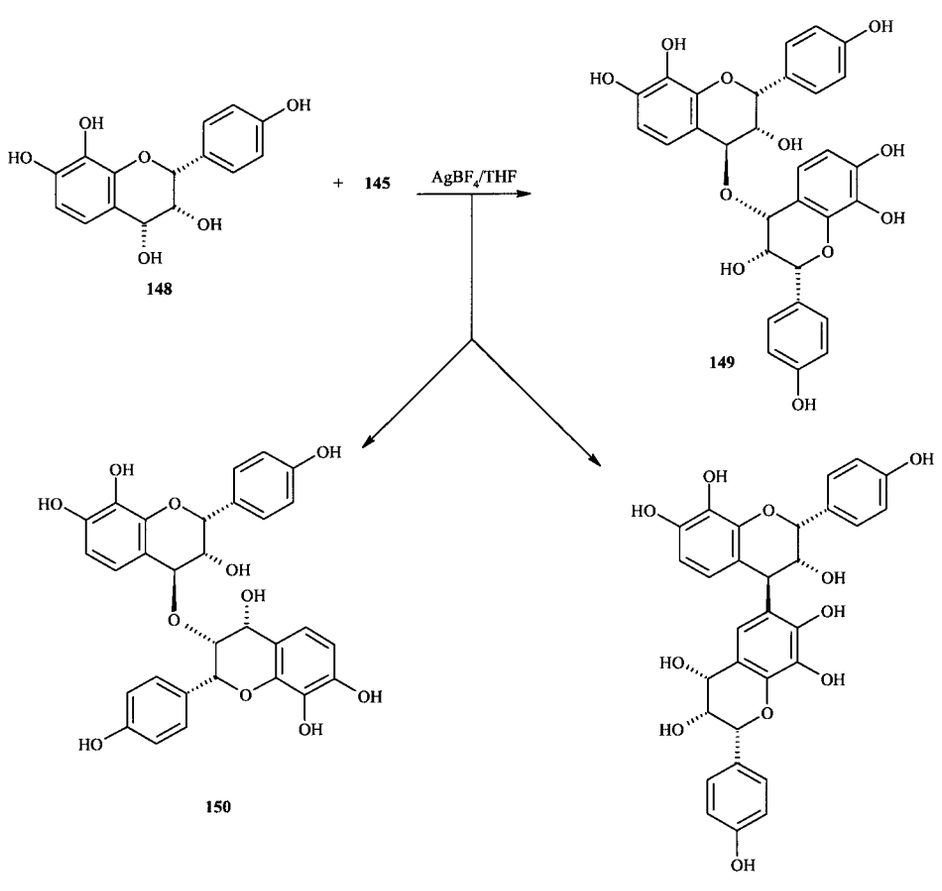
The proanthocyanidin pool in plants usually involves the presence of carbon–carbon bonds linking predominantly flavan-3-ol constituent moieties.[1–5] Such an ensemble of flavan-3-ol units originates *via* electrophilic aromatic substitution of flavan-4-yl carbocations (or their equivalents) presumably 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.[1]

Epioritin-(4β ⟶ 3)-epioritin-4β-ol **143** and epimesquitol-(4β ⟶ 4)-epioritin-4β-ol **144**, the first biflavanoid containing both leucomelacacinidin and leucoteracacinidin units, were isolated from the heartwood of *Acacia caffra*.[86] 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 $AgBF_4$ 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**.[87] The epimesquitol-(4β ⟶ 4)-epioritin-4β-ol **144** and compound **146** were similarly formed when a mixture of 4β-benzylsulfanylepimesquitol **147** and epioritin-4β-ol was treated with $AgBF_4$ 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.[86]

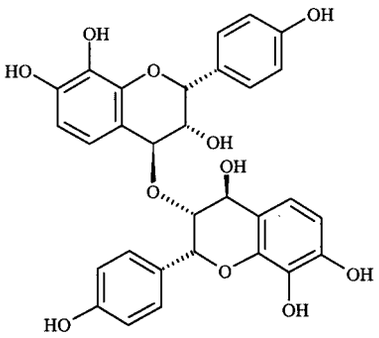
The first triflavanoids possessing both C–C and C–O–C interflavanyl bonds, epioritin-(4β ⟶ 6)-epioritin-(4α ⟶ 4)-



Scheme 15 $AgBF_4$-catalyzed self-condensation of epioritin-4β-ol **145**.



Scheme 16 Synthesis of ether-linked proteracacinidins **149** and **151** and the C–C coupled analogue **151**.



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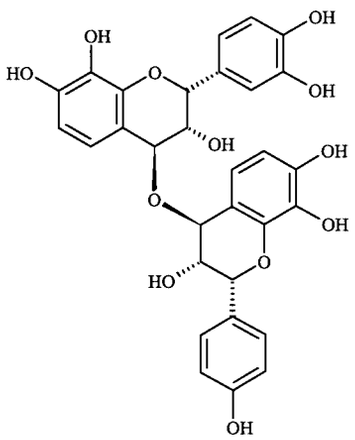

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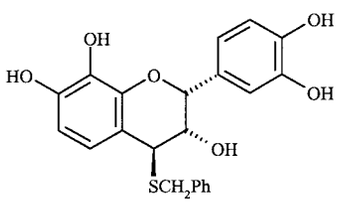

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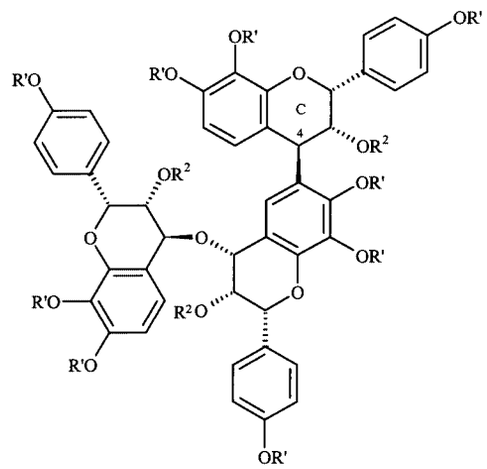
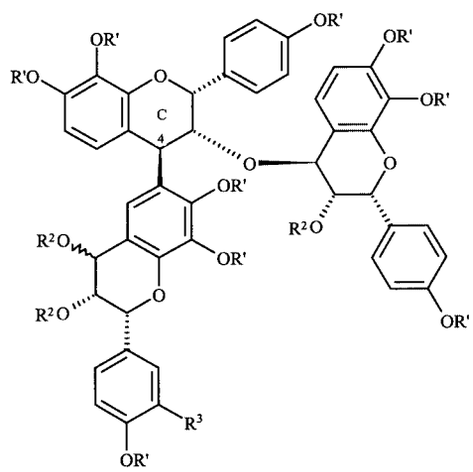
epioritin-4β-ol **152**, epioritin-(4β ⟶ 3)-epioritin-(4β ⟶ 6)-epioritin-4β-ol **154**, and epioritin-(4β ⟶ 3)-epioritin-(4β ⟶ 6)-epimesquitol-4α-ol **156**, as well as the symmetrical dimeric epioritin-(4β ⟶ 4)-epioritin-4β-ol **158** were also identified in the heartwood of *A. caffra*.[88]

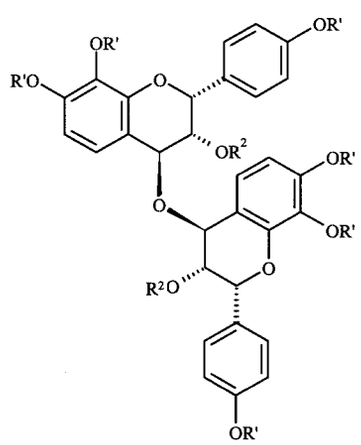
The absence of a second aromatic chromophore in close proximity of the C-4 stereocentre in ether-linked leucoanthocyanidins at both the di- and tri-meric levels precludes assessment of their absolute configuration by chiroptical methods. Trimeric derivatives **153**, **155 and 157** exhibited negative 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 ^{1}Lb transitions of all the constituent units exhibiting 2*R* absolute configuration.[19,20] Positive Cotton effects in the 220–250 nm region were then reminiscent of ^{1}La transitions as well as contributions resulting from the biphenylmethylidene chromophore at C(4)(C).[89,90] 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



152 R' = R² = H
153 R' = Me, R² = Ac

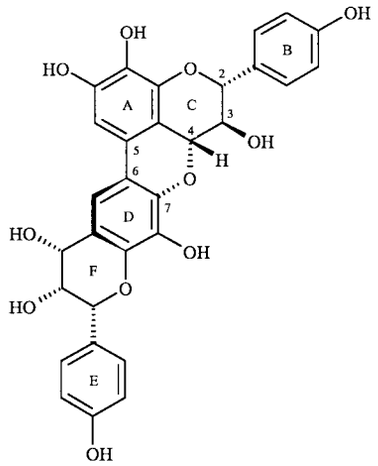


154 ≡ , R' = R² = R³ = H
155 ≡ , R' = Me, R² = Ac, R³ = H
156 ≡ , R' = R² = H, R³ = OH
157 ≡ , R' = Me, R² = Ac, R³ = OMe



158 R' = R² = H
159 R' = Me, R² = Ac

presumably preferentially cleaved due to the anchimeric effect of the *axial* C(3)–OH bond.[87] 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**. The



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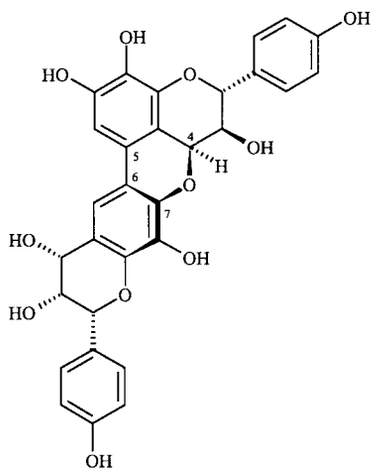

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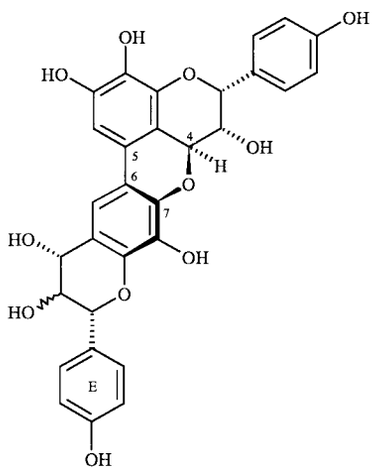

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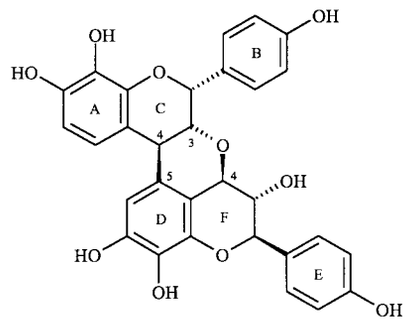
167



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absolute configuration of the flavan-3-ols could then be determined by comparison of their CD spectra[19] with those of authentic samples.

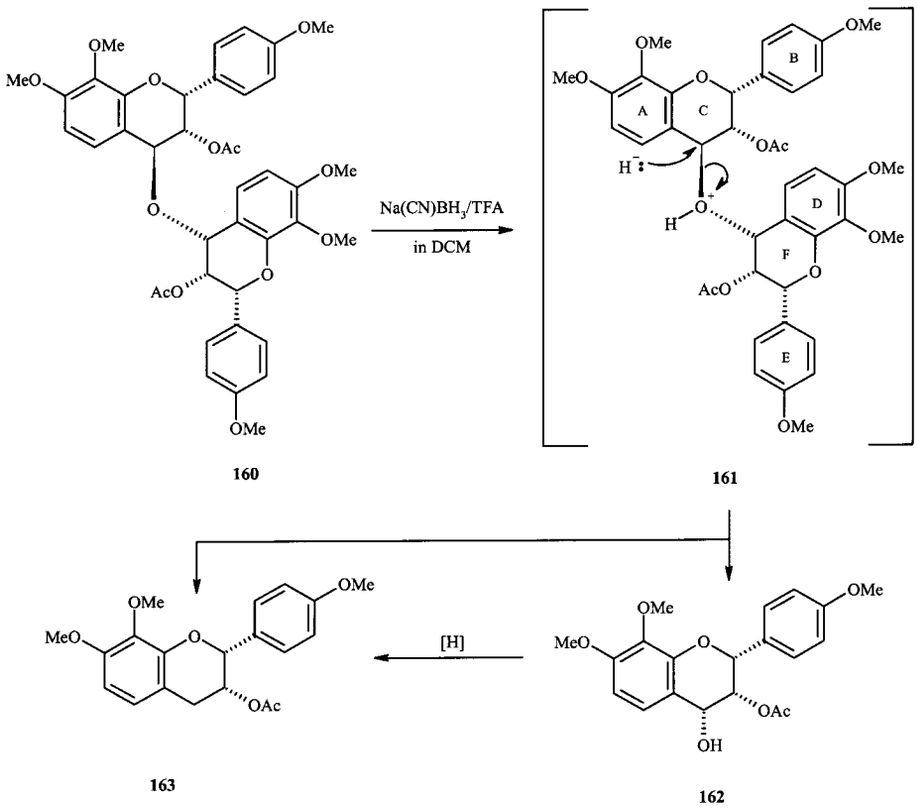
The rare series of doubly-linked proteracacinidin-type oligoflavanoids was extended by identification of four new analogues, oritin-(4α → 7,5 → 6)-epioritin-4α-ol **164**, oritin-(4β → 7,5 → 6)-epioritin-4α-ol **165**, epioritin-(4β → 7,5 → 6)-epioritin-4α-ol **166**, epioritin-(4β → 7,5 → 6)-epioritin-4α-ol **167** and epioritin-(4β → 5,3 → 4)-oritin-4α-ol **168**.[91] 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.[92] 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.[89,90] 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)

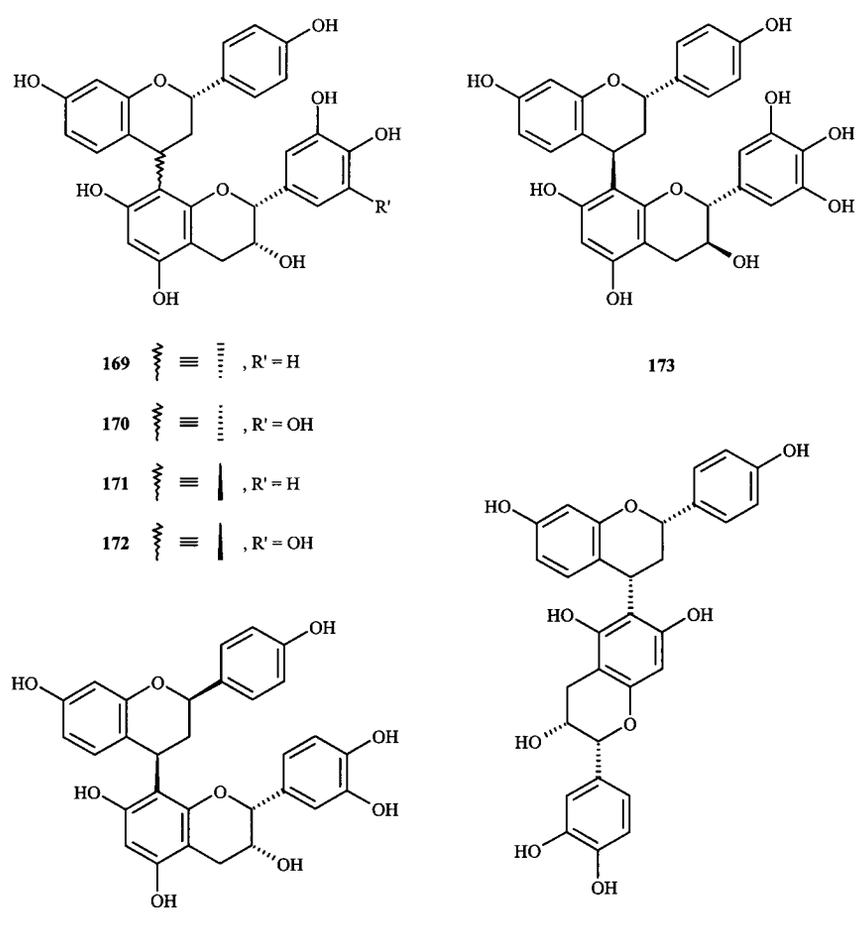
In the previous review,[1] two procassinidin analogues with catechin bottom units were listed under "Proanthocyanidins with flavan chain extender units". The procassinidins represent a rare group of naturally occurring proanthocyanidins and only four compounds have thus far been reported. Seven new analogues 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.[93] These compounds are cassiaflavan-(4α → 8)-epicatechin **169**, cassiaflavan-(4α → 8)-epigallocatechin **170**, cassiaflavan-(4β → 8)-epicatechin **171**, cassiaflavan-(4β → 8)-epigallocatechin **172**, cassiaflavan-(4β → 8)-gallocatechin **173**, *ent*-cassiaflavan-(4β → 8)-epicatechin **174**, and cassiaflavan-(4α → 6)-epicatechin **175**. Synthesis as the permethylaryl acetate derivatives was done by reduction of the flavanone, including the optically pure (2*S*)-di-*O*-methylliquiritigenin, to the flavan-4-ol which then served as electrophile in the Lewis acid ($TiCl_4$) catalyzed coupling with the appropriate flavan-3-ol permethylaryl ether, *e.g.* penta-*O*-methylepi- or -gallocatechin.[93]

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

The bark of *A. petersiana* also afforded three dimers with a

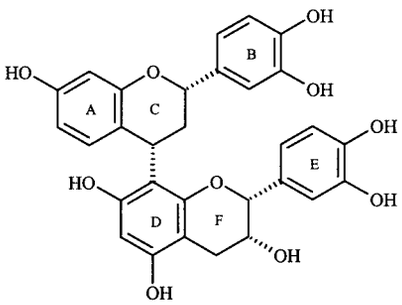


Scheme 17 Reductive cleavage of the C–O–C bond in leucoteracacinidin derivative **160**.



7,3′,4′-trihydroxylation chain extender unit.[94] Owing to the close structural relationship of the ABC moiety, *i.e.* the chain extender unit, in compounds **176**–**178** with the (2*S*)-7,3′,4′-trihydroxyflavanone, butin, the trivial name butiniflavan, was proposed for the (2*S*)-7,3′,4′-trihydroxyflavan ABC unit. *Ent*-butiniflavan is a (2*R*)-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 butiniflavan-(4α → 8)- and (4β → 8)-epicatechins **176** and **177**, and butiniflavan-(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 $TiCl_4$ as Lewis acid.



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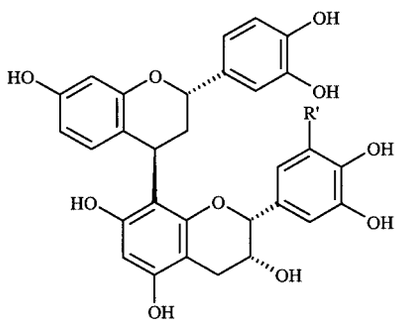


177 R' = H
178 R' = OH

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).[95,96] The genesis of these compounds was explained as follows. Oxidation of theaflavin **179** (Scheme 19) affords the *o*-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 dienophilic 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.[96]

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.[97] 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,[98] while epigallocatechin gallate **12**, a main constituent of green tea, was demonstrated to possess potent tumor necrosis factor-α (TNF-α) release inhibiting activity.[99]

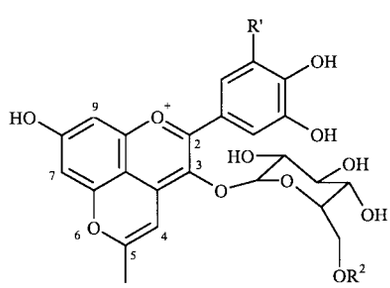
Complexation of the theaflavin group of polyphenols with caffeine is thought to be largely responsible for the formation of tea cream, the precipitate that forms as tea cools. The self-association of theaflavin **179** with caffeine was thus studied using ^{1}H NMR techniques.[100] These studies indicated 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-2*H*-1-benzopyran-3,5,7-triol moieties 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]

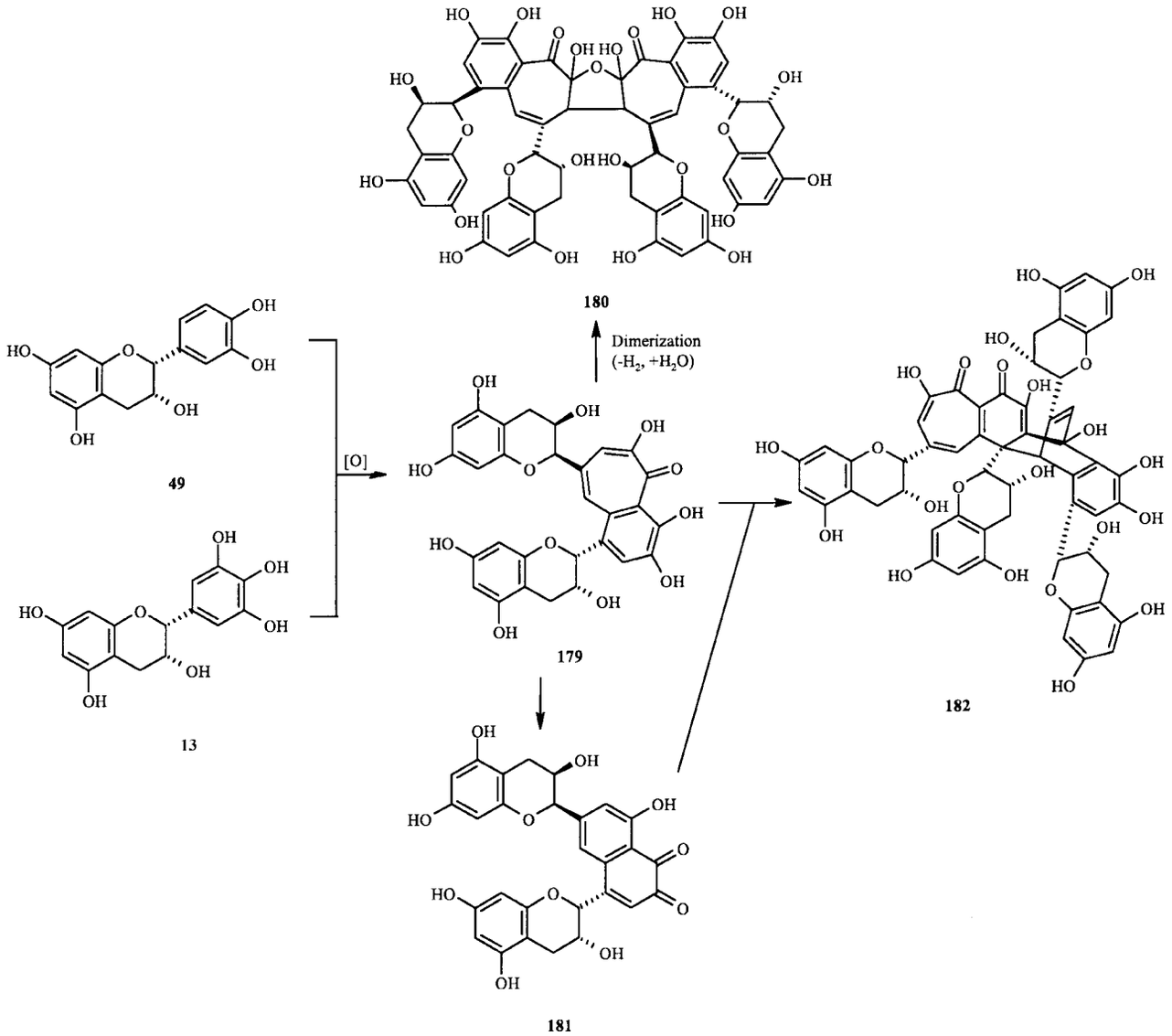
5 Miscellaneous

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.

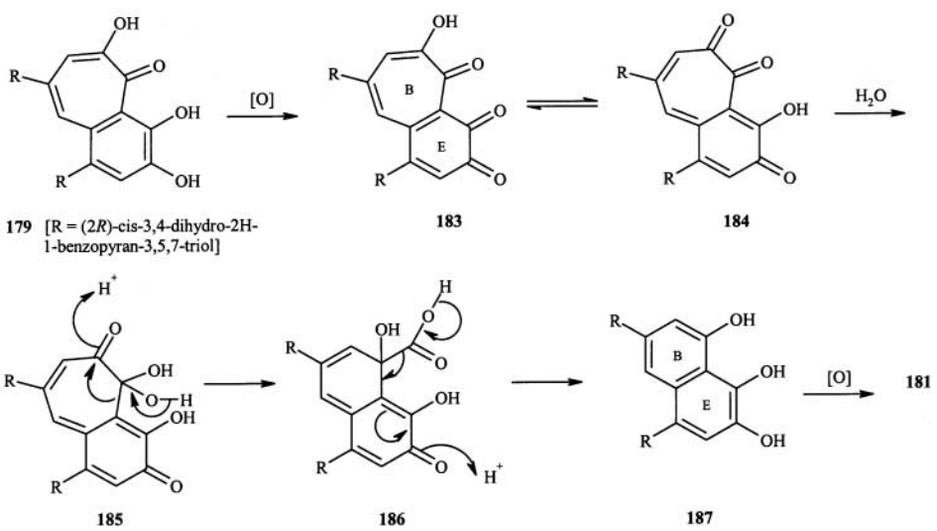
Four new pyranoanthocyanins, pyranocyanins A and B, **188** and **190**, and pyranodelphinidins A and B, **189** and **191**, were isolated from the seed of *Ribes nigrum* (black currant).[103] It was subsequently demonstrated that compounds **188**–**191** were indeed artefacts resulting from oxidative addition of acetone, the solvent used for extraction, to the parent anthocyanins present in black currant seed.[104] The formation of pyranoanthocyanins was explained *via* the sequence outlined in Scheme 20. Thus, the cycloaddition between acetone and a flavylium 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]



188 R' = H, R^2 = rhamnosyl
189 R' = OH, R^2 = rhamnosyl
190 R' = H, R^2 = H
191 R' = OH, R^2 = H



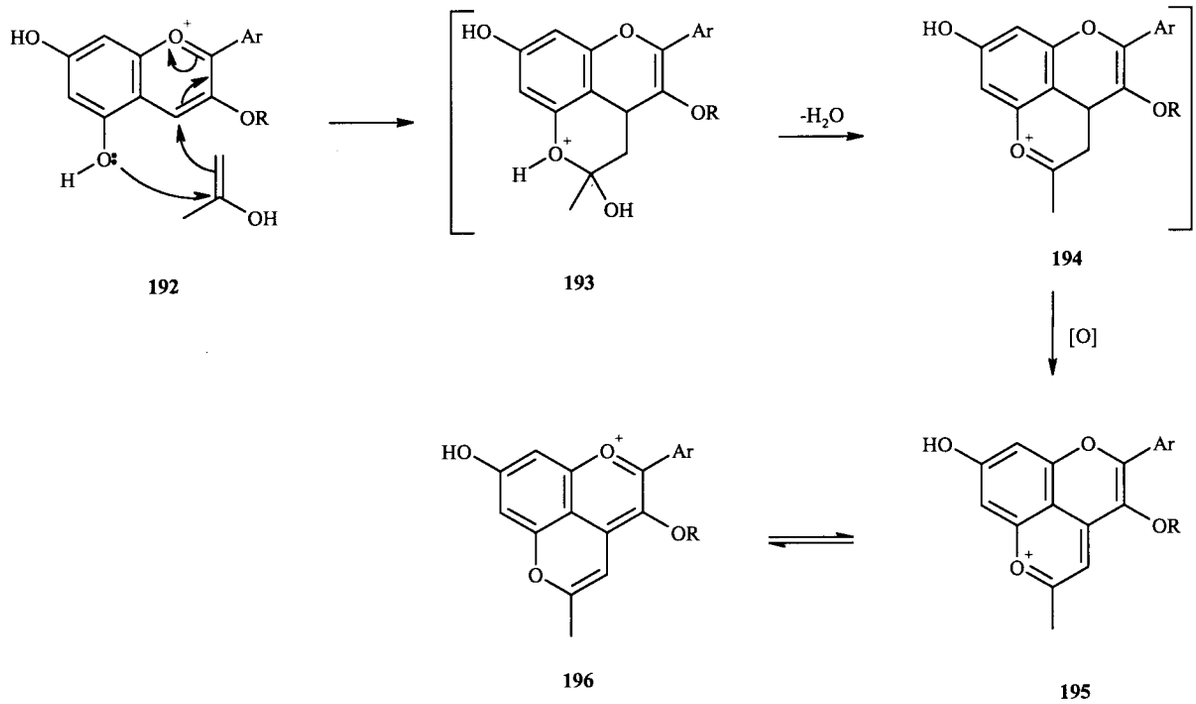
Scheme 18 Oxidation of compounds **49** and **13** using banana fruit homogenate.



Scheme 19 Proposed mechanism for the formation of theanaphthoquinone **181**.

6 HPLC/MS analysis of proanthocyanidins

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).[108,109] Most of these techniques are capable of adequately separating monomers, dimers and trimers, but



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

are unable to resolve the more structurally diverse higher oligomers.[108]

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.[110] 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[111] 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,[112] cocoa (*Theobroma cacao*),[113] quantification of procyanidins in cocoa and correlation to total antioxidant capacity,[114] identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium spp.*),[115] and analysis of polymeric proanthocyanidins from grape (*Vitis vinifera*) seeds.[116,117] 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.[118]

The utility of the HPLC/MS protocol is demonstrated for analysis of the procyanidins from grape seeds.[117] 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.

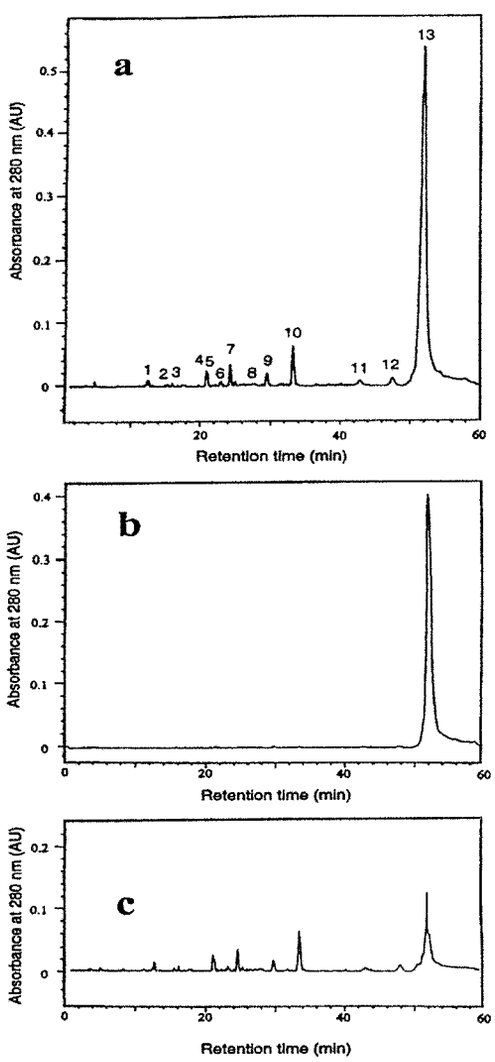


Fig. 1 Reversed-phase HPLC of grape seed extract before ultrafiltration (a), after ultrafiltration through a 3000 Da NMWCO membrane (b), the filtrate after ultrafiltration (c).

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

m/z	Compound[a,b]			
291	P_1			
443		P_1G_1		
579	P_2			
731		P_2G_1		
867	P_3			
1019		P_3G_1		
1155	P_4			
1171			P_3G_2	
1307		P_4G_1		
1443	P_5			
1459			P_4G_2	
1595		P_5G_1		
1731	P_6			
1748			P_5G_2	
1884		P_6G_1		
2021	P_7			
2036			P_6G_2	
2172		P_7G_1		
2188				P_6G_3
2310	P_8			
2324			P_7G_2	
2460		P_8G_1		
2477				P_7G_3
2612			P_8G_2	
2749		P_9G_1		
2902			P_9G_2	

[a] Tentative assignment based on molecular weights. All species tabulated were singly charged. [b] Abbreviations: P, procyanidin; P_2, procyanidin dimer, *etc.*; G, gallate; G_1, monogallate, *etc.*

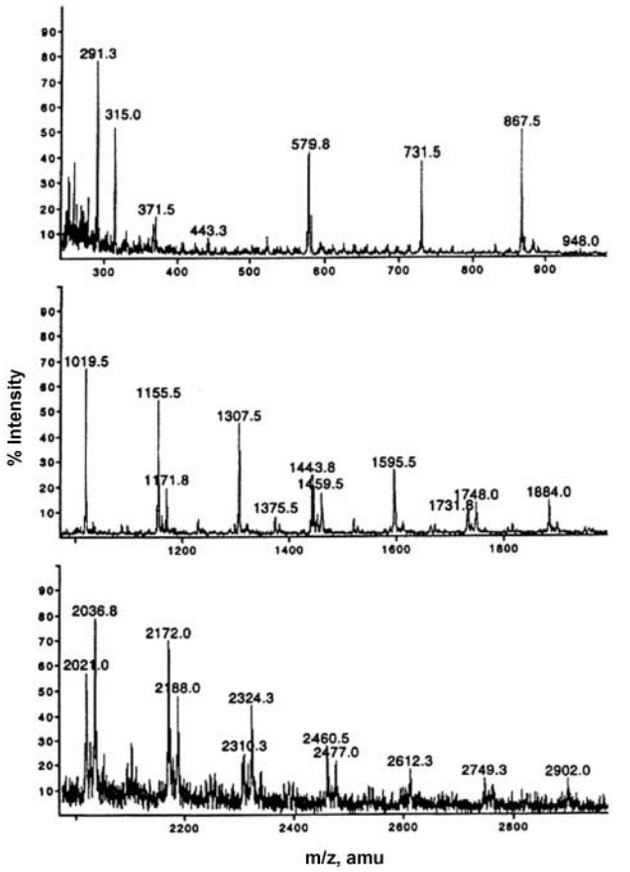


Fig. 2 ESI/MS data for the procyanidin polymer peak of grape seed extracts. Data is represented in three *m/z* ranges: 250–1000, 1000–2000 and 2000–3000.

1(c). Peaks 1–12 in Fig. 1(a) were identified by LC-ESI/MS as gallic acid (1), procyanidin trimer (P_3) (2), procyanidin tetramer (P_4) (3), procyanidin dimer (P_2) (4), procyanidin dimer (P_2) (5), procyanidin trimer (P_3) (6), catechin (7), procyanidin dimer (P_2) (8), procyanidin dimer (P_2) (9), epicatechin (10), monogalloylated procyanidin dimer (P_2G_1) (11), and epicatechin 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.*[113] 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.[112] 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 ^{13}C NMR methodology, as well as the implementation of molecular mechanics and molecular orbital calculations that were discussed in the previous review,[1] 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 polyflavanoids and their association with proteins was also published.[119]

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, antioxidant and radical-scavenging properties.[120] Their tendency to interfere with biological systems results, at least in part, from the characteristic ability to form complexes with other biomolecules.[121] The increasing interest in the use of "natural remedies" and of appropriate diets to control disease and illness 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.[122] A number of other useful contributions pertaining to the nutritional and health promoting properties of the proanthocyanidins and related polyphenols are listed as refs. 123–130. Insofar as the possible modes of action of natural polyphenols present in foodstuffs and beverages, and as constituents of herbal medicines, is concerned there is circumstantial *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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10 References

1 D. Ferreira and X-C. Li, *Nat. Prod. Rep.*, 2000, **17**, 193.
2 L. J. Porter, in *The Flavonoids. Advances in Research Since 1986*, ed. J. B. Harborne, Chapman and Hall, London, 1994, p. 23.
3 D. Ferreira and R. Bekker, *Nat. Prod. Rep.*, 1996, **13**, 411.
4 D. Ferreira, R. J. J. Nel and R. Bekker, in *Comprehensive Natural Products Chemistry*, ed. D. H. R. Barton, K. Nakanishi, O. Meth-Cohn and B. M. Pinto, Elsevier, New York, 1999, vol. 3, p. 747.
5 D. Ferreira, E. V. Brandt, J. Coetzee and E. Malan, *Fortschr. Chem. Org. Naturst.*, 1999, **77**, 21.
6 H. Geiger, in *The Flavonoids. Advances in Research Since 1986*, ed. J. B. Harborne, Chapman and Hall, London, 1994, p. 95.
7 R. Bekker, E. V. Brandt and D. Ferreira, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2535.
8 R. Bekker, E. V. Brandt and D. Ferreira, *Tetrahedron*, 1999, **55**, 10005.
9 R. Bekker, E. V. Brandt and D. Ferreira, *Tetrahedron*, 2000, **56**, 5297.
10 R. W. Hemingway, L. Y. Foo and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1209.
11 *Flavonoids in Health and Disease*, ed. C. Rice-Evans and L. Packer, Dekker, New York, 1997.
12 F. Moyo, B. A. Gashe and R. R. T. Mejinda, *Fitoterapia*, 1999, **70**, 412.
13 S. Piacente, L. Balderrama, N. De Tommase, L. Morales, L. Vargas and C. Pizza, *Phytochemistry*, 1999, **51**, 709.
14 R. J. J. Nel, M. Mthembu, J. Coetzee, H. van Rensburg, E. Malan and D. Ferreira, *Phytochemistry*, 1999, **52**, 1153.
15 M. A. Ramadan, M. S. Kamel, K. Ohtani, R. Kasai and K. Yamasaki, *Phytochemistry*, 2000, **54**, 891.
16 B. Y. Hwang, H. S. Kim, J. H. Lee, Y. S. Hong, J. S. Ro, K. S. Lee and J. J. Lee, *J. Nat. Prod.*, 2001, **64**, 82.
17 M. Cuendet, O. Potterat and K. Hostettmann, *Phytochemistry*, 2001, **56**, 631.
18 O. Korver and C. K. Wilkins, *Tetrahedron*, 1971, **27**, 5459.
19 H. van Rensburg, P. J. Steynberg, J. F. W. Burger, P. S. van Heerden and D. Ferreira, *J. Chem. Res. (S)*, 1999, 450.
20 R. J. J. Nel, H. van Rensburg, P. S. van Heerden and D. Ferreira, *J. Chem. Res. (S)*, 1999, 606 (*M*, 2610).
21 W. Fan, Y. Tezuka, Q. Xiong, M. Hattori, T. Namba and S. Kadota, *Chem. Pharm. Bull.*, 1999, **47**, 1049.
22 H. van Rensburg, P. S. van Heerden and D. Ferreira, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3415.
23 S. Valcic, A. Muders, N. E. Jacobsen, D. C. Liebler and B. N. Timmermann, *Chem. Res. Toxicol.*, 1999, **12**, 382.
24 S. Valcic, J. A. Burr, B. N. Timmermann and D. C. Liebler, *Chem. Res. Toxicol.*, 2000, **13**, 801.
25 C. Cren-Olivé, S. Lebrun, P. Hapiot, J. Pinson and C. Rolando, *Tetrahedron Lett.*, 2000, **41**, 5847.
26 M. S. van Dyk, J. P. Steynberg, P. J. Steynberg and D. Ferreira, *Tetrahedron Lett.*, 1990, **31**, 2643.
27 W. Janse van Rensburg, D. Ferreira, E. Malan and J. A. Steenkamp, *Phytochemistry*, 2000, **53**, 285.
28 E. Young, E. V. Brandt, D. A. Young, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1737.
29 N-E. Es-Safi, H. Fulcrand, V. Cheynier and M. Moutounet, *J. Agric. Food Chem.*, 1999, **47**, 2088.
30 N-E. Es-Safi, H. Fulcrand, V. Cheynier and M. Moutounet, *J. Agric. Food Chem.*, 1999, **47**, 2096.
31 N-E. Es-Safi, C. Le Guernevé, H. Fulcrand, V. Cheynier and M. Moutounet, *J. Agric. Food Chem.*, 1999, **47**, 5211.
32 N-E. Es-Safi, C. Le Guernevé, V. Cheynier and M. Moutounet, *J. Agric. Food Chem.*, 2000, **48**, 4233.
33 N-E. Es-Safi, V. Cheynier and M. Moutounet, *J. Agric. Food Chem.*, 2000, **48**, 5946.
34 N-E. Es-Safi, C. Le Guernevé, B. Labarbe, H. Fulcrand, V. Cheynier and M. Moutounet, *Tetrahedron Lett.*, 1999, **40**, 5869.
35 N-E. Es-Safi, C. Le Guernevé, V. Cheynier and M. Moutounet, *Tetrahedron Lett.*, 2000, **41**, 1917.
36 J. L. Torres and R. Bobet, *J. Agric. Food Chem.*, 2001, **49**, 4627.
37 T. Kori, F. Nanjou, R. Seto and T. Suzuki, *Jpn. Kokai Tokkyo Koho Pat. no. JP 200113169*, 2001.
38 L-Q. Wang, M. R. Meselhy, Y. Li, N. Nakamura, B-S. Min, G-W. Qin and M. Hattori, *Chem Pharm. Bull.*, 2001, **49**, 1640.
39 K. Muramatsu, M. Fukuyo and Y. Hara, *J. Nutr. Sci. Vitaminol.*, 1986, **32**, 613.
40 I. Abe, T. Seki, K. Umehara, T. Miyase, H. Noguchi, J. Sakakibara and T. Uno, *Biochem. Biophys. Res. Commun.*, 2000, **268**, 767.
41 K. Saeki, S. Hayakawa, M. Isemura and T. Miyase, *Phytochemistry*, 2000, **53**, 391.
42 R. Boges-Argáez, L. M. Peña-Rodríques and P. G. Waterman, *Phytochemistry*, 2000, **54**, 611.
43 J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 2000, **56**, 1819.
44 J. W. Clark-Lewis and L. R. Williams, *Aust. J. Chem.*, 1967, **20**, 2152.
45 J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 1999, **55**, 9999.
46 C. Pouget, C. Fagnere, J-P. Basly, H. Leveque and A-J. Chulia, *Tetrahedron*, 2000, **56**, 6047.
47 H. Lou, Y. Yamazaki, T. Sasaki, M. Uchida, H. Tanaka and S. Oka, *Phytochemistry*, 1999, **51**, 297.
48 P. J. Steynberg, A. Cronje, J. P. Steynberg, B. C. B. Bezuidenhoudt, E. V. Brandt and D. Ferreira, *Tetrahedron*, 1997, **53**, 259.
49 M. S. M. Rawat, D. Prasad, R. K. Joshi and G. Pant, *Phytochemistry*, 1999, **50**, 321.
50 D. Prasad, *Fitoterapia*, 2000, **71**, 245.
51 A. L. B. S. Barreiros, J. P. David, L. P. de Queroz and J. M. David, *Phytochemistry*, 2000, **55**, 805.
52 C-M. Ma, N. Nakamura, M. Hattori, H. Kakuda, J-C. Qiao and H-L. Yu, *J. Nat. Prod.*, 2000, **63**, 238.
53 F. Calzada, C. M. Cerda-Garciá-Rojas, M. Meckes, R. Cedillo-Rivera, R. Bye and R. Mata, *J. Nat. Prod.*, 1999, **62**, 705.
54 F. Calzada, R. Cedillo-Rivera, R. Bye and R. Mata, *Planta Med.*, 2001, **67**, 677.
55 K. Kamiya, C. Watanabe, H. Endang, M. Umar and T. Satake, *Chem. Pharm. Bull.*, 2001, **49**, 551.
56 A. B. Howell, N. Vorsa, A. B. DerMarderosian and L. Y. Foo, *New Engl. J. Med.*, 1998, **339**, 1085, and cited references.
57 J. Avorn, M. Monane, J. H. Gurwitz, R. J. Glynn, I. Choodnovskiy and L. A. Lipsitz, *J. Am. Med. Assoc.*, 1994, **271**, 751.
58 E. H. Beachey, *J. Infect. Dis.*, 1981, **143**, 325.
59 L. Y. Foo, Y. Lu, A. B. Howell and N. Vorsa, *Phytochemistry*, 2000, **54**, 173.
60 L. Y. Foo, Y. Lu, A. B. Howell and N. Vorsa, *J. Nat. Prod.*, 2000, **63**, 1225.
61 K. Kondo, M. Kurihara, K. Fukuhara, T. Tanaka, T. Suzuki, N. Miyata and M. Toyoda, *Tetrahedron Lett.*, 2000, **41**, 485.
62 T. Tanaka, K. Kondo and I. Kouno, *Phytochemistry*, 2000, **53**, 311.
63 J-N. Wang, Y. Hano, T. Nomura and Y-J. Chen, *Phytochemistry*, 2000, **53**, 1097.
64 K. N. Matamarova, Z. A. Kuliev, A. D. Vdovin, N. D. Abdullaev and B. M. Murzubraimov, *Chem. Nat. Compd.*, 1999, **35**, 39.
65 A. E. Ismailov, Z. A. Kuliev, A. D. Vdovin, N. D. Abdullaev and B. M. Murzubraimov, *Chem. Nat. Compd.*, 1999, **35**, 33.
66 A. E. Ismailov, Z. A. Kuliev, A. D. Vdovin, N. D. Abdullaev and B. M. Murzubraimov, *Chem. Nat. Compd.*, 1998, **34**, 450.
67 W. Tückmantel, A. P. Kozikowski and L. J. Romanczyk, Jr., *J. Am. Chem. Soc.*, 1999, **121**, 12073.
68 A. P. Kozikowski, W. Tückmantel and C. George, *J. Org. Chem.*, 2000, **65**, 5371.
69 D. H. R. Barton, D. O. Jang and J. Cs. Jaszberenyi, *J. Org. Chem.*, 1993, **58**, 6838.
70 A. P. Kozikowski, W. Tückmantel and Y. Hu, *J. Org. Chem.*, 2001, **66**, 1287.
71 W. Tückmantel, A. P. Kozikowski and L. J. Romanczyk, *Int. Pub. No., WO 00/61547*, 2000.
72 S. Déprez, I. Mila and A. Scalbert, *J. Agric. Food Chem.*, 1999, **47**, 4219.
73 S. Krisa, P. W. Téguo, A. Decendit, G. Deffieux, J. Vercauteren and J-M. Mérillon, *Phytochemistry*, 1999, **51**, 651.
74 B. Nay, V. Arnaudinaud, J-P. Peyrat, A. Nuhrich, G. Deffieux, J-M. Mérillon and J. Vercauteren, *Eur. J. Org. Chem.*, 2000, 1279.
75 B. Nay, J-P. Monti, A. Nuhrich, G. Deffieux, J-M. Mérillon and J. Vercauteren, *Tetrahedron Lett.*, 2000, **41**, 9049.
76 B. Nay, V. Arnaudinaud and J. Vercauteren, *Eur. J. Org. Chem.*, 2001, 2379.
77 V. Arnaudinaud, B. Nay, A. Nuhrich, G. Deffieux, J-M. Mérillon, J-P. Monti and J. Vercauteren, *Tetrahedron Lett.*, 2001, **42**, 1279.
78 V. Arnaudinaud, B. Nay, S. Vergé, A. Nuhrich, G. Deffieux, J-M. Mérillon, J-P. Monti and J. Vercauteren, *Tetrahedron Lett.*, 2001, **42**, 5669.
79 K. Weinges, H. Shick and F. Rominger, *Tetrahedron*, 2001, **57**, 2327.
80 K. N. Matamarova, Z. A. Kuliev, A. D. Vdovin and N. D. Abdullaev, *Chem. Nat. Prod.*, 1998, **34**, 676.
81 Z. A. Kuliev, K. Kh. Kim, A. D. Vdovin, N. D. Abdullaev, Z. A. Khushbaktova and V. N. Syrov, *Chem. Nat. Prod.*, 2000, **36**, 60.
82 J. C. P. de Mello, F. Petereit and A. Nahrstedt, *Phytochemistry*, 1999, **51**, 1105.
83 L. Y. Foo, Y. Lu, A. L. Molan, D. R. Woodfield and W. C. McNabb, *Phytochemistry*, 2000, **54**, 539.
84 J. P. J. Marais, I. Mueller-Harvey, E. V. Brandt and D. Ferreira, *J. Agric. Food Chem.*, 2000, **48**, 3440.
85 C. Lakenbrink, U. H. Engelhardt and V. Wray, *J. Agric. Food Chem.*, 1999, **47**, 4621.

86 L. Bennie, E. Malan, J. Coetzee and D. Ferreira, *Phytochemistry*, 2000, **53**, 785.
87 J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 1998, **54**, 9153.
88 L. Bennie, J. Coetzee, E. Malan and D. Ferreira, *Phytochemistry*, 2001, **57**, 1023.
89 G. G. de Angelis and W. C. Wildman, *Tetrahedron*, 1969, **25**, 5099.
90 J. H. van der Westhuizen, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1220.
91 L. Bennie, J. Coetzee, E. Malan, J. R. Woolfrey and D. Ferreira, *Tetrahedron*, 2000, **57**, 661.
92 J. C. Craig and S. K. Roy, *Tetrahedron*, 1965, **21**, 395.
93 J. Coetzee, L. Mciteka, E. Malan and D. Ferreira, *Phytochemistry*, 2000, **53**, 795.
94 J. Coetzee, L. Mciteka, E. Malan and D. Ferreira, *Phytochemistry*, 1999, **52**, 737.
95 T. Tanaka, Y. Betsumiya, C. Mine and I. Koune, *Chem. Commun.*, 2000, 1365.
96 T. Tanaka, K. Inoue, Y. Betsumiya, C. Mine and I. Koune, *J. Agric. Food Chem.*, 2001, **49**, 5785.
97 N. Subramanian, P. Venkatesh, S. Ganguli and V. P. Sinkar, *J. Agric. Food Chem.*, 1999, **47**, 2571.
98 L. K. Leung, Y. Su, R. Chen, Z. Zhang, Y. Huang and Z-Y. Chen, *J. Nutr.*, 2001, **131**, 2248.
99 S. Okabe, M. Suganuma, Y. Imayoshi, S. Taniguchi, T. Yoshida and H. Fujiki, *Biol. Pharm. Bull.*, 2001, **24**, 1145.
100 A. J. Charlton, A. L. Davis, D. P. Jones, J. R. Lewis, A. P. Davies, E. Haslam and M. P. Williamson, *J. Chem. Soc., Perkin Trans. 2*, 2000, 317.
101 Y-J. Zhang, Y. Betsumiya, A. Matsuo, T. Ueda, T. Tanaka and I. Kouno, *Peptide Sci.*, 1999, 315.
102 V. de Freitas and N. Mateus, *J. Agric. Food Chem.*, 2001, **49**, 940.
103 Y. Li, Y. Sun and L. Y. Foo, *Tetrahedron Lett.*, 2000, **41**, 5975.
104 Y. Lu and L. Y. Foo, *Tetrahedron Lett.*, 2001, **42**, 1371.
105 C. Garcia-Viguera, P. Zafrilla and F. A. Tomas-Barberan, *Phytochem. Anal.*, 1998, **9**, 274.
106 N. Mateus, A. M. S. Silva, J. Vercauteren and V. de Freitas, *J. Agric. Food Chem.*, 2001, **49**, 4836.
107 S. Remy, H. Fulcrand, B. Labarbe, V. Cheynier and M. Moutounet, *J. Sci. Food Agric.*, 2000, **80**, 745.
108 T. Okuda, T. Yoshida and T. Hatano, *J. Nat. Prod.*, 1989, **52**, 1.
109 T. Okuda, T. Yoshida and T. Hatano, in *Chromatographic Science Series, Centrifugal Partition Chromatography*, ed. A. P. Foucault, Dekker, New York, 1995, Vol 68.
110 L. J. Porter, in *Methods in Plant Biochemistry, Vol 1, Plant Phenolics*, ed. P. M. Dey and J. B. Harborne, Academic Press Inc., San Diego, 1989.
111 D. Treutter, *J. Chromatogr.*, 1989, **467**, 185.
112 S. A. Lazarus, G. E. Adamson, J. F. Hammerstone and H. H. Schmitz, *J. Agric. Food Chem.*, 1999, **47**, 3693.
113 J. F. Hammerstone, S. A. Lazarus, A. E. Mitchell, R. Rucker and H. H. Schmitz, *J. Agric. Food Chem.*, 1999, **47**, 490.
114 G. E. Adamson, S. A. Lazarus, A. E. Mitchell, R. L. Prior, G. Cao, P. H. Jacobs, B. G. Kremers, J. F. Hammerstone, R. B. Rucker, K. A. Ritter and H. H. Schmitz, *J. Agric. Food Chem.*, 1999, **47**, 4184.
115 R. L. Prior, S. A. Lazarus, G. Cao, H. Muccitelli and J. F. Hammerstone, *J. Agric. Food Chem.*, 2001, **49**, 1270.
116 B. Gabetta, N. Fuzzati, A. Griffini, E. Lolla, R. Pace, T. Ruffilli and F. Peterlongo, *Fitoterapia*, 2000, **71**, 162.
117 Z. Peng, Y. Hayasaka, P. G. Iland, M. Sefton, P. Høj and E. J. Waters, *J. Agric. Food Chem.*, 2001, **49**, 26.
118 S. Guyot, N. Marnet and J-F. Drilleau, *J. Agric. Food Chem.*, 2001, **49**, 14.
119 R. W. Hemingway, P. J. Steynberg, J. P. Steynberg and T. Hatano, in *Advances in Lignocellulosics Characterization*, ed. D. S. Argyropoulos, Tappi Press, Atlanta, 1999, p. 157.
120 T. De Bruyne, L. Pieters, H. Deelstra and A. Vlietinck, *Biochem. System. Ecol.*, 1999, **27**, 445.
121 E. Haslam, *J. Nat. Prod.*, 1996, **59**, 205.
122 E. Haslam, in *Annual Proceedings of the Phytochemical Society*, Kluwer Academic Press, London, New York, 2001, p. 1.
123 T. De Bruyne, L. Pieters, M. Witvrouw, E. De Clercq, D. Vanden Berghe and A. J. Vlietinck, *J. Nat. Prod.*, 1999, **62**, 954.
124 S. Deprez, C. Brezillon, S. Rabot, C. Philippe, I. Mila, C. Lapierre and A. Scalbert, *J. Nutr.*, 2000, **130**, 2733.
125 A. Scalbert and G. Williamson, *J. Nutr.*, 2000, **130**, 2073S.
126 Y. Lu and L. Y. Foo, *Food Chem.*, 2000, **68**, 81.
127 C. Santos-Buelga and A. Scalbert, *J. Sci. Food Agric.*, 2000, **80**, 1094.
128 P. Cos, M. Calomme, J-B. Sindambiwe, T. De Bruyne, K. Cimanga, L. Pieters, A. J. Vlietinck and D. J. Vanden Berghe, *Planta Med.*, 2001, **67**, 515.
129 H. Kolodziej, O. Kayser, A. F. Kiderlen, H. Ito, T. Hatano, T. Yoshida and L. Y. Foo, *Biol. Pharm. Bull.*, 2001, **24**, 1016.
130 A. F. Kiderlen, O. Kayser, D. Ferreira and H. Kolodziej, *Z. Naturforsch., Teil C*, 2001, **56**, 444.