Tetrahedron Vol. 48, No. 10, pp. 1743-1803, 1992
Printed in Great Britain

0040-4020/92 $3.00+.00
Pergamon Press plc

TETRAHEDRON REPORT NUMBER 308

DIVERSITY OF STRUCTURE AND FUNCTION IN OLIGOMERIC FLAVANOIDS

Daneel Ferreira*, Jan P. Steynberg, David G. Roux, and E. Vincent Brandt.
Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 South Africa

(*Received* 10 *August* 1991)

Contents

1. INTRODUCTION

The proanthocyanidins presumably constitute the most ubiquitous group of all plant phenolics[1]. Their exceptional concentrations in the barks and heartwoods of a variety of tree species have resulted in their commercial extraction with the initial objective of applying the extracts in leather manufacture[2]. The chemistry of those proanthocyanidins which are of commercial significance, and also of others of more academic interest, has represented an unattractive and therefore much neglected area of research[3]. Inhibiting factors have been the complexity of condensed tannin extract composition and the consequent problem of their isolation and purification, the lack of a universal method of both synthesis and of assessing the absolute stereochemistry at the point of the interflavan linkage, the need to contend with the phenomenon of dynamic rotational isomerism about interflavanoid bonds during NMR spectral investigations, and the lack of knowledge regarding the points of bonding at nucleophilic centres of the flavan-3-ol chain extender units.

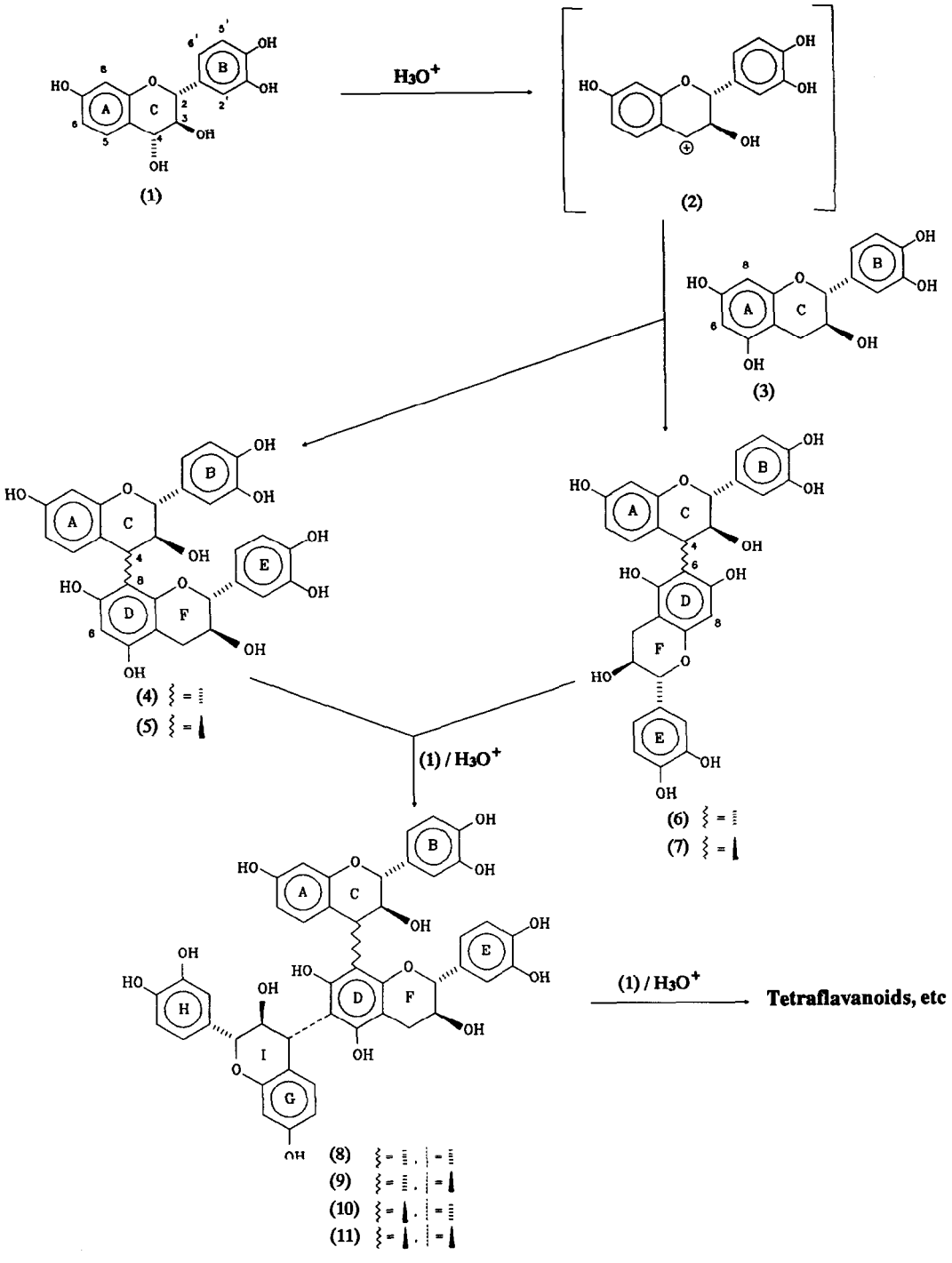
The more significant initial work on oligoflavanoids was therefore inevitably limited mainly to an analytical approach involving biflavanoids[4-7]. Notable exceptions were the earlier attempts by Geissman and Yoshimura[8] and by Weinges and co-workers[9] at synthesising procyanidin biflavanoid derivatives. However, by their very nature these synthetic methods would not permit their extension to higher oligomers. Investigations on the procyanidins were subsequently extended by Haslam and his collaborators[10,11] using analytical and degradative techniques; the latter, amongst others, involving cleavage of the interflavanoid bond of highly condensed tannins of unknown structure with toluene-α-thiol. The resultant C-4 thio ethers were utilized for generating 4-carbocations under acidic conditions in a 'biogenetically patterned' condensation with catechins to form procyanidin biflavanoids[12].

Against this background we embarked on exploring methods of direct synthesis, an approach being based on the premise that flavan-3,4-diols as potential electrophiles, and nucleophilic flavan-3-ols are involved in initiating condensation. A combination of flavan-3,4-diols and the immediate condensation products presumably 'propagates' the condensation sequence. Availability of the synthetic oligomers, identical to those obtained from natural sources, offered the opportunity to study their base-catalyzed transformations, such an approach being pursued in view of the fact that the majority of the industrial applications of condensed tannins involve their dissolution and/or reaction at alkaline pH[13,14]. Generalized principles which have accrued from these studies constitute the subject of this Report.

2. SYNTHESIS OF OLIGOFLAVANOIDS

2.1 Selection of precursors to synthetic oligoflavanoids

In those flavanoid metabolic pools which possess the potential for condensed tannin formation, flavan-3,4-diols, *e.g.* (1), when considered as *p*-hydroxybenzyl alcohols, represent structural units capable of generating C-4 carbocations (2)[15] under mild acidic conditions. These may subsequently be trapped *via* interaction with the potent nucleophilic centres of the ubiquitous flavan-3-ols[15], *e.g.* (3), exhibiting *meta*-substituted A-rings (Scheme 1) to form predominantly the (4α,8)- and (4β,8)-biflavanoids (4) and (5), and to a lesser extent also

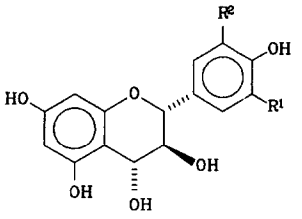


Scheme 1. General route to the formation of profisetinidin oligoflavanoids

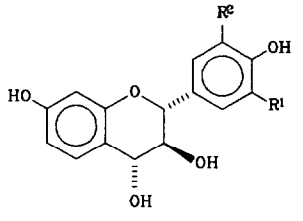
the (4,6)-regiomers (6) and (7)[16]. Substitution at the remaining and more potent nucleophilic site of the D-ring compared to that of the A-ring of these biflavanoids by carbocation (2) would then lead to the 'branched' or 'angular' triflavanoids (8) - (11)[17,18] which may subsequently serve as the precursors to tetraflavanoids[19,20], and eventually also to the higher oligomers (*vide infra*).

2.2 Flavan-3,4-diols as incipient electrophiles

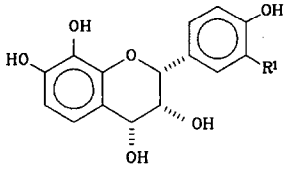
The flavan-3,4-diols or leucoanthocyanidins, *via* their C-4 carbocations *e.g.* (2), (*cf.* Scheme 1), serve as a source of the chain extender units in our semi-synthetic approach to oligoflavanoids. The stability of these carbocations is dependent on the degree of delocalization of the positive charge over the A-ring. From simple chemical concepts it may be predicted that such delocalization will be most effective for flavan-3,4-diols with phloroglucinol-type A-rings (12) - (14), intermediate in efficiency for resorcinol-type leuco-compounds (15) -



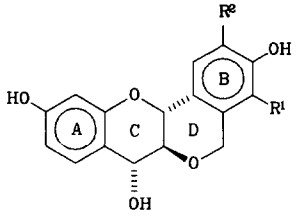
(12) $R^1=H, R^2=OH$
(13) $R^1=R^2=OH$
(14) $R^1=R^2=H$



(15) $R^1=H, R^2=OH$
(16) $R^1=R^2=OH$
(17) $R^1=R^2=H$



(18) $R^1=OH$
(19) $R^1=H$

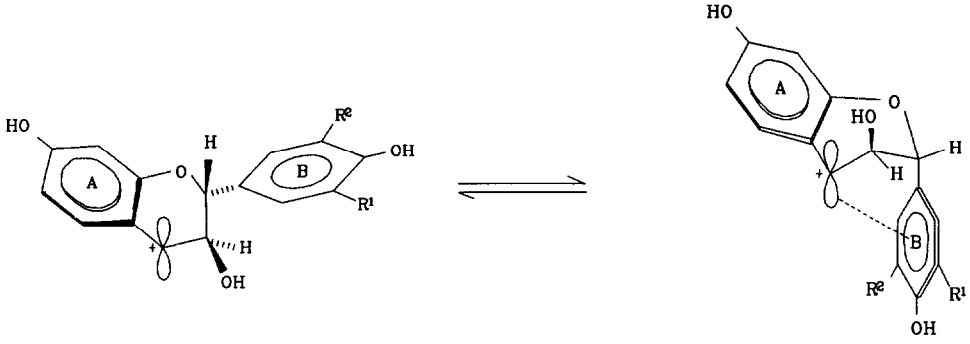


(20) $R^1=H, R^2=OH$
(21) $R^1=OH, R^2=H$

(17), and still less effective for pyrogallol-type (-)-melacacidins (18) and (-)-teracacidins (19). These concepts provide a simple rationale for the striking instability of leucocyanidins (12), leucodelphinidins (13), and leucopelargonidins (14), and hence their absence from natural sources containing oligomers derived from them. This contrasts with the stability and wide distribution of the natural 5-deoxy analogues (15) - (19).

The potential of the B-ring to contribute towards stabilizing C-4 carbocations of type (23) *via* an A-conformation (26) has been overlooked for a long time. First proposed by Brown[21], recognised by us on

several occasions[22-24], and formally designated A-conformer by Porter[25], this represents a half-chair/sofa conformation for the pyran ring in which the 2-aryl group occupies an axial-(26) as opposed to the 'customary' equatorial orientation in the E-conformer (23). Such a profound effect of the B-ring to additionally stabilize



(22) $R^1 = R^2 = OH$
(23) $R^1 = H, R^2 = OH$
(24) $R^1 = R^2 = H$

(25) $R^1 = R^2 = OH$
(26) $R^1 = H, R^2 = OH$
(27) $R^1 = R^2 = H$

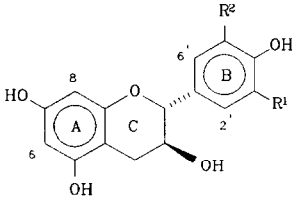
C-4 carbocations *via* an A-conformation was strikingly demonstrated by the different rates of condensation observed for (+)-leucorobinetinidin (16)[26], (+)-mollisacacidin (15)[26], and (+)-guibourtacacidin (17)[27]. Owing to the conformational mobility of the pyran heterocycle, benzylic carbocations of type (2) may hence be additionally stabilized by charge donation from the B-ring. The more electron-rich pyrogallol function in the (+)-leucorobinetinidin carbocations [(22) ↔ (25)] is more effective than the pyrocatechol functionality in (+)-mollisacacidin analogues [(23) ↔ (26)] and the mono-oxygenated moiety in the (+)-guibourtacacidin ions [(24) - (27)] hence leading to condensation rates decreasing in the order (16) > (15) > (17).

(+)-Peltogynol (20) and (+)-mopanol (21) which also possess the potential for C-4 carbocation formation, are non-reactive under conditions which readily promote aromatic substitutions with flavan-3,4-diols as electrophiles[28]. Forcing conditions are required for promoting condensations of (20) and (21) with nucleophilic phenols and the reactions are characterized by low yields[28,29]. The increased energy requirements for these condensation reactions similarly result from the C-rings of these compounds being restricted to an (*E*) C-3 sofa conformation of type (22) by the D-ring hence eliminating contributions by an A-conformer of type (25) towards a decrease in the activation energy[29].

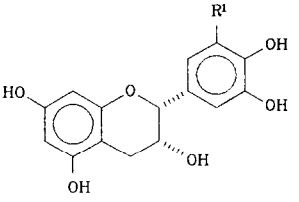
The different classes of condensed tannins are grouped according to the phenolic substitution pattern of the flavan-3,4-diol which served as the source of the chain extender unit, *e.g.* procyanidin (3′,4′,5,7-tetrahydroxy), prodelphinidin (3′,4′,5,5′,7-pentahydroxy), profisetinidin (3′,4′7-trihydroxy), prorobinetinidin (3′,4′,5′,7-tetrahydroxy), proguibourtinidin (4′,7-dihydroxy), proteracacidins (4′,7,8-trihydroxy), and promelacacidins (3′,4′,7,8-tetrahydroxy) (see ref. 30 for nomenclature of oligoflavanoids and also below). The vast majority of the naturally occurring flavan-3,4-diols are found in the wood and bark of the Leguminosae and particularly in the Acacia species. Their distribution is summarized in refs. 1 and 31.

2.3 Nucleophilic flavanoids

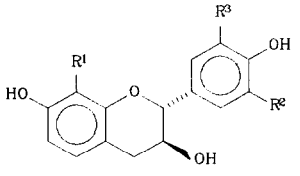
The biomimetic pool from which oligoflavanoids with $C_4(sp^3)$ - $C_{6/8}(sp^2)$ interflavanyl linkages originate, presumably contains a variety of potential nucleophilic units. Despite this, the majority of these metabolites are constituted of a flavan-3-ol as repeating unit, presumably originating from a flavan-3,4-diol entity (*vide supra*), and a terminal 'lower' unit comprising a nucleophilic C-4 deoxy flavan-3-ol with phloroglucinol A-ring. Most prominent amongst these are the procyanidins[6,10,12] with their (+)-catechin (28) and (-)-epicatechin (31)



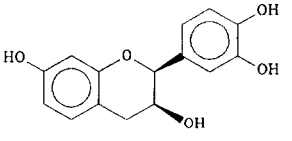
(28) $R^1 = OH$, $R^2 = H$
(29) $R^1 = R^2 = OH$
(30) $R^1 = R^2 = H$



(31) $R^1 = H$
(32) $R^1 = OH$



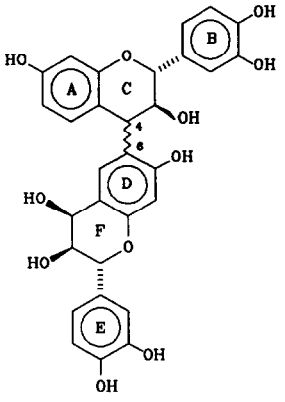
(33) $R^1 = R^3 = H$, $R^2 = OH$
(34) $R^1 = H$, $R^2 = R^3 = OH$
(35) $R^1 = R^2 = OH$, $R^3 = H$



(36)

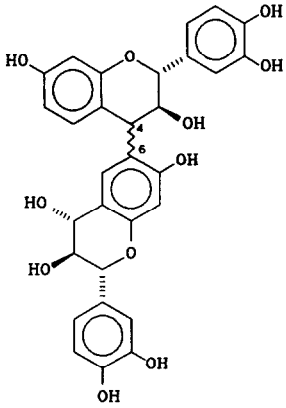
constituent units, the profisetinidins[5,18,32] based on (-)-fisetinidol (33), its enantiomer (+)-fisetinidol, and (+)-catechin (28) and (-)-epicatechin (31)[33], the prorobinetinidins[26,34] composed of (-)-robinetinidol (34), (+)-catechin (28), and (+)-gallocatechin (29) entities, and several other groups with limited distribution[35-40], but also possessing a lower flavan-3-ol unit with C-4 deoxy heterocycle. Flavan-3-ols with phloroglucinol A-rings, *e.g.* (+)-catechin (28), would be expected to represent stronger nucleophilic substrates than their resorcinol counterparts, *e.g.* (-)-fisetinidol (33).

Flavanoids possessing C-4 carbonyl functions exhibit reduced nucleophilicities of their aromatic A-rings[2]. By the same token the inductive effect of the 4-hydroxyl function of flavan-3,4-diols or of the C-4 carbocation resulting from its protonation, reduces their innate tendency for self-condensation[28,41]. Examples where the heterocycles of the terminal 'lower' units are oxygenated at C-4, are restricted to four dimeric (37) - (40) and a single trimeric profisetinidin (41) with terminal 3,4-diol function from the heartwood of *Acacia mearnsii*[4,41], and the profisetinidins, (42) - (44) with constituent dihydroflavonol DEF-units from *Burkea africana*[42].



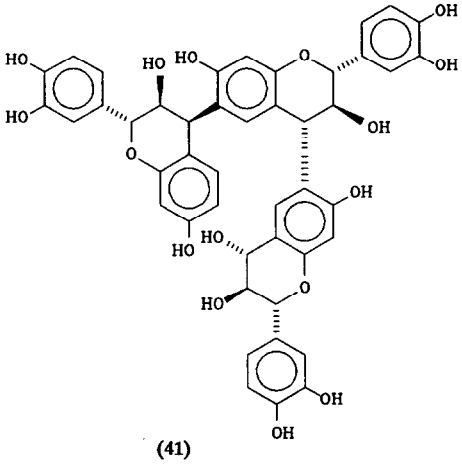
(37) ⌇ = ⋮

(38) ⌇ = |

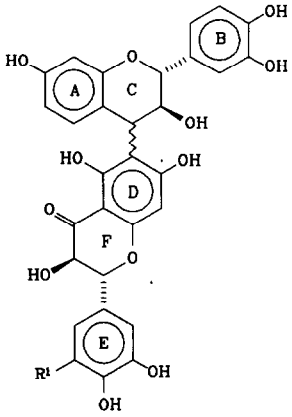


(39) ⌇ = ⋮

(40) ⌇ = |



(41)



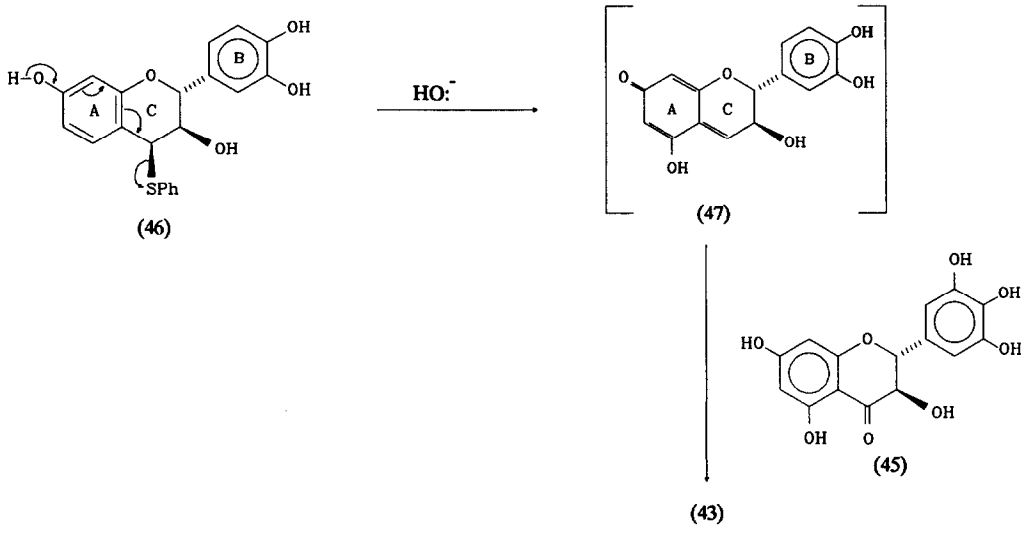
(42) ⌇ = ⋮ , R^1 = H

(43) ⌇ = ⋮ , R^1 = OH

(44) ⌇ = | , R^1 = OH

Acid-induced self-condensation of (+)-mollisacacidin (15) afforded biflavanoids (38) and (40) and the triflavanoid (41) in very low yields together with high molecular condensates. The conditions required for self-condensation are generally more drastic[41] than those permitting the facile condensation of the flavan-3,4-diol with its flavan-3-ol analogue, (-)-fisetinidol (33)[16,43]. The more prolonged or drastic conditions required for initial 'dimerization' of flavan-3,4-diol (15) to biflavanoids (37) - (40) should subsequently result in the preferential and accelerated condensation with the 'upper' ABC units of products to form higher condensates. Such a conjecture is supported by the uncontrollable nature of self-condensation of the flavan-3,4-diol which leads to high condensates rather than to oligomers of intermediate mass. Efforts to couple (+)-mollisacacidin (15) and (+)-ampelopsin (45) under acidic conditions aimed at the formation of profisetinidins (43) and (44) invariably failed[42]. The (-)-fisetinidol-(4α,6)-(+)-ampelopsin (43) was eventually synthesized in 0.85% yield

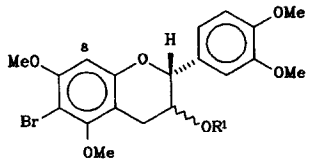
by condensation of 4β-phenylthio-(-)-fisetinidol (46) and (+)-ampelopsin (45) in alkaline buffer at pH 9 according to a protocol recently developed by Hemingway and Foo[44,45]. Thio-ether (46) presumably serves as a precursor to an A-ring quinone-methide (47) which is then trapped *via* interaction with the phenolic A-ring of (+)-ampelopsin (45).



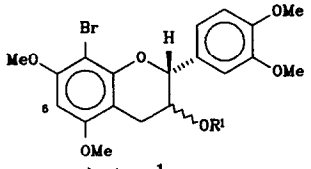
2.4 Bonding positions at nucleophilic centres

One of the more important problems which hampered progress of the chemistry of condensed tannins for a long time, was differentiation between the alternatives of C4-C8 and C4-C6 [*cf.* structures (4) and (6), Scheme 1] interflavanoid links in those instances where the 'lower' terminal flavan-3-ol unit is composed of a substituted (+)-catechin or (-)-epicatechin. The solvent shift method for methoxyl function developed by Pelter *et. al.*[37,46], although applicable to the methyl ethers of biflavonoids, *i.e.* compounds with 3- or 4-carbonyl function, was less reliable when applied to the methyl ether acetates of biflavanoids*, *e.g.* profisetinidin (4), often leading to ambiguous or erroneous conclusions. In what might now be regarded as a classic approach, Hundt and Roux[47] synthesized both 6- and 8-bromo derivatives (48) and (50) of (+)-catechin, and after differentiation[48] by X-ray crystallography, these were converted *via* lithio intermediates into analogues bearing 6- and 8-substituents which possess both electron withdrawing (COOH) and donating (OH, CH_2OMe) properties. Study of the absolute values of the chemical shifts of these compounds in $CDCl_3$ indicated that H-8 consistently resonates at a significantly lower field than H-6 without overlap, and also that differential values devolving mainly upon shifts of the *axial* H-2, provided criteria for differentiating between 6 and 8 substitution. A similar approach

* *Owing to the complexity of the metabolic pool of heartwoods containing 5-deoxy (A-ring) oligoflavanoids, successive purification as phenols, methyl ethers, and as methyl ether acetates is often the only way to achieve the desired degree of purity*



(48) ⦚ - ▌, $R^1 = CH_2Ph$
(49) ⦚ - ⦙, $R^1 = Ac$

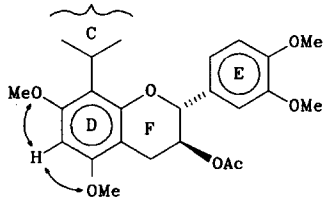


(50) ⦚ - ▌, $R^1 = CH_2Ph$
(51) ⦚ - ⦙, $R^1 = Ac$

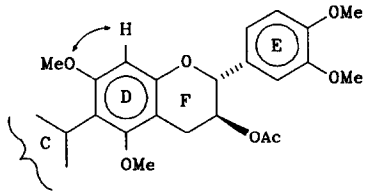
indicated that the same principles also applied to the 6- and 8-bromo-(-)-epicatechin methyl ether acetates (49) and (51)[49]. Subsequent work on various classes of biflavanoids which are based on (+)-catechin (28) and related units with phloroglucinol-type D-rings[16,28] as terminal 'lower' units, has demonstrated these criteria to be reliable provided that they are used in conjunction and also circumspectly.

By contrast, bonding to flavan-3-ols of the resorcinol-type, *e.g.* (-)-fisetinidol (33) and (+)-epifisetinidol (36) occurs preferentially at the least hindered 6-position[16]. Such allocation follows from singlets representing H-5 and -8 present in the aromatic region of ^1H NMR spectra of analogous biflavanoids at high magnetic fields.

Differentiation of (4,6)- and (4,8)-regiomers, *e.g.* the heptamethyl ether diacetates of (4) and (6), based on the absolute values of chemical shifts of 6- and 8-H(D) is, however, both temperature- and solvent dependent thus limiting its general utility. Nuclear Overhauser effect (NOE) difference spectroscopy (^1H NMR) has recently[50] been elegantly applied to distinguish between the C-8 (52) and C-6 (53) substituted (+)-catechin moieties of oligoflavanoids; NOE association of the residual proton with either two methoxy groups (6-H →



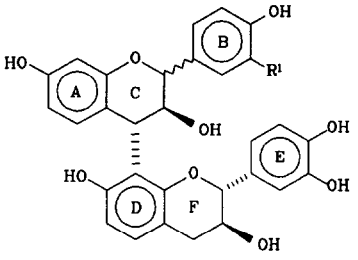
(52)



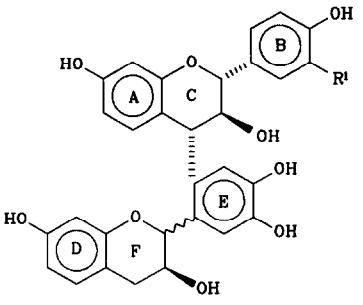
(53)

5,7-OMe) confirming 8-C-(52) or with one methoxy group (8-H → 7-OMe) indicative of 6-C-linked units (53).

Despite the remarkable preference of flavan-3-ols with resorcinol-type A-rings to be electrophilically substituted at C-6[16], analogues where C-8 (A-ring) and C-6 (B-ring) of (-)-fisetinidol (33) and (+)-epifisetinidol (36) served as nucleophilic centres, have recently been identified. Amongst these are the (4α,8)-linked profisetinidins (54) and (55) and the (+)-guibourtinidol-(4α,8)-(-)-fisetinidol (56)[51] and the C - E-ring profisetinidins (57) and (58)[52] and proguibourtinidin (59)[51] from the heartwood of *Colophospermum mopane*.



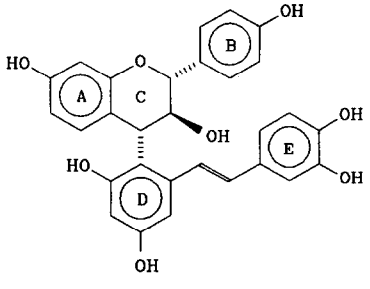
(54) ⟨ = ⟩ , R^1 = OH
(55) ⟨ - ⟩ , R^1 = OH
(56) ⟨ = ⟩ , R^1 = H



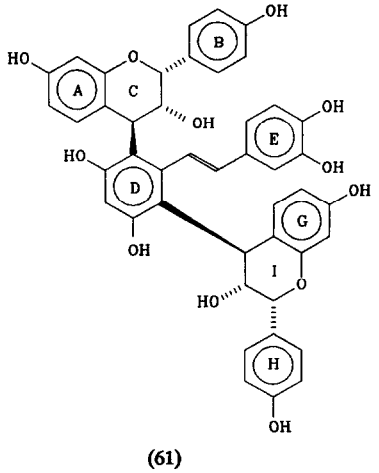
(57) ⟨ = ⟩ , R^1 = OH
(58) ⟨ - ⟩ , R^1 = OH
(59) ⟨ = ⟩ , R^1 = H

The extensive utilization of ^{1}H NMR decoupling experiments, using the benzylic 2- and 4-H resonances as reference signals, in conjunction with NOE measurements played a key role in the structural elucidation of metabolites (54) - (59) as their phenolic methyl ether diacetates. These techniques also facilitated determination of the structure of a unique (+)-catechin-(2,2′)-(+)-taxifolin biflavanoid[53] in which the B-ring of (+)-catechin is preferentially involved in coupling which presumably indicates phenol oxidation in contrast to the more commonly encountered intermediacy of C-4 carbocations.

It should be emphasized that the oligoflavanoids exhibiting 'abnormal' coupling patterns, *e.g.* (54) - (59) are usually encountered in natural sources which do not possess significant concentrations of flavan-3-ols with phloroglucinol-type A-rings or which are devoid of flavan-3-ols. The latter situation prevails in the heartwood



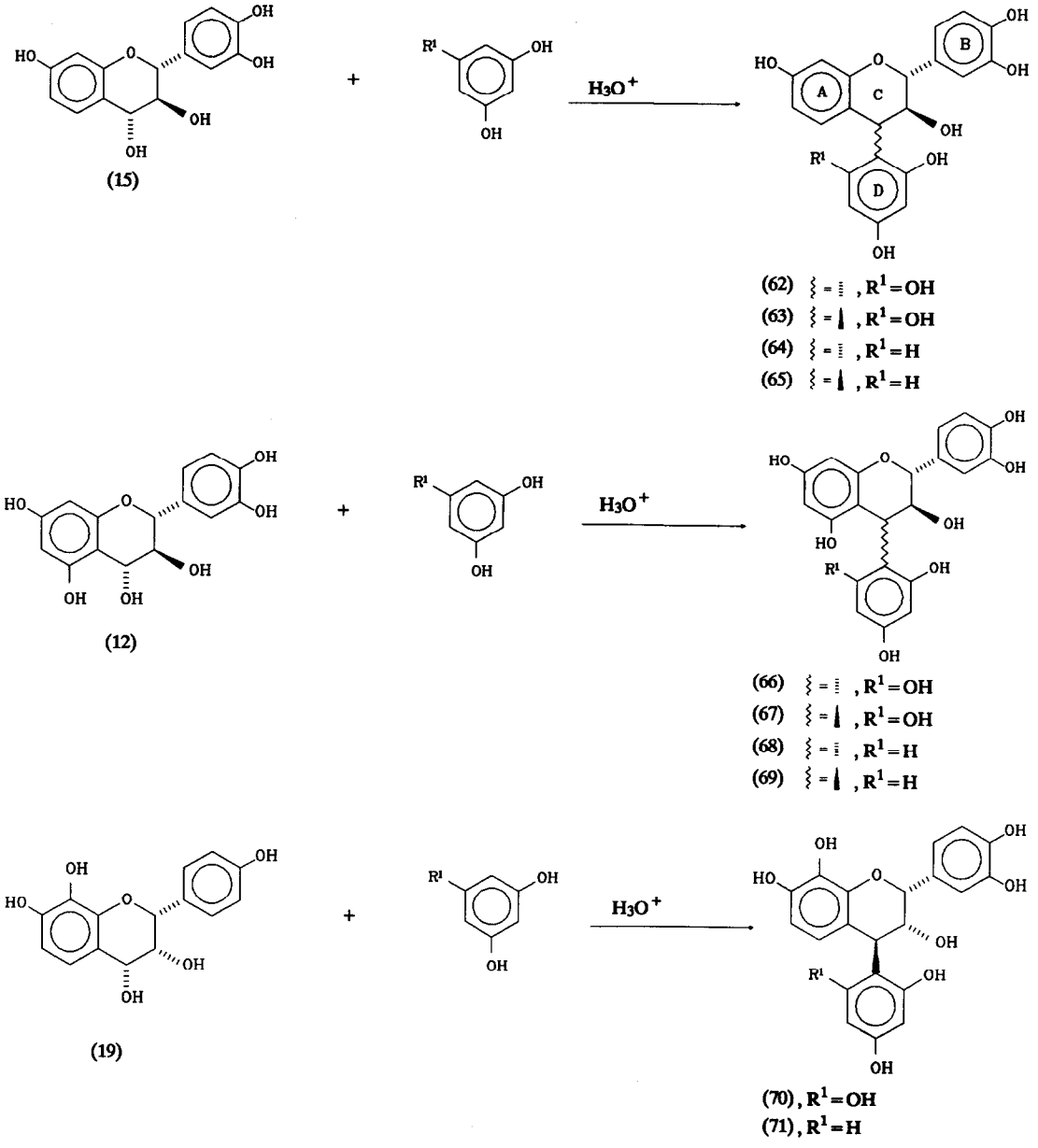
(60)



(61)

of *Guibourtia coleosperma* where stilbenes replaced flavan-3-ols as nucleophiles in the biosynthetic sequence leading to a series of (+)-guibourtinidol-stilbene bi- and tri-flavanoids, *e.g.* (60) and (61)[54].

2.5 Conditions for C-C interflavanoid bonding, stereochemical course of condensation, and methods for determining the absolute configuration at C-4.

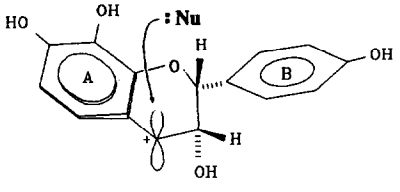


Scheme 2. Stereochemical course of condensation of flavan-3,4-diols with phloroglucinol and resorcinol

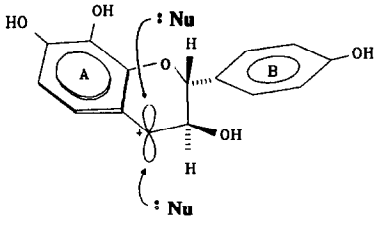
The experimental conditions which would permit the formation of C-4 - C-6 or -8 interflavanoid links between flavan-3,4-diols and flavan-3-ols had to be sufficiently mild to avoid self-condensation of the flavan-3,4-diol, to avoid anthocyanidin formation as a side reaction, to permit stereochemical conclusions, and to allow assessments regarding the regioselectivity of condensation. Minimum requirements for the condensation of flavan-3,4-diols exhibiting absolute configurations at C-2 and -3 representative of the chain extender units in condensed tannins, and the highly nucleophilic phenols, phloroglucinol and resorcinol were examined (Scheme 2) to subsequently serve as models for the different classes of oligoflavanoids[55,56]. The generation of carbocations, *e.g.* (2), or A-ring quinone-methides[57], *e.g.* (47), from typical flavan-3,4-diols and their trapping by nucleophilic phenolic nuclei, were found to proceed under mild acidic conditions (0.1M HCl), at ambient temperatures (20-25°C), and over short periods (2-4 h) with a minimum of side reactions. Under these conditions coupling reactions of 5,7-dihydroxyflavan-3,4-diols, *e.g.* (12) are presumably under thermodynamic control[58] in contrast to the kinetic regulation[55,56] of coupling involving 5-deoxy (A-ring) analogues, *e.g.* (15). Acid conditions are furthermore beneficial as regards ensuring maximum stability of pyrocatechol or pyrogallol B-ring functionality towards oxidation.

Thus, substitution at C-4 of flavan-3,4-diols with 2,3-*trans* configuration, *e.g.* (+)-2,3-*trans*-3,4-*trans*-flavan-3,3′,4,4′,7-pentaol (15) and its 5-oxy analogue (12), both with (2*R*,3*S*,4*R*) absolute configuration, proceeds stereoselectively to afford 3,4-*trans*-[(62), (64), (66), and (68)] and 3,4-*cis*-4-arylflavan-3-ols [(63), (65), (67), and (69)] in the proportions of *ca.* 1.5-2:1. By contrast both phloroglucinol and resorcinol are captured with complete stereoselectivity by the carbocation generated from (-)-2,3-*cis*-3,4-*cis*-teracacidin [(19):2*R*,3*R*,4*R*] to give the 2,3-*cis*-3,4-*trans*-4-arylflavan-3-ols (70) and (71) with inversion of configuration at C-4.

Assuming that carbocationic intermediates possess sofa conformations, nucleophilic attack on the ion with (2*R*,3*R*)-2,3-*cis* configuration (72) proceeds from the less hindered 'upper' side presumably with neighbouring group participation of the 3-*axial* hydroxyl in an E-conformation and by the 2-*axial* B-ring in an A-conformation (*vide supra*), whereas reaction with a 2,3-*trans* carbocation (73) is directed preferentially from the less hindered



(72) : Complete stereoselectivity



(73) : Moderate stereoselectivity

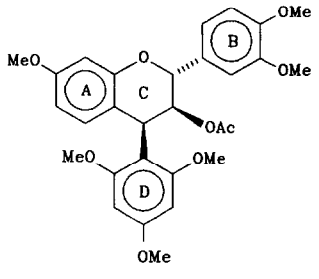
'lower' side, *i.e.* reaction proceeds with a moderate degree of stereoselectivity. It should be noted that the terms stereoselectivity and stereospecificity have thus far been used incorrectly when describing the interactions of intermediates (72) and (73) with nucleophilic phenolic moieties (*cf.* refs. 3, 31, 55, and 56). Furthermore, the controversy[1] regarding the intermediacy of a C-4 carbocation (72) or an A-ring quinone methide[57] (47) in the acid-mediated condensation of flavan-3,4-diols with nucleophiles is actually irrelevant since C-4 is in either species sp^2 hybridized with similar heterocyclic ring geometry thus resulting in the same stereochemical course

of the coupling step. The formation of A-ring quinone methide intermediates, nevertheless, constitutes a viable mechanism for the condensation of 4-substituted flavans over a wide range of pH values[44,45,57].

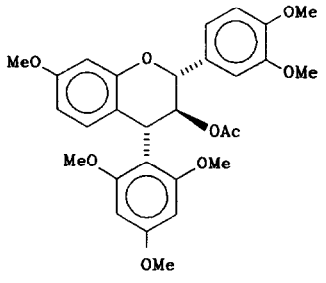
Since flavan-3,4-diols and nucleophilic flavan-3-ols with phloroglucinol- or resorcinol-type A-rings, as well as analogous bi- and triflavanoid condensation products accompany tannins, the aforementioned mechanisms involving phenols should accurately predict the configuration at C-4 of all constituent 2,3-*trans* and 2,3-*cis* flavanoid units in natural condensed tannins. Principles similar to those advanced here apparently also govern the stereochemistry of reactions of flavan-3,4-diols with sulphur[21,59] and oxygen nucleophiles[60], and of the solvolysis of (2*R*,3*R*)-2,3-*cis* procyanidins in the presence of thiols, phloroglucinol, or flavan-3-ols[10,12,61].

The reactions of (2*R*,3*S*,4*R*)-(+)-leucocyanidin (12) are more highly directed to the 3,4-*trans* isomers (66) and (68)[16,62] owing to greater steric restraint due to the 5-hydroxy group. Coupling of (+)-leucocyanidin and (+)-catechin gave the 4-linked dimers with no evidence for any 3,4-*cis* dimers[63]. This latter result was consistent with the common occurrence of (4α,8)-bis-(+)-catechin, (+)-catechin-(4α,8)-(-)-epicatechin, and their (4α,6)-isomers in lower yields from plants containing procyanidins. Recently, however, the synthesis and natural occurrence in low proportions of procyanidins exhibiting 2,3-*trans*-3,4-*cis* linkages have been demonstrated[64-67] (see also section 3.1).

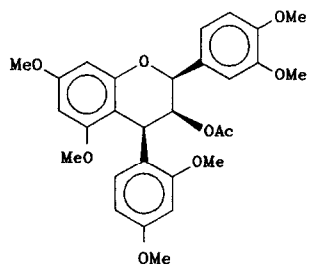
Apart from defining the ideal conditions for biomimetic condensations, the derivatives of the optically pure 4-arylflavan-3-ols in Scheme 2 offered the opportunity to formulating a chiroptical rule which defines the absolute configuration at C-4 of flavanoid units of this type and hence in biflavanoids and higher oligomers. The CD bands of the 4-arylflavan-3-ols and other proanthocyanidins are much more intense than those of their constituent flavan units because of the close proximity of the A- and D-ring chromophores (Snatzke's second chiral sphere[68]) in contrast to the more remote locality of the A- and B-ring chromophores in monomeric



(74)



(75)



(76)

flavans (Snatzke's third chiral sphere). Thus, the absolute configuration of the interflavanoid bond could be correlated with the sign of the CD band near 230 nm (probably an ^1L_a transition), a positive sign being correlated with a 4ß (74) and negative with a 4 (75), configuration, regardless of the configuration of the rest of the molecule[16,55,56]. In much the same way, Haslam and his co-workers[69] demonstrated that the CD spectra of

procyanidin oligomers possess a short wavelength couplet centred near 200 nm. A positive couplet was correlated with a 4β and a negative couplet with a 4α interflavanoid bond configuration. The CD method thus supplemented their previous indirect method based on ^{13}C NMR chemical-shift differences[12].

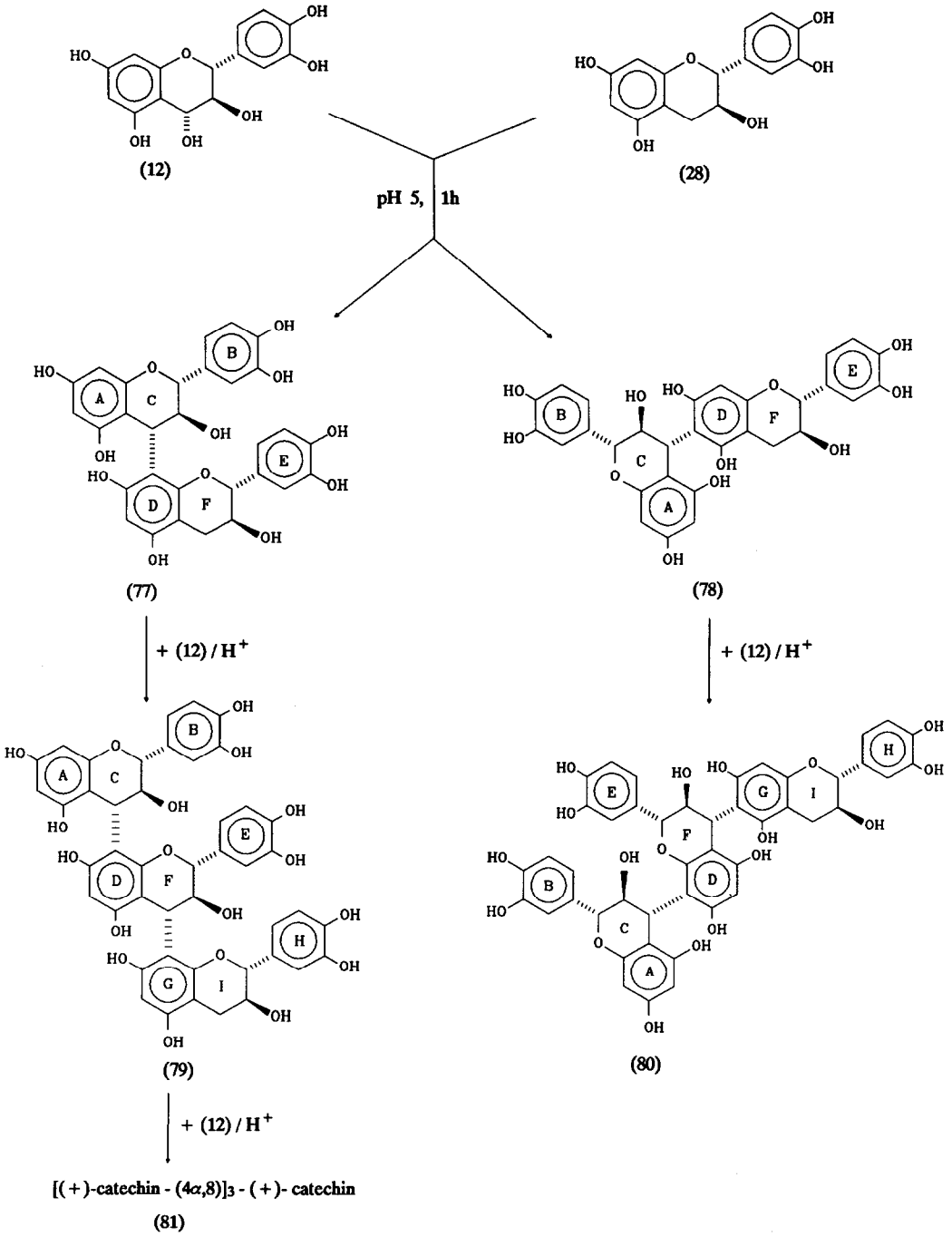
However, 4-arylflavan-3-ol derivatives with 2,3-*cis*-3,4-*cis* configuration of their C-rings, *e.g.* (76) and also some with all-*trans* configurations, do not obey the aromatic quadrant rule[70], hence leading to exceptions[71] to the aforementioned observations. Analogues which do not conform to this otherwise simple rule also exhibit 'abnormal' ^{1}H NMR coupling constants which have been ascribed to heterocyclic boat conformations[71]. Owing to the high energy requirements[25] involvement of a boat conformation must, however, be rejected. Such deviations in coupling constants and also the exceptions to the aromatic quadrant rule are at present more accurately explained in terms of an equilibrium between E- and A-conformers[25] (*vide supra*) of the heterocycle of 4-arylflavan-3-ols and related compounds (see section 6).

3. Biomimetic synthesis of the different classes of oligoflavanoids

Acid-catalyzed reactions to produce flavan-4-carbocations or A-ring quinone-methides, either from flavan-3,4-diols or from interflavanoid bond cleavage of oligomeric/polymeric proanthocyanidins, that react with the A-rings of flavan-3-ols to produce oligomeric proanthocyanidins according to the principles outlined above, have been so successfully employed that they have been called biomimetic syntheses[16,58,72,73]. Typical examples are now selected to illustrate the basic differences between the general course of condensations involving carbocations/quinone-methides derived from flavan-3,4-diols with phloroglucinol-type A-rings on the one hand and those originating from diols with resorcinol-type rings with (+)-catechin as common substrate.

3.1 Procyanidins

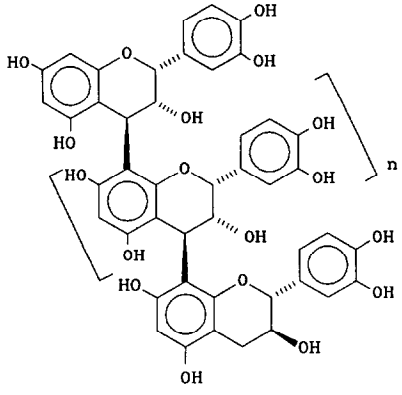
Condensation of molar equivalents of (+)-leucocyanidin (12), generated *in situ* from (2*R*,3*R*)-2,3-*trans*-(+)-taxifolin[16,62], with (+)-catechin (28) in aqueous medium at pH 5 gives predominantly (4α,8)- but also (4α,6)-bis-(+)-catechins (77) and (78) (10:1 ratio) together with their 'linear' [(+)-catechin-(4α,8)]$_2$-(+)-catechin and (+)-catechin-(4α,6)-(+)-catechin-(4α,8)-(+)-catechin triflavanoid analogues (79) and (80), and the [(+)-catechin-(4α,8)]$_3$-(+)-catechin tetraflavanoid homologue (81) (12:3 ratio) (Scheme 3)[63]. The oligomers were characterized as their phenolic methyl ether acetates. Complete consumption of the flavan-3,4-diol and recovery of *ca.* 45% of (+)-catechin were observed. From this and other parallel condensations (*e.g.* 1:5 molar ratio), a number of significant conclusions are possible regarding stereo- and regio-selectivity and also the reaction rate under these conditions. Regio- and stereo-selectivity follow from the established stability-selectivity relationship, *i.e.* the relatively stable C-4 carbocation derived from diol (12) is formed rapidly but reacts selectively because of such stability. Steric considerations favour substitution (see also above) at C-8 on (+)-catechin, thus ensuring 'linearity', in contrast to 'angularity' resulting from 6,8-disubstitution, in subsequent condensations.



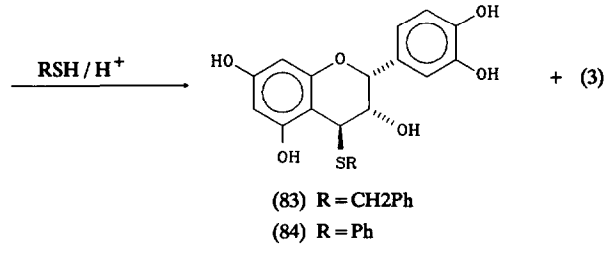
Scheme 3. Condensation sequence of (+)-leucocyanidin with (+)-catechin and with resultant procyanidins.

Equally significant is the apparent preference of the electrophile for bi- and triflavanyl procyanidins once condensation is initiated. This implies that with generation of competing substrates, the nucleophilicities of the oligomeric procyanidins, *e.g.* (77) are superior to that of (+)-catechin. Enhanced stabilization of the transition state by hyperconjugation during substitution of oligomers has been invoked to rationalize such a phenomenon[43,64]. However, the biological implications of these observations are that under the weakly acidic conditions prevailing in plants, leucocyanidins (and also leucodelphinidins and leucopelargonidins) are presumably subject to a transient existence; the resultant procyanidin (and also prodelphinidin and propelargonidin) (*cf.* ref. 1 for a summary of naturally occurring procyandins, prodelphinidins, and propelargonidins) oligomers invariably represent a high average degree of condensation; and that the extent of (4,6)-coupling is small compared with (4,8)-links. Since an increased molar proportion (*e.g.* 1:5) of (+)-catechin offered during the above *in vitro* condensations places increasing emphasis on lower mass oligomers[64], one might venture the prediction that in any given natural situation the initial and also continued stoicheiometric balance between electrophile and the nucleophiles should determine the degree of condensation of the oligomeric products.

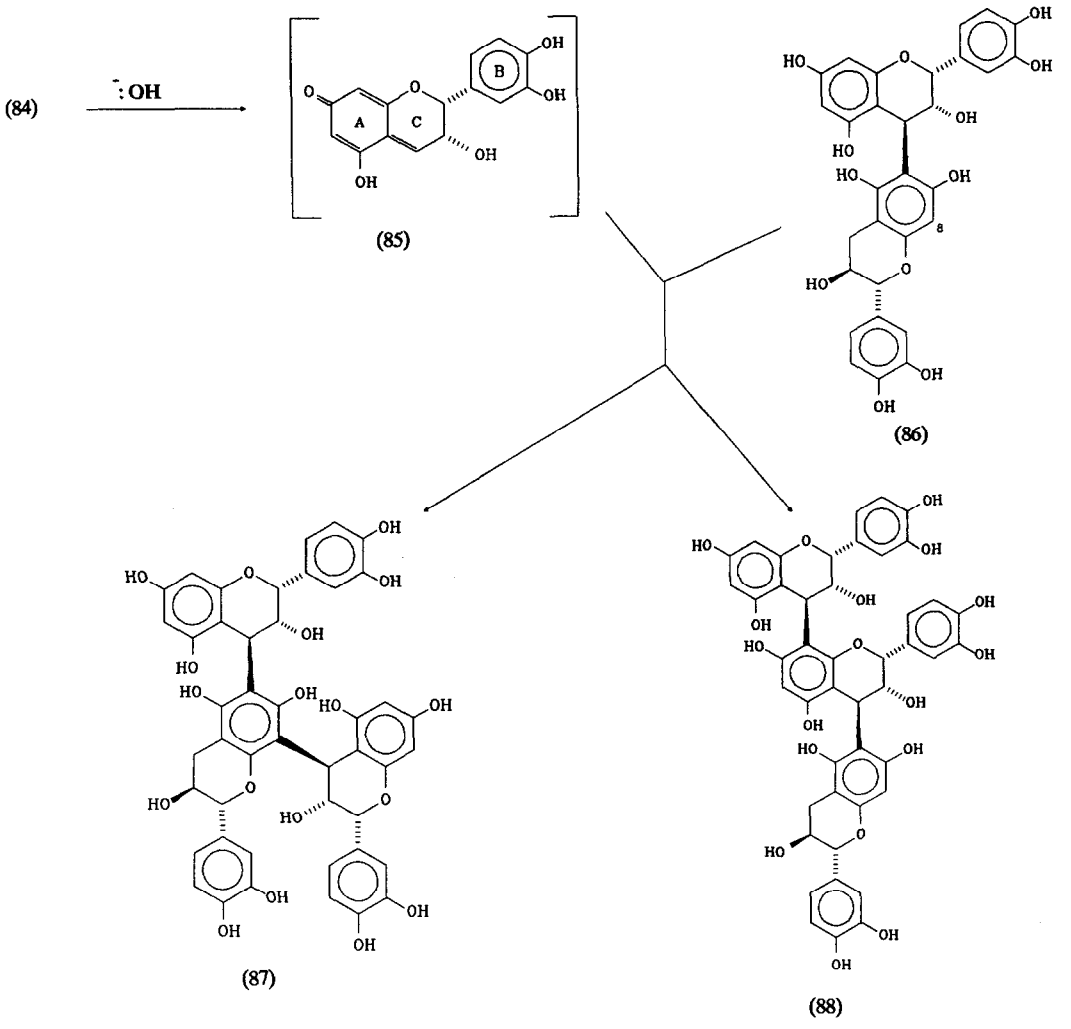
The absence of a flavan-3,4-diol- or dihydroflavonol analogue of (-)-epicatechin in natural sources, has hampered the semi-synthetic approach towards the structural elucidation of procyanidins with (2*R*,3*R*)-2,3-*cis* constituent units. This problem has been circumvented *via* acid-catalyzed thiolysis [toluene-α-thiol[10-12] or benzenethiol[21,74]] of oligomeric procyanidins, *e.g.* (82), with (-)-epicatechin chain extender units, and the



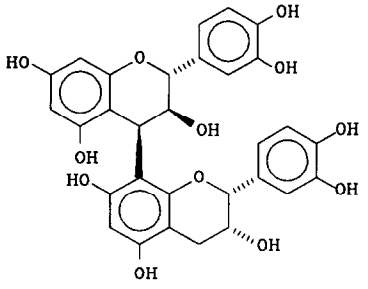
(82)



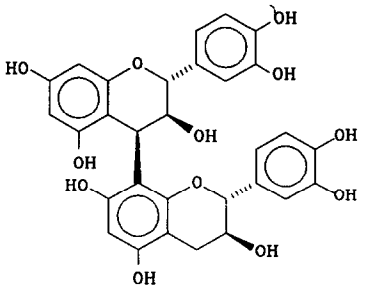
subsequent utilization of the C-4 thio-ethers (83) and (84) as electrophiles in condensation with appropriate phenolic nucleophiles[12,44,45]. Conversion of thio-ether (84) into the A-ring quinone-methide (85) under mild basic conditions and subsequent trapping by (-)-epicatechin-(4β,6)-(+)-catechin (86), led to synthesis of the first 'branched' procyanidin trimer (87) in higher yield than the linear analogue (88), suggesting that naturally occurring procyanidin polymers may be highly branched[45] despite the fact that such analogues have not yet been encountered in natural sources. The synthesis of the first procyanidins with 3,4-*cis* configuration, *e.g.* (89)[64-66], has, however, similarly preceded the first recognition[67] of the (4β,8)-bis-(+)-catechin (90) in Nature.



The utility of the aforementioned approaches towards the synthesis of oligomeric procyanidins is, however, limited by the lability of the interflavanyl bond under both acidic[75-77] and basic[44] conditions. The ability to carry out condensations in mild alkaline solutions nevertheless presents considerable advantages for the synthesis of procyanidins. At pH levels below 9 the interflavanoid bond is sufficiently stable hence allowing Foo and Hemingway[45] to synthesize the first example of a 'branched' trimer (87) (see above).



(89)



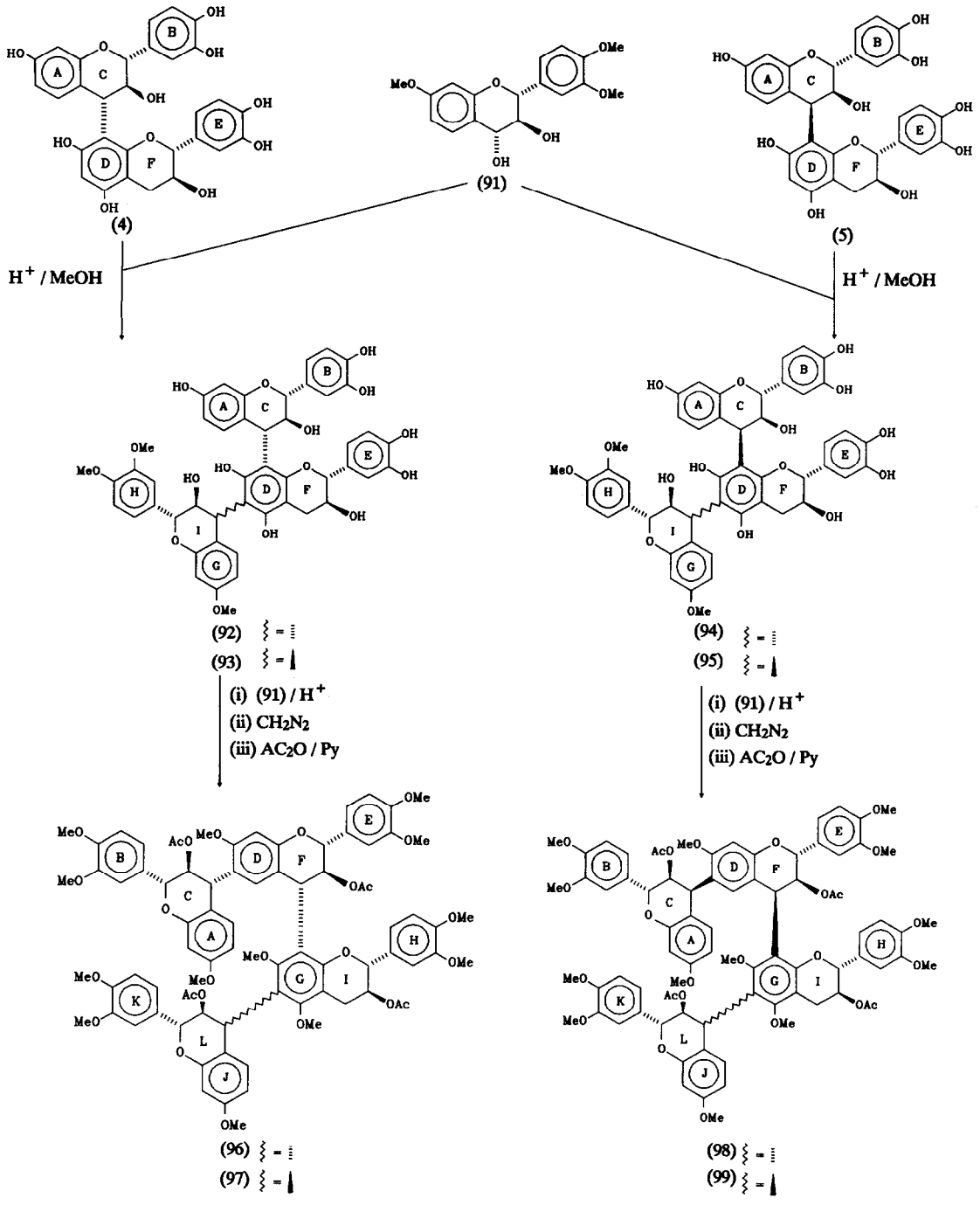
(90)

3.2 Profisetinidins and prorobinetinidins

The following principles are relevant to the prorobinetinidins[26] and profisetinidins[18,78] as found in the well-known wattle ('mimosa') and 'quebracho' extracts (*Acacia* and *Schinopsis* spp.) used commercially for leather tanning. The C-4 carbocationic intermediates (2) derived from flavan-3,4-diols with resorcinol-type A-rings, *e.g.* (+)-mollisacacidin (15), are less stable than those of the leucocyanidin-type with a consequent reduction in the degree of regio- and stereo-selectivity during substitution on (+)-catechin and a completely different emphasis in the composition of biflavanoids resulting from condensation on a 1:1 molar basis (Scheme 1). Thus, under optimized conditions (4,8)-(4) and (5) relative to (4,6)-coupling (6) and (7) occurrs in the proportion of 4:1 and 3,4-*trans* relative to 3,4-*cis* isomers in the ratio* of 3:2. However, judging by products isolated from certain natural tannin extracts[26] initial *in vivo* condensations related to the above are highly regioselective [at C-8 of (+)-catechin] and stereoselective (mainly represented by 3,4-*trans* profisetinidin and prorobinetinidin substituents), while in others[79] the composition of the biflavanoid mixture approximates that obtained by synthesis as above. This significant difference presumably indicates that condensation is enzymatically controlled in 'living' wattle bark containing chlorophyll, but partly the product of an ageing process as in the heartwoods of slow-growing *Schinopsis* and *Rhus* spp.

Furthermore, in wattle bark the C-8 and -6 positions on (+)-catechin (28) and (+)-gallocatechin (29) appear to be substituted successively, judging from extract composition[26], giving 'angular' triflavanoids (8) - (11) which represent key intermediates to further condensation. This stage may be simulated *in vitro* by simply offering a 2:1 ratio of the flavan-3,4-diol to (+)-catechin. Since both 'strongly nucleophilic' positions on the A-ring of the flavan-3-ol are now substituted, continued coupling involving the more weakly nucleophilic C-6 positions on the (-)-fisetinidol substituents must slow down significantly. The next step should thus be 'rate determining' in the formation of 'angular' tetraflavanoids (Scheme 4). Such anticipated behaviour, therefore, contrasts with the principle of accelerated 'linear' condensation with increasing mass apparent in the case of the procyanidin oligomers (*cf.* Scheme 3).

* *Ratios are those of the phenolic heptamethyl ether diacetates isolated*



Scheme 4. Synthesis of natural profisetinidin tetraflavanoids as their tridecamethyl ether tetra-acetate derivatives

The problem of the course of continued substitution on the 'angular' profisetinidin triflavanoids (8) - (11) by the appropriate C-4 carbocation generated from (+)-mollisacacidin presents difficulties since monocondensation on either of the 6- and 8-coupled (-)-fisetinidol units (*i.e.* C-6 of the A- and G-rings) would lead to 16 possible tetraflavanoids[79,80] which cannot be readily differentiated by ^{1}H NMR spectroscopy. However, an indication of further 6-linkage to one of the (-)-fisetinidol substituents on (+)-catechin was evident from the 500 MHz spectrum of the tridecamethyl ether triacetate of only one of four profisetinidin tetraflavanoids from the heartwood of *Acacia mearnsii*[19]. Owing to the phenomenon of dynamic rotational isomerism[4,5,12,81-83] about the interflavanoid bonds, none of the remaining three tetraflavanoids from the same source gave sharp spectra hence indicating the necessity of a concise synthesis capable of differentiating between substitution at C-6 of the A- or G-rings in the transformation of tri- to tetra-flavanoid.

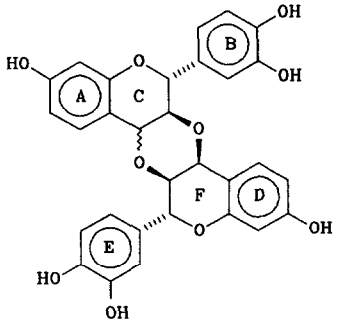
Synthesis of these natural tetraflavanoids was performed[19] *via* the readily available[84] free phenolic (-)-fisetinidol-(4α,8)- and (4β,8)-(+)-catechins (4) and (5) and tri-*O*-methyl-(+)-mollisacacidin (91) (Scheme 4). The initial condensation results in exclusive attack on the phloroglucinol-type D-ring to produce the four partially methylated angular trimers (92) - (95). These were further reacted with the flavan-3,4-diol to give the four tetrameric methyl ether acetates (96) - (99) following methylation and acetylation. The second condensation must, of course, occur exclusively on the sole phenolic A-ring site available. The final condensations occurred with a high degree of regiospecificity which may be attributed to asymmetric induction[19]. Comparison of the ^{1}H NMR data at 500 MHz of the synthetic analogues (96) - (99) with those of the corresponding derivatives of the natural products from *Acacia mearnsii* provided unambiguous proof of their identity. Such a comparison was substantiated by synthesis of positional isomers in which the bi- and mono-flavanoid units on (+)-catechin are reversed[80] hence eliminating the doubts raised by Porter[1] regarding the integrity of the structures of the profisetinidin tetraflavanoids (96) - (99)

The tetraflavanoid derivative (98) and its diastereomer [(2*S*,3*R*) absolute configuration of the ABC-, DEF-, and JKL-(+)-fisetinidol units] from the heartwood of *Rhus lancea* (karee)[78,85], and three synthetic analogues[80] have exceptional conformational stability, resulting in unusually sharp ^{1}H NMR spectra at 500 MHz and permitting definition[86,87] of the conformations of these compounds by nuclear Overhauser difference spectroscopy. The unique thermodynamic stability of their dominant (85-90% abundance) conformers is attributed to the combined effects of the relative configurations of constituent flavanyl units, to steric repulsion by functional groups *ortho* to interflavanyl bonds, and to steric inhibition of mobility about interflavanyl bonds due to partial overlap of terminal units.

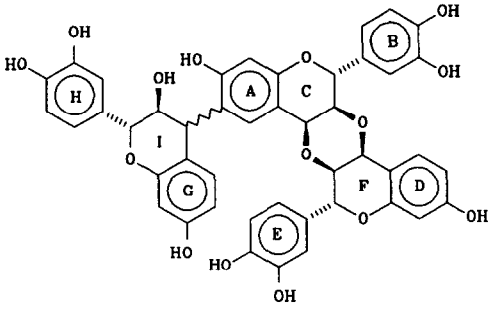
Besides the presence of minor proportions of the aforementioned profisetinidins, the bark of *A. mearnsii* contains predominantly prorobinetinidin-type oligoflavanoids[2,3]. These metabolites are based on either a (+)-catechin (28) or (+)-gallocatechin (29) chain terminating unit. Their synthesis[16,26] *via* reaction of the flavan-3-ols and (2*R*,3*R*,4*S*)-2,3-*trans*-3,4-*trans*-flavan-3,3',4,4',5',7-hexaol [(+)-leucorobinetinidin] (16) are governed by the same principles as those outlined above for the profisetinidins.

A variety of profisetinidin biflavanoids with a chain terminating unit other than (+)-catechin has also

been identified. Amongst these are the dioxane-linked[88,89] dimers (100), (101), and trimers (102), and (103), the methyl ethers of which have been synthesized through boron trifluoride catalyzed condensations of (+)-mollisacacidin tri-*O*-methyl ether (91) (see also ref. 90 for an alternative approach to analogues of this type); the (-)-fisetinidol-(4α,8)- and (4β,8)-(-)-epicatechins[33] from the heartwoods of three species of the Caesalpiniodeae; the four dimeric (37) - (40) and a single trimeric analogue (41) with terminal flavan-3,4-diol



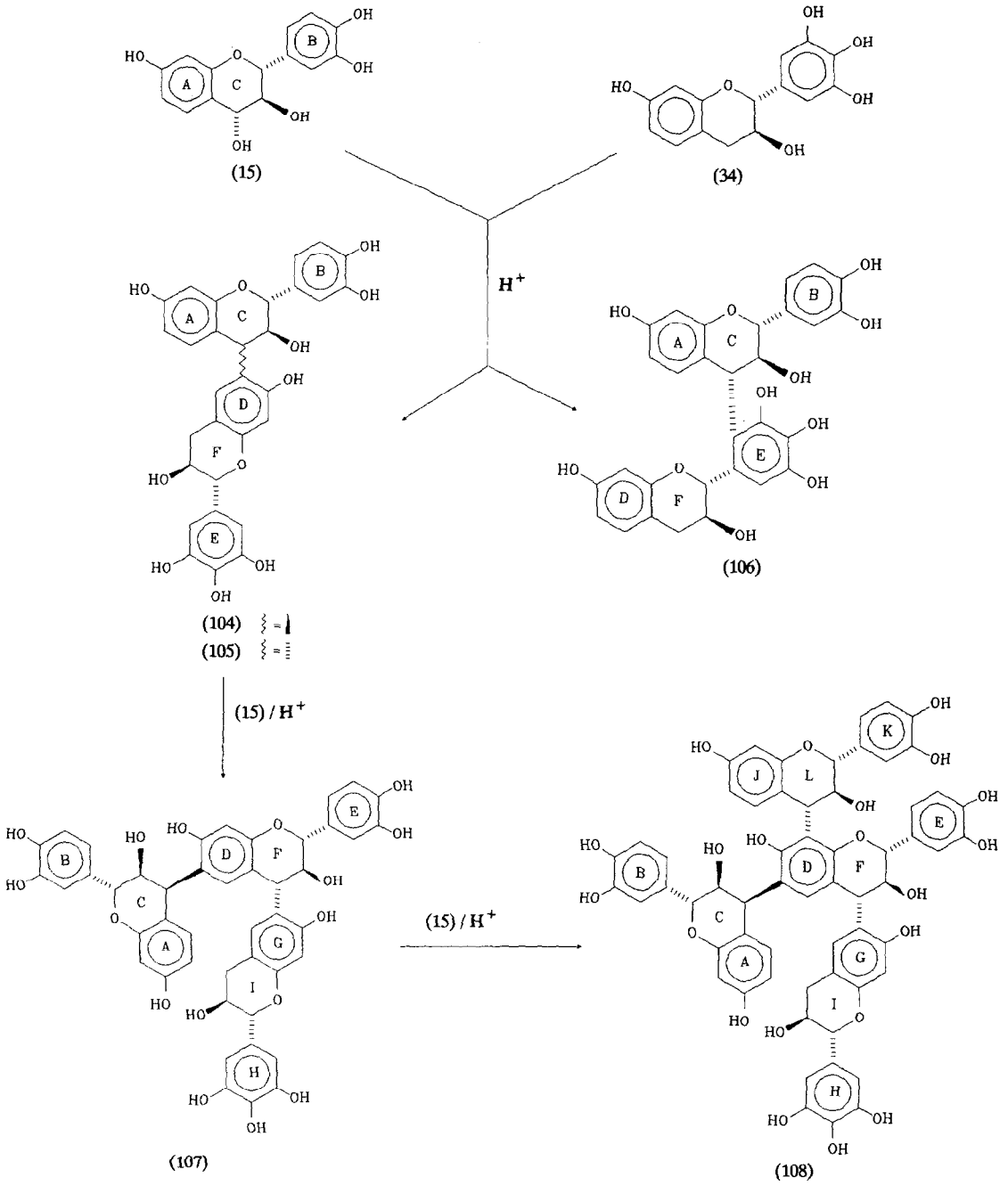
(100) ⦚ = ⦙
(101) ⦚ = |



(102) ⦚ = ⦙
(103) ⦚ = |

function[4,41]; those with constituent dihydroflavonol units (42) - (44)[42]; and a series of bis-fisetinidols involving both (-)-fisetinidol-(33) and (+)-epifisetinidol-(36) moieties coupled *via* C-6, C-8, or C-6 (B-ring) of the chain terminating fisetinidol unit[51,52], *e.g.* compounds (54) - (59)*; and a single (-)-fisetinidol-(4β,6)-(-)-robinetinidol (104)[42] from the heartwood of *Burkea africana*. The synthesis[42,91] (Scheme 5) of the latter compound represents an interesting variation on the general theme since it offered the first opportunity of establishing the course of coupling with a flavan-3-ol in which the nucleophilicity of the B-ring is comparable to that of the resorcinol A-ring. Acid-mediated coupling of (+)-mollisacacidin (15) and (-)-robinetinidol (34) afforded the (-)-fisetinidol-(4β,6)- and (4α,6)-(-)-robinetinidol dimers (104) and (105), and the unique (-)-fisetinidol-(4α,2′)-(-)-robinetinidol (106), representing the first *in vitro* example where the B-ring of the flavan-3-ol serves as nucleophile competing with the resorcinol A-ring in coupling with the flavan-3,4-diol derived C-4 carbocationic intermediate. The (4α,6)-analogue (105) presumably serves as 'activated' nucleophile in genesis of the triflavanoid (107) and the latter subsequently for the 'branched' tetraflavanoid (108). These phenomena are explicable by invoking both steric and conformationally dependent hyperconjugative effects which preferentially operate in the (-)-fisetinidol-(4α,6)-(-)-robinetinidol (105) and presumably also dictate the course of coupling of (+)-mollisacacidin with respectively (-)-fisetinidol (33)[43] and (+)-epifisetinidol (36)[91].

* *All efforts to induce coupling at C-8 or C-6 (B-ring) of fisetinidol units have hitherto failed*[51,52]

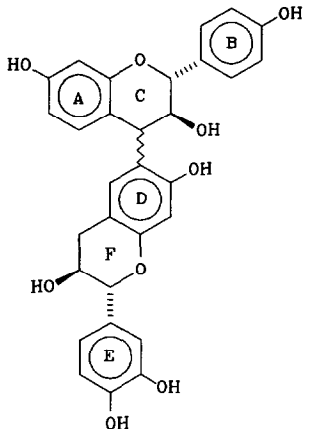


Scheme 5. Acid mediated condensation of (+)-mollisacacidin and (-)-robinetinidol

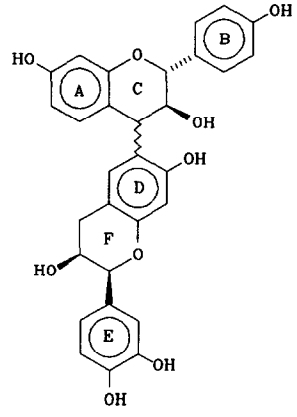
3.3 Proguibourtinidins

Pro- and leuco-guibourtinidins with their 4',7-dihydroxy phenolic functionality represent a relative rare group of condensed tannins which, while occurring as minor components in Australian *Acacia* species[32], predominate in the Southern African species *Guibourtia coleosperma*[54,92], *Julbernardia globiflora*[37], and *Acacia luederitzii*[93,94]. Notable amongst these compounds are those analogues that carry a 3,3',4,5'-tetrahydroxystilbene terminating unit *e.g.* proguibourtinidins (60) and (61) from *G. coleosperma*[54] which are synthetically available by substituting the nucleophilic flavan-3-ol by the tetrahydroxystilbene in acid-mediated coupling with the appropriate guibourtacacidin.

The number of naturally occurring proguibourtinidins has recently been extended considerably by identification of a series of analogues with the 5-deoxy flavan-3-ols, (-)-fisetinidol and (+)-epifisetinidol as the chain terminating units. Besides the (+)-guibourtinidol-(4α,8)- and (4α,6')-(-)-fisetinidols (56) and (59)[51,52], the heartwood of *Colophospermum mopane* also contains the (4,6)-coupled analogues (109) - (112)[27]. These



(109) ⦚ = ⦙
(110) ⦚ = |



(111) ⦚ = ⦙
(112) ⦚ = |

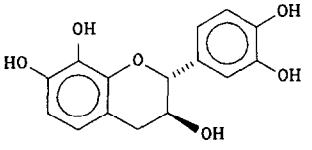
compounds were difficult to synthesize by the usual protocol presumably as a result of the low nucleophilicity of the flavan-3-ol and, more important, of reduced reactivity of the flavan-3,4-diol owing to the poor ability of the mono-oxygenated B-ring to stabilize an intermediate carbocation *via* an A-conformation (27) (see section 2.2).

A guibourtinidol-epiafzelechin dimer was recently isolated from *Cassia fistula* sapwood for which a (4α,8)-interflavanyl linkage was assumed but not proven[95].

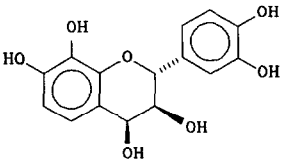
3.4 Proteracacidins and promelacacidins

Whilst considerable progress has been made on the chemistry and structures of proanthocyanidins based on the phloroglucinol and resorcinol A-ring flavanoids, those oligomers with pyrogallol-type A-rings remain largely unexplored. Although the flavan-3,4-diols (-)-melacacidin (18), its C-4 epimer, (-)-isomelacacidin, and (-)-teracacidin (19) are present in a large number of *Acacia* species[32,96], their corresponding proanthocyanidin oligomers are more sparsely populated. The additional hydroxyl function at C-8 presumably renders the aromatic A-ring less able to react as nucleophile for condensation with C-4 carbocations[16] or quinone-methides[44,45] or, alternatively, 8-hydroxylation might counteract electron release from the 7-hydroxyl group, thus reducing the tendency of flavan-3,4-diols (18) and (19) to form C-4 carbocations or A-ring quinone-methide intermediates which are considered essential for initiating condensation. These considerations led to suggestions[3,97] that on electronic grounds oligomers composed of pyrogallol-type A-ring moieties are unlikely to exist and that the polymers that co-occur with these flavan-3,4-diols are probably oxidation products.

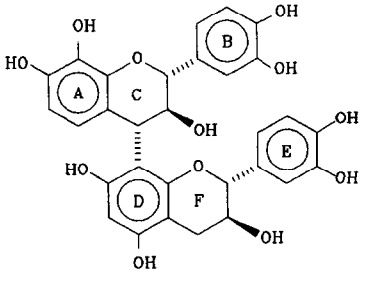
Several studies[55,56,98] have, however, since demonstrated that the flavan-3,4-diols (18) and (19) are susceptible to facile condensation with phenolic nuclei under mild acidic conditions to give 4-arylflavan-3-ols of types (70) and (71) (*cf.* Scheme 2), such a phenomenon suggesting that the formation of natural proanthocyanidins of the 7,8-dihydroxyflavanoid pattern is not chemically prohibited. These observations have subsequently led to identification[50,99] of three $C(sp^3)$ - $C(sp^2)$ biflavanoids (115), (116), and (117) with constituent 3,3',4',7,8-pentahydroxyflavan moieties from *Prosopis glandulosa* (mesquite) where they co-exist with the



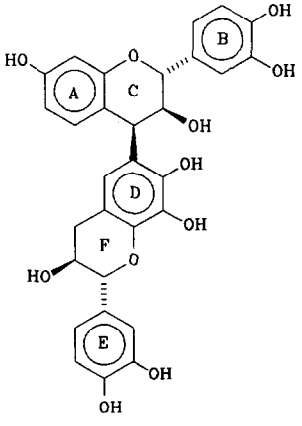
(113)



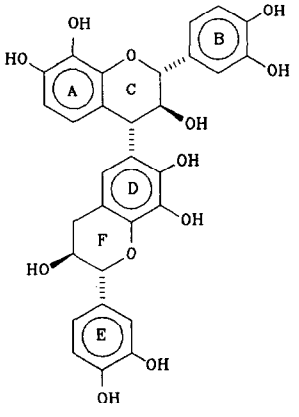
(114)



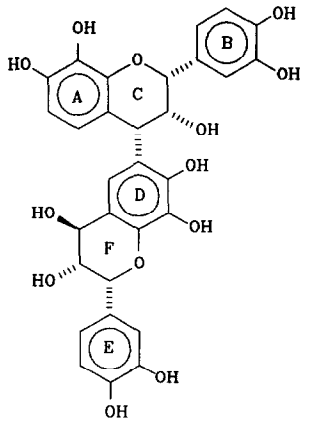
(115)



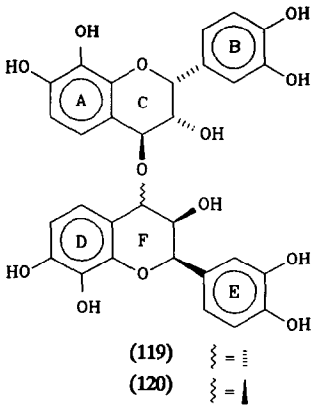
(116)



(117)



(118)

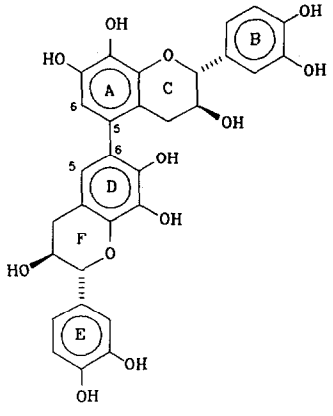


(119) ⦚ = ⦚

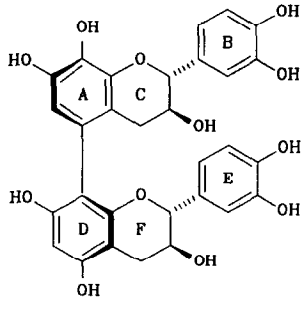
(120) ⦚ = ⦙

related flavan-3-ol (113) and flavan-3,4-diol (114), and the (+)-2,3-*cis*-3,3′,4′,7,8-pentahydroxyflavan-(4,6)-isomelacacidin (118)[38], and the unique (4-*O*-4)-linked biflavanoids (119) and (120)[100] from the heartwood of *Acacia melanoxylon*. The natural occurrence of these promelacacidins clearly demonstrates that the pyrogallol-type A-ring is sufficiently reactive for nucleophilic condensation and also to facilitate C-4 carbocation formation from an associated flavan-3,4-diol hence initiating the formation of proanthocyanidin oligomers, the (4-*O*-4)-coupled analogues (119) and (120) further extending the phenomenon of heterogeneity of the interflavanyl linkage among natural proanthocyanidin dimers. No natural counterparts for the two synthetic proteracacidins[16] based on (+)-catechin have hitherto been recorded.

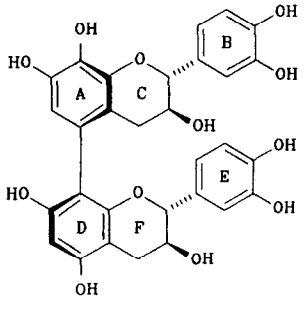
In the heartwood of *P. glandulosa* the promelacacidins (115) and (117) are accompanied by a series of oxidative coupled analogues (see section 4) with (+)-mesquitol (113) serving as a key precursor[50]. Amongst these are the (5,6)-bis-(+)-mesquitol (121), the atropisomeric (+)-mesquitol-(5,8)-(+)-catechins (122) and



(121)

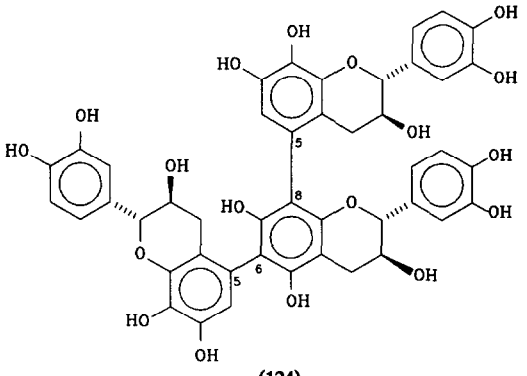


(122)



(123)

(123) with respectively *S* and *R* absolute configuration at the $C_{(sp^2)}$-$C_{(sp^2)}$ interflavanyl linkages, and the four atropisomeric (5,6:5,8)-*m*-terphenyl-type triflavan-3-ols of general structure (124). Oligomeric structures were

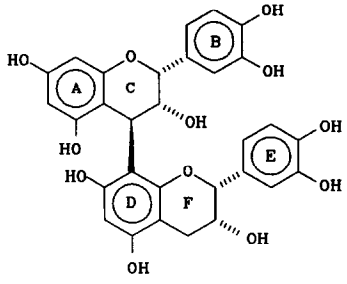


(124)

confirmed by biomimetic oxidative coupling[50,101] involving (+)-mesquitol and (+)-catechin, and by NOE difference spectroscopy, the latter technique elegantly allowing definition of the absolute configuration at the point of the interflavanyl linkage in the phenolic methyl ether acetates of the (5,8)-biphenyl-type biflavan-3-ols (122) and (123)[50], and of the four *m*-terphenyl analogues of type (124)[102].

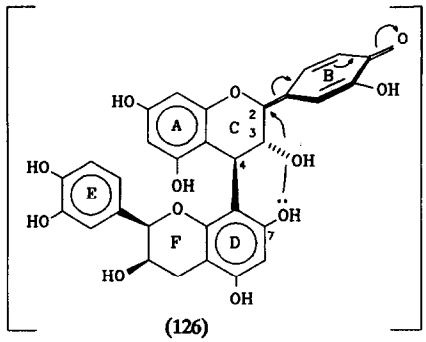
3.5 A-Type proanthocyanidins

Proanthocyanidin A-2 (127) [(-)-epicatechin-(2β → 7, 4β → 8)-(-)-epicatechin] was first isolated by Mayer *et. al.* from the seed of *Aesculus hippocastanum*[103]. The structure was deduced by Haslam and his collaborators *via* spectroscopic and chemical evidence[11] and has, more recently, been unequivocally established by X-ray crystallography[104]. A variety of proanthocyanidins possessing the doubly-linked unit of either (2β,4β)-(127) or (2α,4α)-configuration (128) has since been reported[6,11,105-112]. Constituent units other than (+)-catechin and (-)-epicatechin have also been encountered, *e.g.* a flavonol[113], a flavan C-ring[108], (-)-epigallocatechin[113] and the afzelechins[105,108,114] (*cf.* ref. 1 for a reasonable comprehensive summary of known A-type procyanidins). As with the procyanidins and also other classes of condensed tannins, the group of Nishioka made considerable contributions to the chemistry of the A-type analogues, especially regarding those oligomers containing both A- and B-type linkages, *e.g.* triflavanoid (130) (*cf.* refs. 110-114 and refs. cited therein).



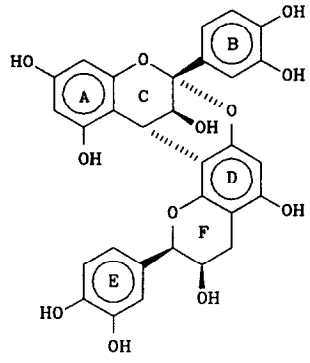
(125)

[O]

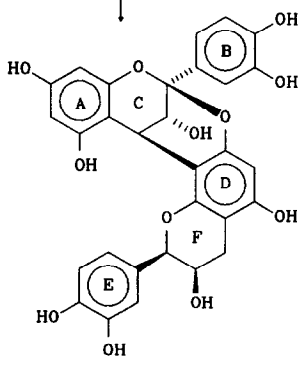


(126)

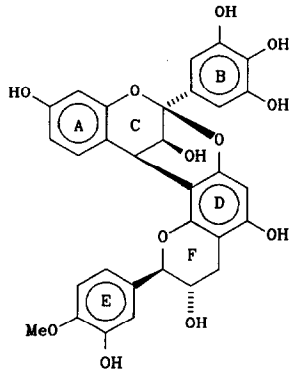
7-OH(D) → C-2 (*si*-face)



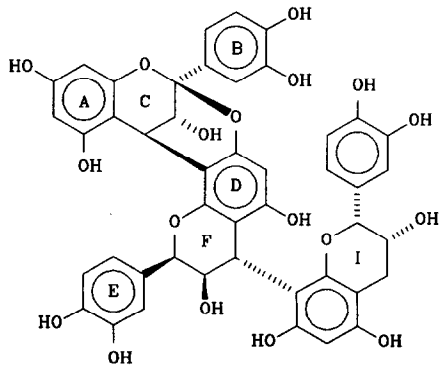
(128)



(127)



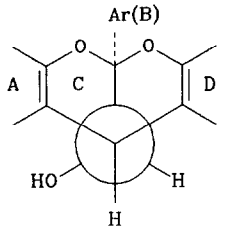
(129)



(130)

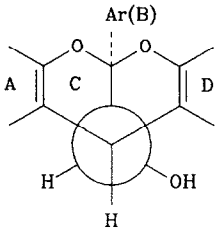
Owing to the close structural relationship between proanthocyanidin A-2 (127) and procyanidin B-2 (125), Porter[1] has proposed a biosynthetic pathway for the conversion of B- to A-type procyanidins which involves an enzyme mediated hydroxylation at C-2 (C-ring) of (125). Despite the considerable progress in the semi-synthetic approach towards condensed tannins over the last fifteen years, similar efforts aimed at the oxidative conversion of B- to A-type procyanidins are much more limited. These methods are restricted to the use of $H_2O_2/NaHCO_3$[111-113] and molecular oxygen[115,116], both sets of conditions, however, giving low yields of the A-type proanthocyanidins. It seems reasonable to assume that the transformation of *e.g.* procyanidin B-2 (125) into the A-type analogue (127) involves the oxidative removal of hydride ion at C-2(C) as the initial step. The nature of the oxidizing species is, however, not clear when using oxygen. Although this reagent may effect the transformation (125) → (126)[117] it seems more reasonable that the prevailing conditions induce oxidation of the *o*-dihydroxy functionality of the pyrocatechol B- or E-rings to an *o*-quinone which subsequently serves as oxidant for the conversion (125) → (126).

Proanthocyanidins with A-type linkages invariably display $^3J_{HH}$ = 3-4 Hz[11] for 3- and 4-H (C-ring), a phenomenon which by reference to X-ray data for procyanidin A-2 (127) and ^{13}C NMR comparisons, has consequently been accepted to indicate 3,4-*trans* relative configuration for all known compounds in this class of naturally occurring condensed tannins. The recent synthesis of the first A-type analogue (129) with 3,4-*cis* configuration of the C-ring[116], however, indicated that these compounds exhibit identical ^1H NMR coupling constants ($J_{3,4}$ = 3.5 Hz) irrespective of the relative configurations of their C-rings. Consideration of the structure of the prorobinetinidin related compound (129) with 3,4-*cis* configuration in conjunction with the conformational rigidity of the bicyclic ring system indicates very similar dihedral angles between 3- and 4-H(C) in both 3,4-*trans* (131) and 3,4-*cis* (132) homologues which should thus lead to almost identical coupling



(131)

(3,4)-*trans*



(132)

(3,4)-*cis*

constants for these protons. A method based on the selective ^1H NOE association of 3-H(C) permitting such a differentiation in the A-series of (4,8)-linked proanthocyanidins was also described[116].

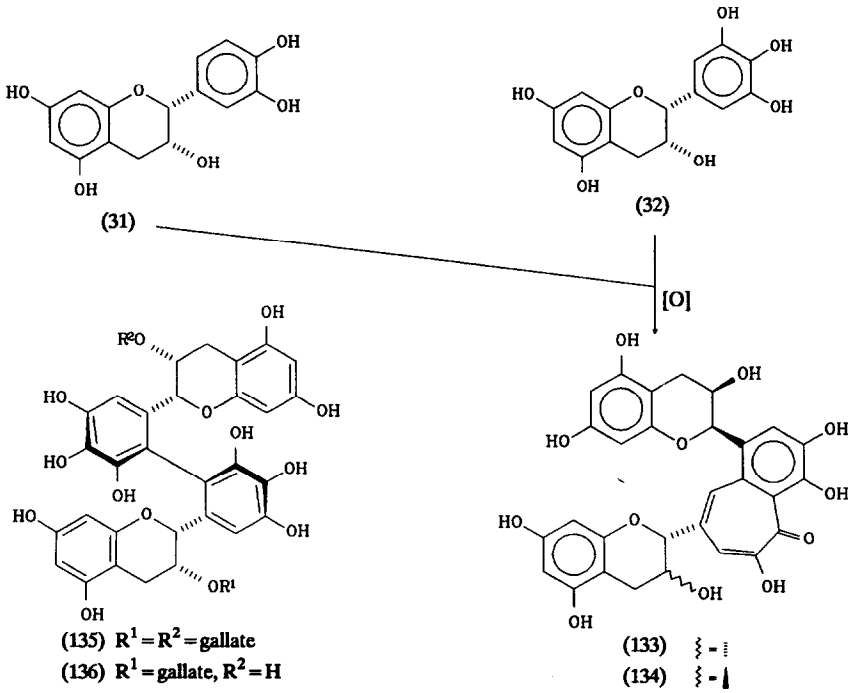
4. Flavan-3-ol Oligomers *via* Phenol Oxidative Coupling

Although oxidative coupling of flavonoids is an established natural phenomenon affecting mainly flavones and flavanones[31,118], participation by flavan-3-ols in this mode of condensation is less common. Examples of

the latter involve (2′,8)-coupling of (+)-catechin *via* the respective B- and A-rings, giving two biphenyl-type 'dehydrodicatechins', a trimer, and also higher oligomers[119-122]. They have been prepared[119] by enzymic dehydration of (+)-catechin while bi- and ter-flavan-3-ol analogues, the latter based on a single biphenyl link, have been encountered in the bark of *Quercus robur*[123,124].

Of special interest amongst this class of flavan-3-ol oligomers is the theaflavins and thearubigins which play such a key role in the quality of black tea[125]. These compounds do not occur in the green leaf, but are formed during the black tea manufacturing process by enzymatic oxidative transformation of some of the flavan-3-ols present in the fresh green leaf. Considerable clarification of the details of the oxidative conversions have followed from the work of several groups, particularly that of Ollis and his co-workers. The principal components of the green tea leaf are (-)-epicatechin (31) and (-)-epigallocatechin (32), and their respective 3-*O*-gallate esters. These precursors are then transformed into the theaflavin and thearubigin type pigments of black tea during 'fermentation' which involves a random enzyme catalyzed oxidative coupling process[126-128].

The polymeric thearubigins have been recognized as being in part polymeric proanthocyanidins[75,126]. The theaflavin fraction on the contrary comprises a considerable number of compounds including theaflavin (133), its 3-*O*- and 3′-*O*-monogallates, and corresponding *O,O*-digallate, and theaflavic and isotheaflavic acids[129]. Theaflavin may be considered to be formed by oxidative coupling of (-)-epicatechin (31) and (-)-epigallocatechin (32) in a normal type of benztropolone synthesis[130,131]. The recent isolation[132] of theasinensins A and B (135) and (136), a pair of dimeric (-)-epigallocatechin gallates, in which the flavan-3-ol are linked by a (2′,2′)-biphenyl bond, gives credence to the proposals regarding the genesis of theaflavin (133)



and isotheaflavin (134)[133]. Both the theasinensins and the atropisomeric (+)-mesquitol-(5,8)-(+)-catechins (see section 3.4) possess characteristically intense CD bands expected for biaryl compounds[50,98,132]. The chirality of the biaryl unit in both theasinensins A and B is (*S*), the same as that for ellagitannins[134]. Theasinensins C-G and a related compound, oolongtheanin, have since then been reported, the biphenyl bond in some of these also exhibiting (*R*)-chiralities[135].

5. Miscellaneous Oligoflavanoids and Techniques for Structural Elucidation.

The present Report focuses on some of the more important principles which govern the chemistry of oligomeric flavanoids and is not intended to be a comprehensive treatment of all known compounds. Detailed accounts of the known oligomeric proanthocyanidins and their distributions in plants have recently been compiled[1,31,136-138] and will not be repeated here.

The predominant part of our contributions towards condensed tannin chemistry comprises a study of analogues possessing 5-deoxy chain extender units, *e.g.* the profisetinidins, prorobinetinidins, and the proguibourtinidins. Owing to the inherent complexity of the phenolic mixtures of plant species containing these structural types, the major part of this research deals with the methyl ether acetates of the oligomeric proanthocyanidins since the additional chromatographic stages offered by consecutive methylation and acetylation are often prerequisites for sample purity. This has resulted in our heavy reliance on ^{1}H NMR techniques[139] and to the partial neglect of the very powerful ^{13}C NMR method.

^{13}C NMR spectroscopy has proved to be one of the most generally useful techniques for the study of proanthocyanidin structure[11,12,140-146], especially as the phenols usually provide good quality spectra, whereas their ^{1}H NMR spectra are considerably broadened due to proton-exchange processess. The ^{13}C NMR spectra of oligomers reveal such information as the A- or B-ring substitution pattern, the relative stereochemistry of the C-ring, and in favourable cases, the position of interflavanyl linkages. Extensive compilations of spectra, especially for procyanidins, have appeared[144,145] while the presence of *O*-gallates, *O*-glycosides, or cinchonain-type functionality may be deduced *via* the elegant papers of Nonaka and Nishioka (*cf.* ref. 1 and refs. cited therein).

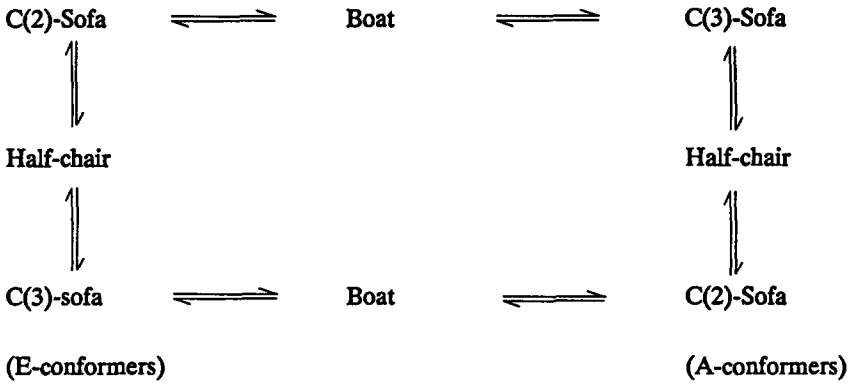
Fast atom bombardment mass spectrometry (FAB-MS) has become a powerful tool for studying the structures of biopolymers, FAB and similar forms of mass spectrometry have the potential for achieving the same degree of importance in the elucidation of proanthocyanidin structure currently accorded ^{1}H- and ^{13}C-NMR spectroscopy. Their applications in this regard were recently thoroughly reviewed[147].

6. CONFORMATION OF OLIGOFLAVANOIDS

Conformational analysis of oligoflavanoids is in principle concerned with the conformation of the pyran

heterocycle and with the phenomenon of conformational isomerism owing to restricted rotation about the interflavanyl linkage. The advent of ^{1}H NMR spectroscopy enabled Clark-Lewis and collaborators[148] to propose C-ring conformations approximating a half-chair, with the B-ring in an equatorial position, for a series of flavans with phenolic groups protected by methylation and with various heterocyclic ring substituents. Numerous ^{1}H NMR investigations have since then borne out these findings. More recently X-ray crystallographic studies of (-)-epicatechin (31)[149], the 8-bromo-3',4',5,7-tetramethyl ether derivatives of (+)-catechin[48] and (-)-epicatechin[138], and (+)-leucocyanidin (12)[150] have been published which generally support the NMR conclusions.

Porter, Mattice and co-workers[25] have considered the factors that influence the equilibration of the C-ring of flavan-3-ols and demonstrated that it may be described by the equilibrium:



where E- and A-conformers[151] are those with the B-ring equatorial or axial respectively. Figure 1 [25] depicts the ground-state energy conformations which may be adopted by the flavan heterocycle with the hatched line indicating the projection of the A-ring. Figure 2 [25] gives the relative stereochemistry of groups at C-2 and C-3

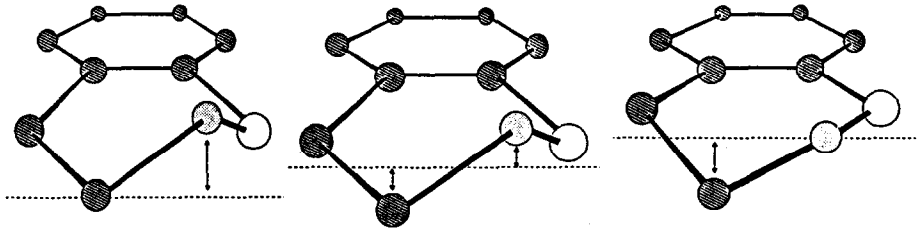


Figure 1.

for the E- and A-conformations of (+)-catechin (28) [(137) and (138)] and (-)-epicatechin (31) [(139 and (140)]. The conformations are viewed in the sense indicated by the arrow in structures (28) and (31), and the solid line in (137) - (140) represents the A-ring plane

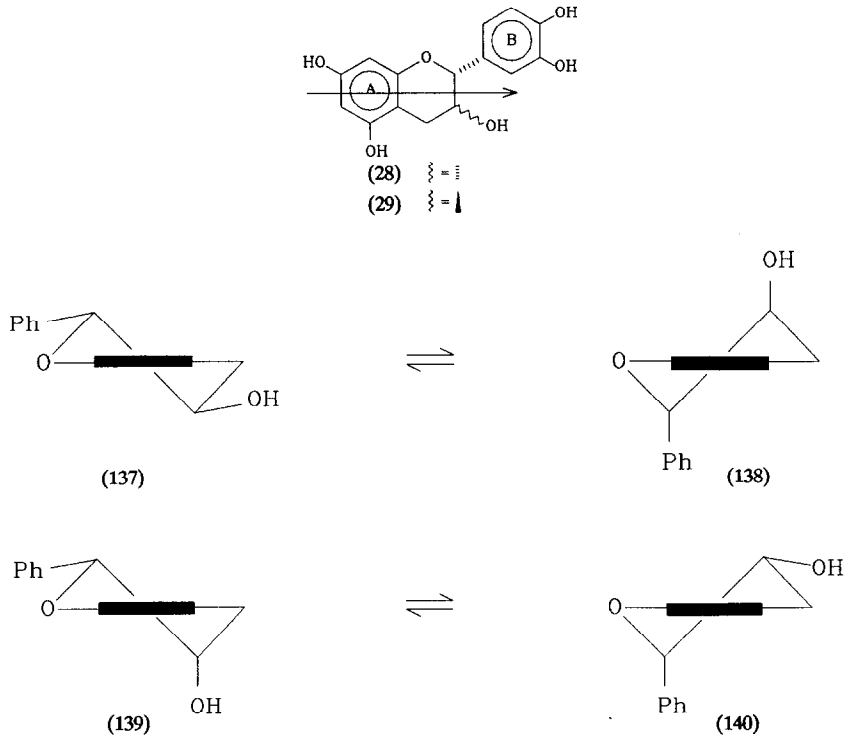


Figure 2.

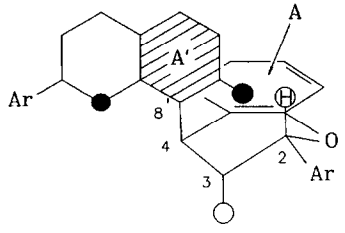
The boat conformation represents the high-energy transition state for the interconversion of E- and A-conformers. An unequal conformational energy for these conformers is manifested by an unequal population of the two states, the one with lower energy being populated to a greater extent. ^{1}H NMR measurements in conjunction with theoretical calculations[25] demonstrated that the E:A ratio for (+)-catechin (28) and (-)-epicatechin (31) were 62:38 and 86:14 respectively. Acetylation of the 3-hydroxy group stabilized the A-conformation and altered the ratio to 48:52.

Substitution at C-4 by a hydroxy or aryl substituent strongly favours the E-conformation owing to minimization of 1,3-diaxial interactions and the *pseudo*-allylic or A(1,3)-strain effect[25]. It was also concluded that the favoured E-conformers of (+)-catechin-4 and (-)-epicatechin-4 units would adopt C(2)-sofa and half-chair conformation, respectively as minimum energy species.

The profound effect of the dynamic equilibrium between E- and A-conformers (*cf.* the aforementioned conformational itinerary) on the dihedral angles of heterocyclic protons and hence their ^{1}H NMR coupling constants is obvious. Prior to the pioneering work of Porter and Mattice[25] the observation of 'abnormal'

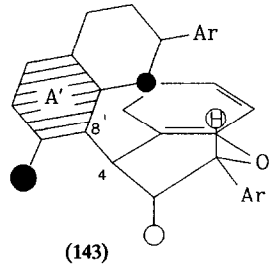
$^3J_{H,H}$-values for C-4 substituted flavan-3-ols with 2,4-*cis* arrangement of substituents has often led us to wrongly assume a preferred boat or twisted boat conformation for these and other analogues exhibiting atypical coupling constants and Cotton effects in their CD spectra (*cf.* refs. 2, 28, 71, and 84). The 'abnormal' heterocyclic $^3J_{H,H}$-values are now considered to be representative of the biased equilibrium (on the NMR time scale) between E- and A-conformers in solution.

(4,8)-Linked procyanidin dimers possess detectable conformational isomers resulting from steric interactions in the vicinity of the interflavanoid bond[12,83] which exhibit two sets of ^{1}H NMR signals[145] and



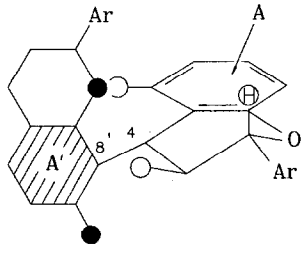
(141)

2,3-*cis*



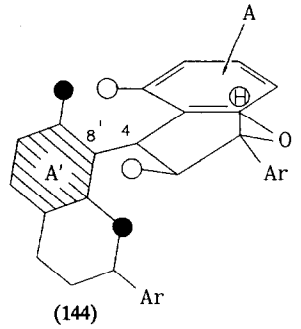
(143)

2,3-*cis*



(142)

2,3-*trans*



(144)

2,3-*trans*

heterogeneous fluorescence decay[152]. Conformations (141) and (142) of the 2,3-*cis*- and *trans* dimers respectively, correspond to that in which the C-4 proton eclipses the aromatic A-ring of the lower flavanoid unit, with the bulky C-2 and C-4_a substituents occupying positions of least steric interaction with the lower flavan unit. In $CDCl_3$, conformation (144) predominates in 2,3-*trans* dimer peracetates whereas conformers (141) and (143) occur in nearly equal proportions for 2,3-*cis* dimer deca-acetates[12,145]. These models have also led to proposal[153] of a transition polarization diagram to account for the sign of the short-wavelength CD couplet of dimeric procyanidins.

Interest in the proanthocyanidin polymers is increasing because of their potential as a renewable source of useful chemicals[31], their probable use by plants as a defense mechanism[154], and their formation of complexes with a variety of naturally occurring and synthetic polymers[155-158]. Owing to the purported importance of the conformation about the interflavanoid bond towards these phenomena, Mattice and co-wor-

kers have of late launched an intensive investigation to an understanding of the conformations of the dimeric procyanidins and also the higher polymers (*cf.* ref. 159 and refs. cited therein).

7. Phlobatannins *via* Base-catalyzed conversions of oligoflavanoids

7.1 Introduction

Condensed tannins are often extracted and/or allowed to react at alkaline pH in the course of manufacture of speciality polymers such as tannin-based adhesives. These preparations invariably exhibit increased acidity and lower reactivity towards aldehydes than those obtained by neutral-solvent extraction[160-162], phenomena which have been attributed to the presence of catechinic acid-type rearrangement products[160,163]. With the exception of some reactions of monomeric flavan-3-ols[145,162-166], and a few flavan derivatives with good leaving groups at C-4[44,45], only a few authoratative papers on the base-catalyzed transformations of oligoflavanoids have been published[167,168]. These studies have largely focussed on the effects of external nucleophiles on intramolecular rearrangements[168] and on the lability of the interflavanyl bond and pyran ring[167] of polymeric procyanidins at high pH values.

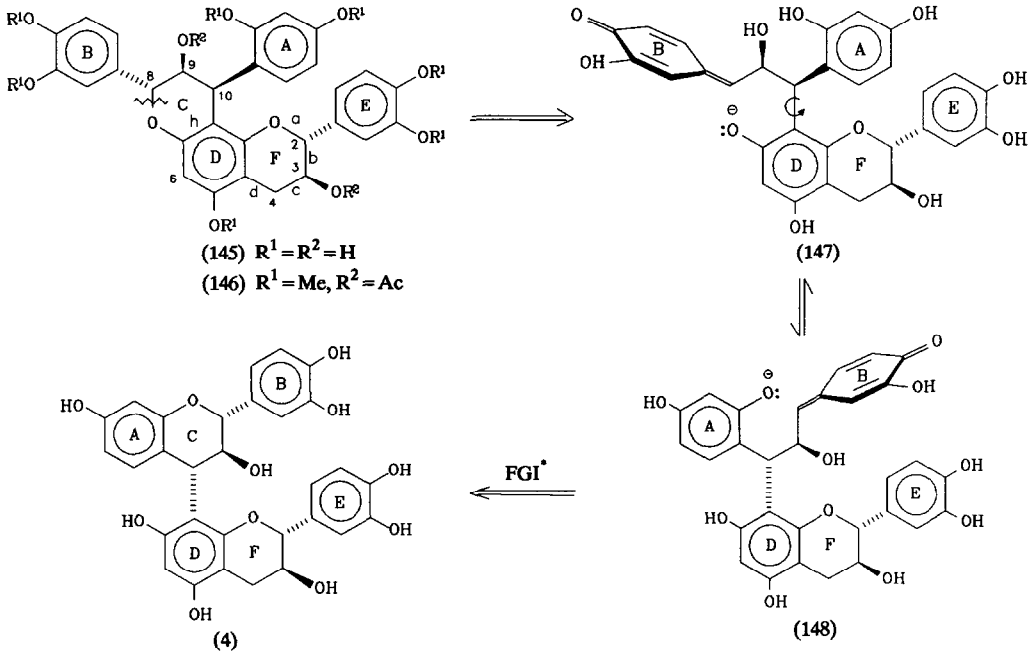
The natural occurrence of a novel class of C-ring isomerized condensed tannins, termed phlobatannins, has recently been demonstrated[169]. The structure of the functionalized 8,9-*trans*-9,10-*cis*-3,4,9,10-tetrahydro-2*H*,8*H*-pyrano[2,3-*h*]chromene (145) and those of related analogues were established by application of NOE difference spectroscopy to their phenolic methyl ether acetates, *e.g.* (146). In each instance, NOE associations of 2-OMe(A) with 3-H(A) and of 4-OMe(A) with both 3-H(A) and 5-H(A) indicate the 'liberation' of resorcinol moieties from C-ring heterocycles, compared with involvement in the presumed (-)-fisetinidol-(4,8)-(+)-catechin precursor (*vide infra*). In addition, the ^{1}H NMR spectra of the derivatives are characterized by the typical absence of the effects of dynamic rotational isomerism at ambient temperatures.

Initial identification of the pyran-rearranged profisetinidins was succeeded by the recognition of additional novel members[22,40,170] of this class of condensed tannins from the heartwoods of *Colophospermum mopane, Guibourtia coleosperma* and *Baikiaea plurijuga*. The usual methods of differentiating regio-isomeric biflavanoids, *i.e.* those based on the absolute chemical shifts of 'residual' D-ring singlets of methyl ether acetates in $CDCl_3$ at ambient temperatures[47], and by observation of NOE associations between the hydrogen- and methoxy protons of the D-ring[50], are less reliable for the methyl ether acetates of analogous phlobatannins. When taken in conjunction with the assignment of the absolute configuration, these problems prompted us to embark on a synthetic program to unequivocally define the structures of the pyran-rearranged metabolites.

Phlobatannin (145) and its C-2 (C-ring) epimer were previously obtained in minute yields from the acid-induced transformation of (-)-fisetinidol-(4α,8)-(+)-catechin (4)[84]. Consideration of the simple retrosynthetic sequence, (145) ⇒ (147) ⇔ (148) $\xrightarrow{\text{FGI*}}$ (4), when taken in conjunction with the facile epimerization at C-2 of flavan-3-ols under basic conditions[145,164,166], indicates that the conversion of the (-)-fisetinidol-(4α,8)-(+)-catechin (4) to the phlobatannin (145) might be more feasible at alkaline pH. These features initiated an

* *Functional group interconversion.*

investigation into the behaviour of a series of proanthocyanidin oligoflavanoids under mild basic conditions with a view to applying the observed principles to the synthesis of their related pyran-rearranged analogues.



(145) $R^1=R^2=H$
(146) $R^1=Me, R^2=Ac$
(147)
(4)
(148)

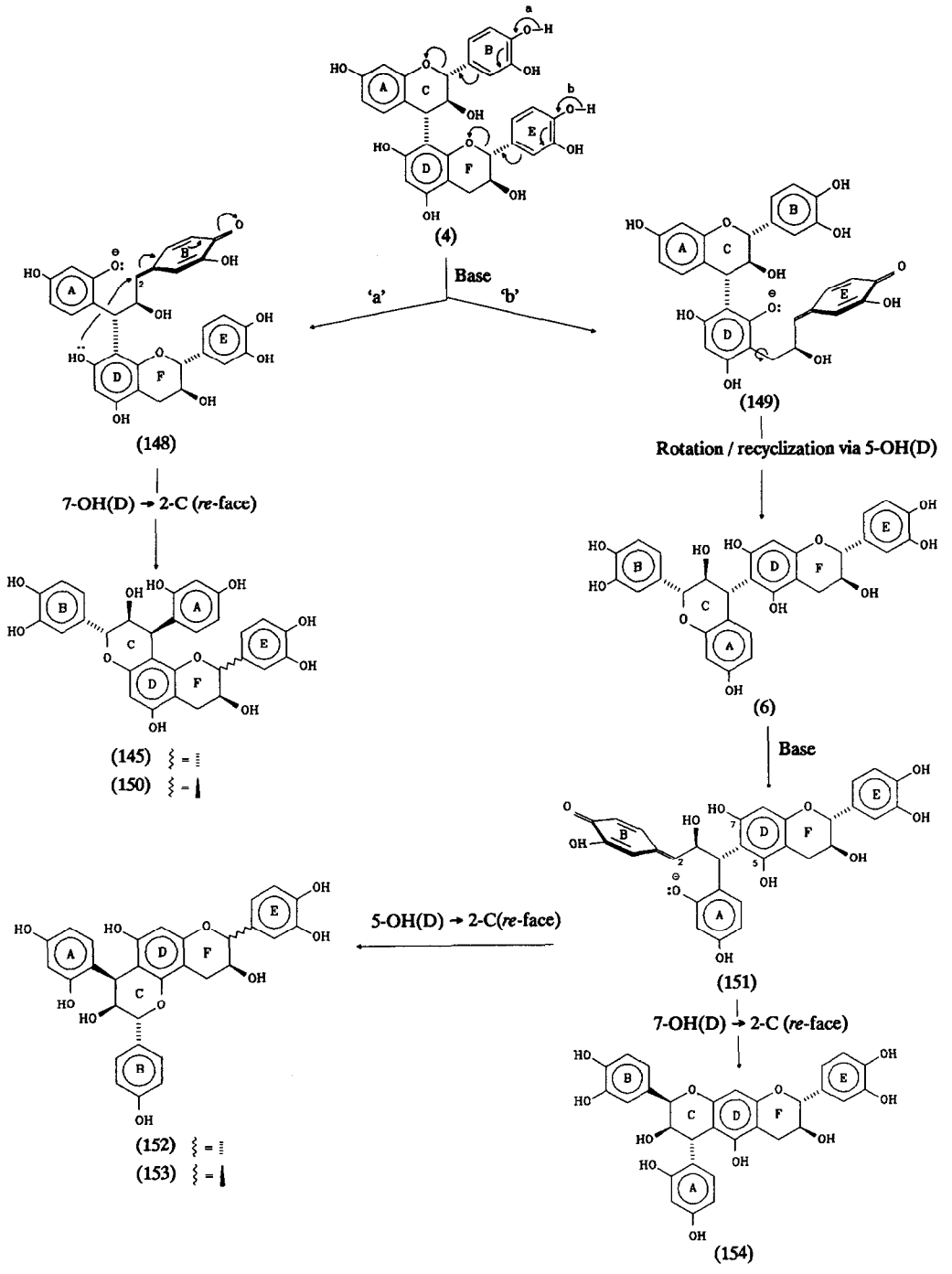
* *Functional group interconversion.*

7.2 Base-catalyzed Rearrangement of Profisetinidin-type Oligoflavanoids

7.2.1 Fisetinidol-(+)-catechin biflavanoids

Treatment of the (-)-fisetinidol-(4α,8)-(+)-catechin (4)[84] with 0.025M Na_2CO_3-0.025M $NaHCO_3$ buffer (pH 10) for 5 hours at 50°C in a nitrogeneous atmosphere*, *i.e.* conditions similar to those applied by Freudenberg[164] for epimerization at C-2 of (+)-catechin *via* intermediate fission of the heterocycle, gave significant (*ca.* 75%) conversion into five products of C-ring isomerization (Scheme 6)[40,171]. These comprise the anticipated tetrahydropyrano[2,3-*h*]chromene (145) ($J_{8,9}$ 10.0; $J_{9,10}$ 6.0 Hz) as a product of C-ring isomerization of biflavanoid (4); its C-2 (F-ring) epimer (150) representing conversion of the (+)-catechin moiety of (4) into (+)-epicatechin; the corresponding [2,3-*f*]-isomeric pair (152) and (153), and a single [3,2-*g*]-regiomer (154).

* *No attempt was made to rigorously exclude oxygen.*

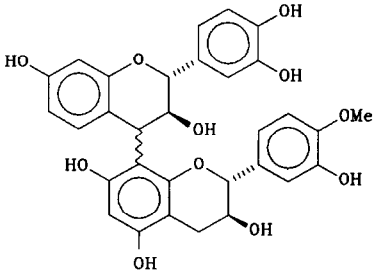


Scheme 6. Base catalyzed formation of tetrahydropyranochromenes (145), (150) and (152) - (154) from (-)-fisetinidol-(4α,8)-(+)-catechin (4). Quinone methides (148), (149), and (151) are postulated and have not been isolated

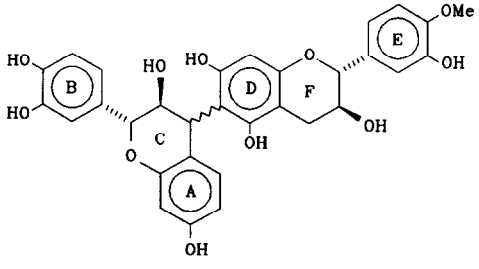
Substitution of resorcinol A-ring by phloroglucinol D-ring functionality presumably occurs *via* a B-ring quinone-methide (148)[166]. Recyclization involving 7-OH(D) requires rotation about the $C_{(3)}$-$C_{(4)}$ bond which will invariably lead from the 3,4-*trans* to a 3,4-*cis* configuration. Dreiding models indicate preference for attack at the *re*-face (C-2) in the quinone-methide (148) and thus for retention of absolute configuration at C-2(C) [C-8(C) in phlobatannin (145)] for biflavanoids of the 2*R*-series with 3,4-*trans* configuration, *e.g.* (4) and (6). An E-ring quinone-methide (149) could undergo rotation about the $C_{(3)}$-$C_{(4)}$ bond and recyclization *via* either 5-OH(D) or the D-ring phenoxide ion thus simultaneously achieving the observed regio- and configurational isomerizations.

In contrast to the lability of the interflavanyl bond in procyanidin biflavanoids[44,45], *e.g.* (77) (Scheme 3) under basic conditions, the corresponding linkage in profisetinidin (4) is more stable as was evidenced by the generation of only small quantities of (+)-catechin. Since oxygen is a prerequisite for the base-catalyzed C-2 epimerization of (+)-catechin and (-)-epicatechin[165], the mechanism for the genesis of the B- and E-ring quinone-methides in Scheme 6 is an oversimplification and should involve, more correctly, the initial formation of a radical anion at the pyrocatechol ring(s) (*cf.* ref. 172). As this will not change the stereochemical course of the pyran-rearrangement reactions, the route to the quinone-methides in Scheme 6 will be retained for the sake of simplicity.

To prevent the side reactions associated with an E-ring quinone-methide (149), biflavanoid precursors of type (4) had to be protected selectively at 4-OH(E). This was done by selective methylation [173] of (+)-catechin, and subsequent acid-catalyzed coupling of the 4′-*O*-methyl-(+)-catechin and (+)-mollisacacidin (15) to give the (4,8)- and (4,6)-(-)-fisetinidol-(+)-catechin mono-*O*-methyl ethers (155) - (158)[40] following chroma-



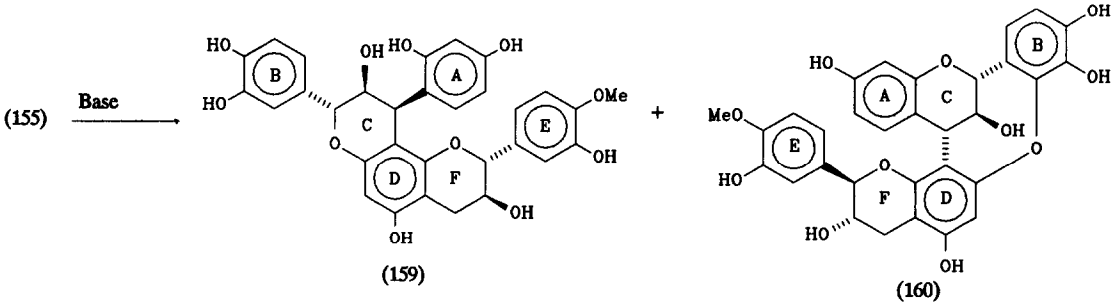
(155) ⟨ = ⟩
(156) ⟨ = |



(157) ⟨ = ⟩
(158) ⟨ = |

tography on Sephadex LH-20 and Fractogel TSK HW-40(S)[174] in ethanol.

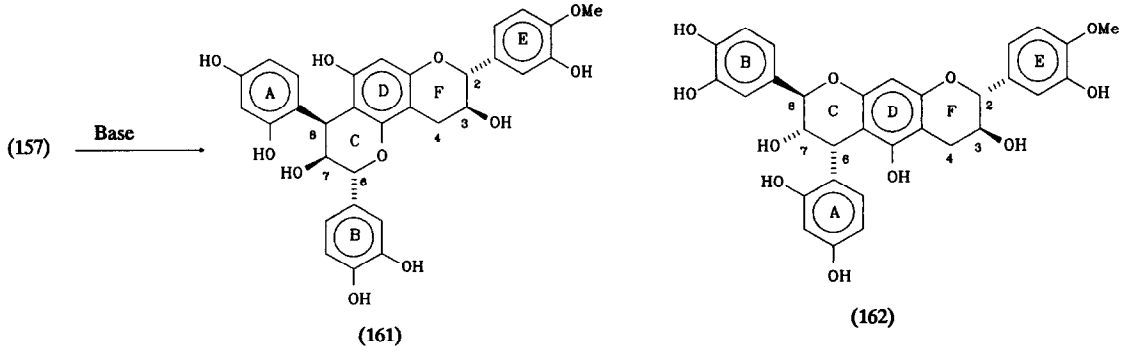
Treatment of the (-)-fisetinidol-(4α,8)-(+)-catechin-*O*-methyl ether (155) with base (pH 10) as above and subsequent chromatography on Sephadex LH-20 gave the 8,9-*trans*-9,10-*cis*-tetrahydropyrano[2,3-*h*]chromene (159) in 58% yield. The phlobatannin (159), resulting from highly stereoselective ring isomeriza-



tion with retention of the configuration at C-2 in (155), is accompanied by small amounts of a dehydro-(-)-fisetinidol-(+)-catechin (160) representing the alternative mode of cyclization *via* C-6(B) in a quinone-methide of type (148) followed by oxidative removal of hydride ion during workup. A computer simulated 3D perspective of (160) indicates a cuplike conformation involving the A-, B-, C-, D-, and eight-membered rings thus resembling those of the calixarenes, which were recently established by X-ray analysis[175].

The presence of the dehydro analogue (160) provides indirect evidence for the proposed quinone-methide mechanism. This compound may, however, originate alternatively *via* the oxidative formation of a B-ring *o*-quinone which could then substitute the B-ring quinone-methides of type (148) as internal electrophile. All efforts to trap an intermediate of type (148) with powerful nucleophiles such as phenyl sulphide- and selenide ions invariably failed. Absence of products resulting from a migrating flavanyl moiety in the 'protected' biflavanoid (155) confirms the conjecture regarding the mechanisme of such a migration in the 'uncontrolled' synthesis.

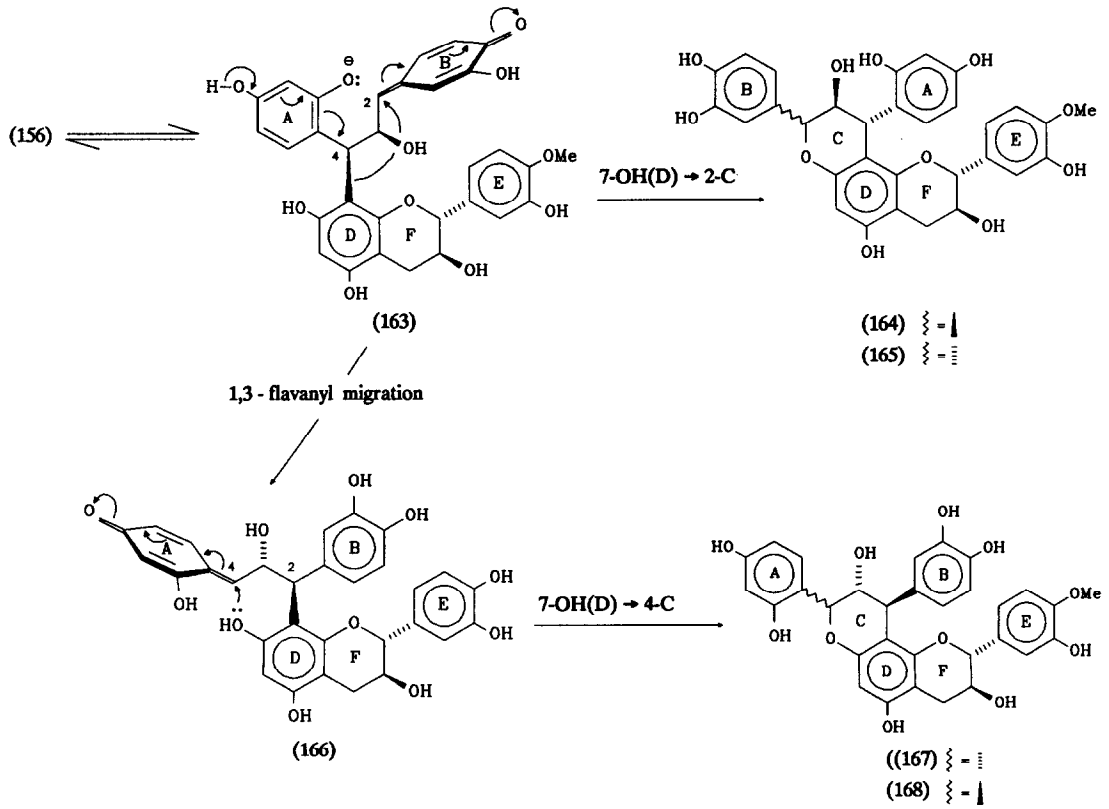
Base treatment of the (-)-fisetinidol-(4α,6)-(+)-catechin-*O*-methyl ether (157) afforded the anticipated products of pyran rearrangement *i.e.* the 6,7-*trans*-7,8-*cis*-tetrahydropyrano[2,3-*f*]chromene (161) and the [3,2-*g*]regiomer (162) as the minor product. The apparent preference for ring isomerization of biflavanoid



(157) involving 5-OH(D) and C-2 in an intermediate quinone-methide of type (151) presumably reflects a solvent dependent preferred interflavanyl conformation favouring participation of 5-OH(D) in the cyclization step. Such an assumption is in line with the observation that the two rotational isomers at the interflavan bond are unevenly populated in the procyanidins[176].

Similar treatment of the (-)-fisetinidol-(4β,8)-(+)-catechin-*O*-methyl ether (156) afforded a mixture comprising four ring-isomerized products[22,170]. These include the 8,9-*cis*-9,10-*trans*- and 8,9-*trans*-9,10-*trans*-tetrahydropyrano[2,3-*h*]chromenes (164) and (165) ($J_{8,9}$ *ca.* 1.0, 9.5; $J_{9,10}$ *ca.* 2.0, 8.0 Hz) as well as an additional pair of *cis-trans*- and all-*trans* analogues (167) and (168) with ^{1}H NMR characteristics ($J_{8,9}$ ca. 1.0, 7.0; $J_{9,10}$ 2.0, 6.0 Hz for the heptamethyl ether diacetates) closely resembling those of the former pair of compounds. Prominent NOE associations between 8-H(C) and 6-H(A) in the *cis-trans* heptamethyl ether diacetate of (164) not only confirmed this configuration and thus differentiated it from a *cis-cis* arrangement but also indicated a preferred sofa conformation for the C-ring in which the resorcinol moiety at C-10 occupies a near-axial (α) orientation.

Extensive NOE-, spin decoupling-, and 2D-heteronuclear correlation experiments[22,170] indicated an

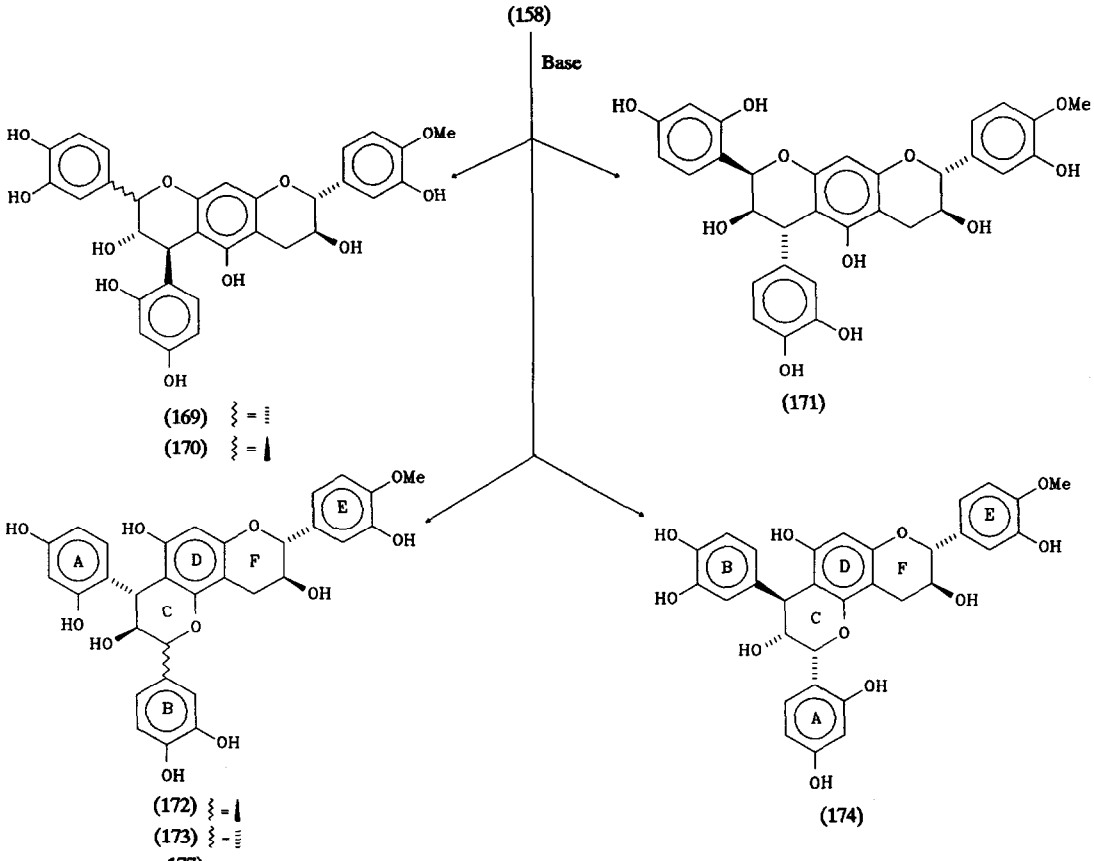


interchange of the resorcinol A- and pyrocatechol B-rings in compounds (167) and (168) relative to the positions of these rings in the 'normal' isomers (164) and (165). A notable feature in the ^{1}H NMR spectra of the groups (164), (165), and (167), (168) is a conspicuous deshielding of 6-H(A) [$\Delta\delta$ -0.74 and -0.80 for the heptamethyl ether diacetates of (167) and (168), respectively] in the latter pair relative to its chemical shift in the derivatives of the *cis-trans*- and all-*trans* isomers (164) and (165). Such a feature is a very useful characteristic of all phlobatannins belonging to the classes (167) and (168) (see also below).

Monitoring of the base-catalyzed transformation of the profisetinidin biflavanoid (156) indicated that it serves as direct precursor to both groups of phlobatannins[22,170]. Formation of the pair (164) and (165) may be rationalized by stereoselective recyclization involving 7-OH(D) and both *re*- and *si*-faces in quinone-methide (164). The novel conversion (156) → (167) + (168) is explicable in terms of initial migration of the (+)-catechin moiety to the *re*-face at C-2 in quinone-methide (163). Stereoselective pyran recyclization of quinone-methide (166) generates the tetrahydropyrano[2,3-h]chromenes (167) and (168), enantiomerically related to (164) and (165) with respect to their C-rings.

The heptamethyl ether diacetates of analogues (164) and (165) exhibit intense negative Cotton effects in the 220-240 nm. region of their CD spectra[22,170] hence indicating a 10α-aryl substituent and *R*-configuration at this chiral centre[56,69,71]. The same derivatives of the ring interchanged analogues (167) and (168) showed similar CD characteristies as those above, thus presumably reflecting similar 9*S*,10*R* absolute configuration for ring C. Such a contradiction may result from significant contributions of A-conformers (F-ring)[25] reversing the sign of the low-wavelength Cotton effect for 10β-aryl groups. The 9*R*,10*S* absolute configurations depicted in formulations (167) and (168), and thus unambiguous proof for the inversion of the absolute configuration at C-9 associated with the mechanism leading to the ring interchange, were confirmed by similar transformations on appropriate 4-arylflavan-3-ol model compounds[24] where the structural features adversely affecting the sign of the low-wavelength Cotton effect are absent.

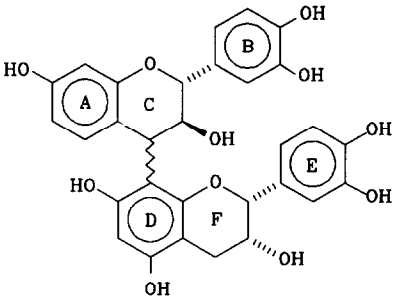
Principles similar to those advanced above also govern the base-catalyzed conversion of the (-)-fisetinidol-(4β,6)-(+)-catechin-*O*-methyl ether (158) affording four ring-isomerized products with *cis-trans*- (169), (171), (172), and (174), and two with all-*trans* configuration (170) and (173) of their C-rings. Differentiation of these and other[177,178] tetrahydropyrano[2,3-*f*]- and [2,3-*h*]chromenes and the regio-isomeric [3,2-*g*]-analogues is effected by NOE experiments on the heptamethyl ether diacetates which indicate selective association of the D-ring singlet and the methoxy group of this ring for the [2,3-*f*]- and [2,3-*h*]-isomers only. The latter groups are accordingly distinguished by the selective NOE association of the D-ring methoxy and the C-ring proton adjacent to the resorcinol moiety for the [2,3-*f*]-analogues only. Confirmation of the tetrahydropyrano[2,3-*h*]chromene arrangement for those analogues having 2α- and 10β-aryl groups or *vice versa*, are available *via* NOE association of 10-H(C) with 2- and 6-H(E)[22,40,169,170].



Application[177] of the same protocol to the (+)-fisetinidol-(+)-catechins (2*S*,3*R* absolute configuration of their C-rings) revealed similar behaviour to those described here for the (-)-fisetinidol-(+)-catechins. Thus, whereas 'upper' 2,3-*trans*-3,4-*trans*-flavan-3-ol units are susceptible to slower but highly stereoselective pyran rearrangement, those moieties with 2,3-*trans*-3,4-*cis* configuration react stereoselectively and are furthermore subject to interchange of resorcinol A- and pyrocatechol B-rings. It seems reasonable to suggest that the rate-determining step in these ring isomerizations involves reversible generation of a C-ring quinone-methide of type (163). In 3,4-*cis* biflavanoids, *e.g.* (156), 7-OH(D) is favourably orientated to anchimerically assist cleavage of the O-C_2 bond, thus enhancing both the rate of quinone-methide formation and pyran rearrangement of 3,4-*cis*-flavan-3-ol moieties. Once formed, quinone-methides derived from 3,4-*trans*-flavan-3-ol units are favourably aligned for rapid and highly stereoselective recyclization *via* 7-OH(D). The near-axial (+)-catechin unit in 3,4-*cis* quinone-methides, *e.g.* (163), would 'relax' to a more equatorial orientation, thus facilitating stereoselective pyran recyclization with preference for attack of 7-OH(D) at the *si*- and *re*-faces in the 2*R*- and 2*S*-series of profisetinidins, respectively. This would presumably result in sufficient life-times to allow for secondary rearrangements to the A/B-ring interchanged products. Owing to the proper alignment of the sp^3 bonding orbital at C-4 and the *p*-orbital at C-2 in quinone-methide (163), the latter compounds may well originate *via* a concerted mechanism.

7.2.2 (-)-Fisetinidol-(-)-epicatechins

Additional members[178] of this unique class of natural condensed tannins, representing the products of C-ring isomerization of (-)-fisetinidol-(4α,8) and (4β,8)-(-)-epicatechin profisetinidins[33] have since been obtained from the same sources as those mentioned above. The protocol of selectively protecting the



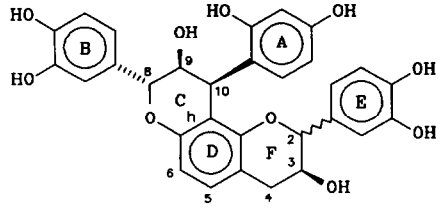
(175) ⟨ = ⟩

(176) ⟨ = ⟩

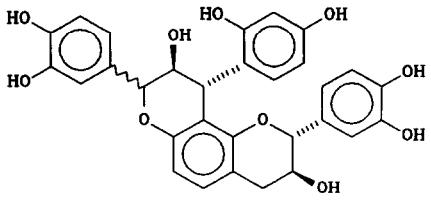
biflavanoids (175) and (176) at 4-OH(E), however, failed as a result of the preferential formation of the 3′-*O*-methyl of (-)-epicatechin under conditions which gave reasonable yields of 4′-*O*-methyl-(+)-catechin[40]. These differences in behaviour of (+)-catechin and (-)-epicatechin may be attributed to an increase in the pK_a of 3′-OH in the latter relative to 4′-OH in both flavan-3-ols[179]. The principles developed here nevertheless facilitated full definition of the structures of the natural products and also the unravelling of the complex mixtures obtained by base-mediated transformation of the profisetinidins (175) and (176)[178]

7.2.3 Bis-fisetinidols

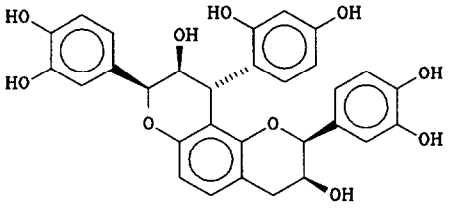
The rare (4,8)-bis-fisetinidols (54) and (55) and C - E-ring coupled analogues (57) and (58) are accompanied in the heartwood of *Colophospermum mopane*[51,52] by a series of related 5-deoxytetrahydropyrano[2,3-*h*]chromenes (177) - (181), and the first C-ring isomerized homologue (182) derived from a putative C - E-ring linked profisetinidin (183). Similar to the established biomimetic route to the 5-oxygenated tetrahydropyrano-[2,3-*h*]chromenes (*vide supra*), (4,8)-bis-fisetinidols of types (57) and (58) presumably served as precursors to homologues (177) - (181) *via* an appropriate B-ring quinone-methide[51]. Genesis of the 2,4-diaryl-6(2-benzopyranyl)-dihydrochromene (182) may similarly be explained *via* the transformation (183) ⇔ quinone-methide (184) → (182). Owing to the synthetic inaccessibility of (4,8)- and C - E-ring coupled bis-fisetinidols, no *in vitro* evidence for the formation of metabolites (177) - (182) could hitherto been obtained.



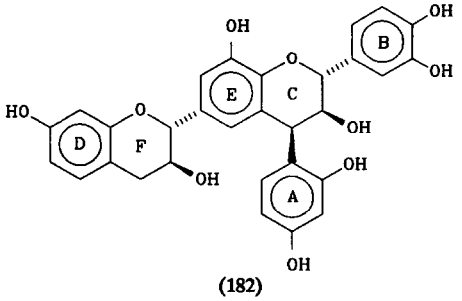
(177) ⦚ = ⦚
(178) ⦚ = ▲



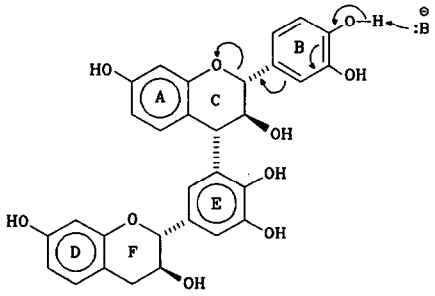
(179) ⦚ = ⦚
(180) ⦚ = ▲



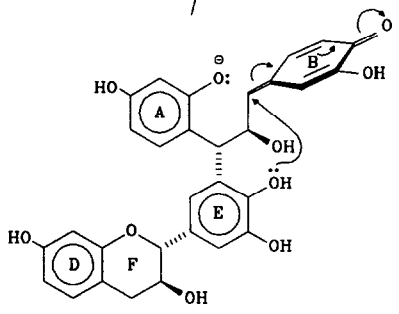
(181)



(182)

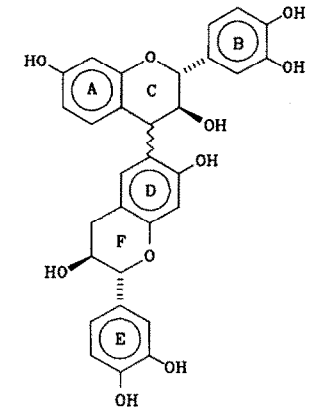


(183)

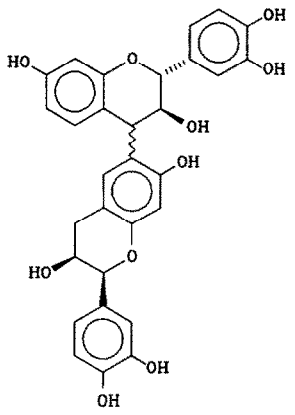


(184)

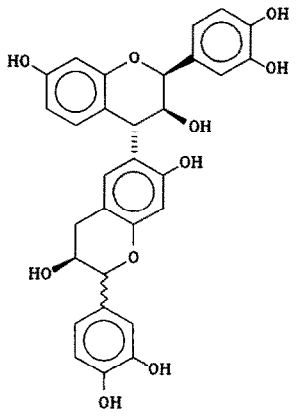
However, in the same natural source the (4,6)-bis-fisetinidols (185) - (190) co-exist with the related tetrahydropyrano[3,2-*g*]chromenes (191) - (194)[180]; the facile synthetic access[16] to bis-fisetinidols (185) - (188) permitting structural confirmation[180] of the novel C-ring isomerized metabolites (191) - (194) by the synthetic protocol applicable to this class of natural condensed tannins.



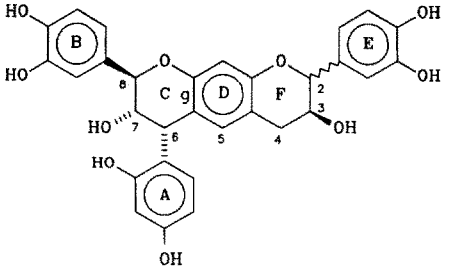
(185) ⦚ = ⦚
(186) ⦚ = ▌



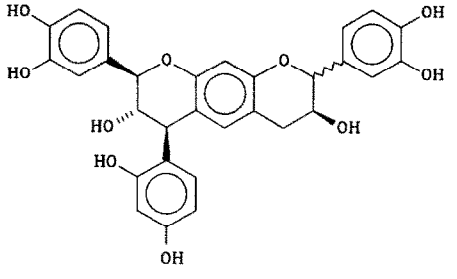
(187) ⦚ = ⦚
(188) ⦚ = ▌



(189) ⦚ = ⦚
(190) ⦚ = ▌



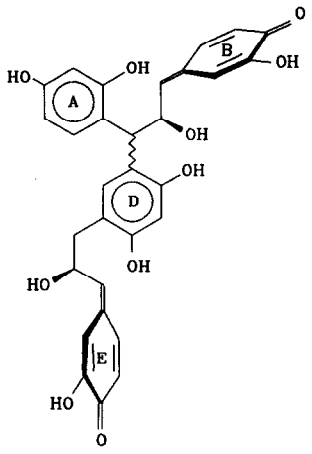
(191) ⦚ = ⦚
(192) ⦚ = ▌



(193) ⦚ = ⦚
(194) ⦚ = ▌

The ring isomerized metabolites (177) - (182) and (191) - (194) apparently originate from precursors in which the nucleophilicities of the phenolic rings effecting isomerization are of comparable [*e.g.* for (185) → (191)] or lower [for (183) → (182)] magnitude than those of the rings acting as leaving groups. Pyran rearrangement presumably leads to a decrease in conformational energy by partial removal of steric effects caused by mutual rotation of bulky groups about the interflavanyl bond of the biflavanoid precursors. Generation of the conformationally more stable product, *e.g.* (177), with its relative planar central 'core' (CDF tricyclic system) presumably provides the main impetus for these C-ring isomerizations rather than the effect of different nucleophilicities of the participating phenolic rings as was initially postulated[169].

A conspicuous feature of the C-ring isomerization reactions of the (4α,6)- and (4β,6)-bis-(-)-fisetinidols (185) and (186), is the high incidence of epimerization at C-2 of both the C- and F-rings[51]. This strongly indicates the intermediacy of quinone-methides (195) and (196) which would not only permit pyran rearrangement but also dual epimerization hence leading to (4,6)-bis-fisetinidols of types (189) and (190). The mild basic



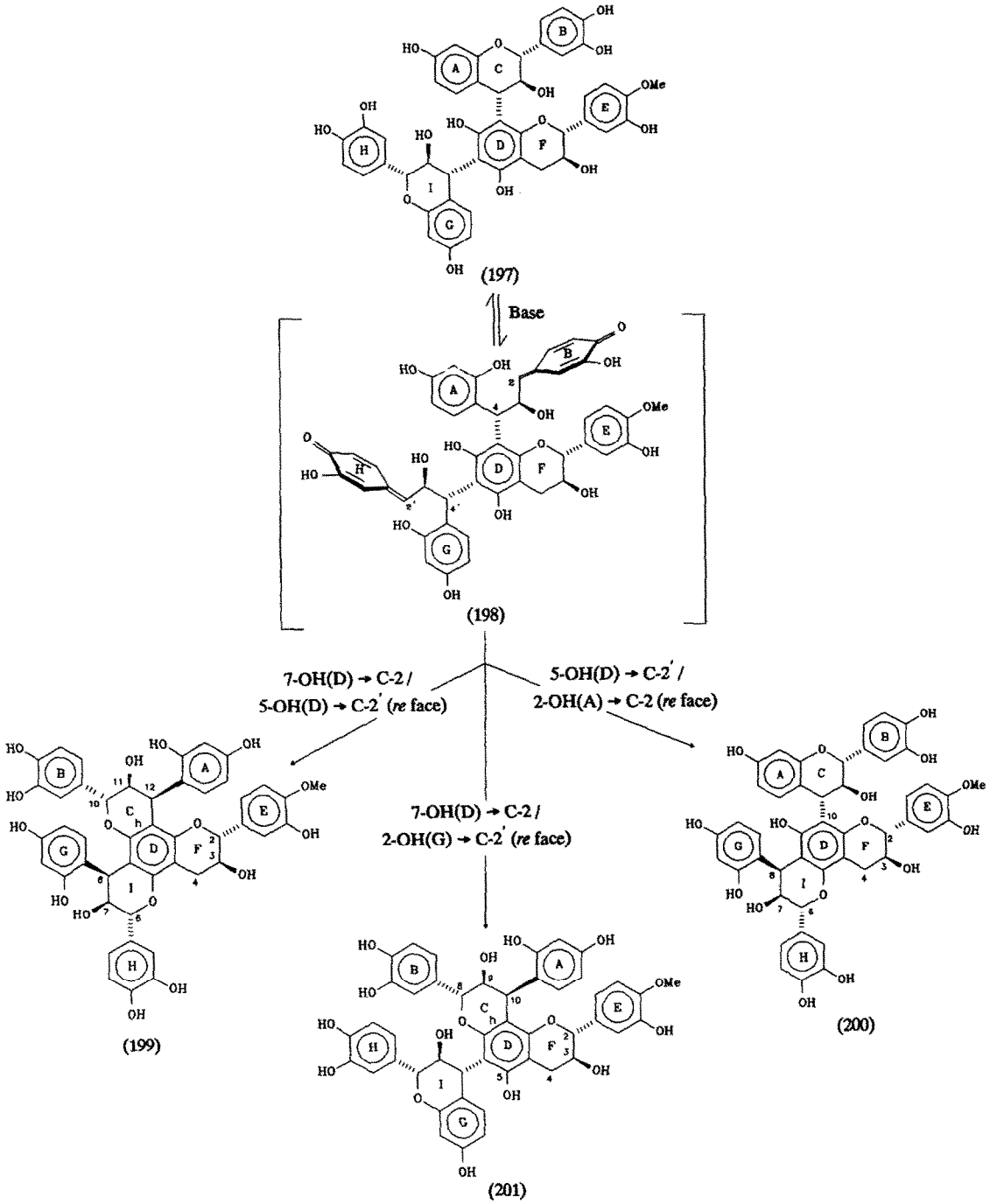
(195) ⦚ = ⦙
(196) ⦚ = |

conditions effecting these transformations presumably closely match those prevailing in Nature. These conditions may then also explain the vast number of compounds in the metabolic pool of *Colophospermum mopane* exhibiting a C-2 epimeric relationship to the predominant (-)-fisetinidol monomeric precursor[29,51,52,180].

7.2.4 Bis-(-)-fisetinidol-(+)-catechin triflavanoids

The concise approach to the synthesis of pyran-rearranged profisetinidins based on the selective generation of B-ring quinone-methides was extended to the first formation of the naturally occurring[169] hexahydrodipyranochromene and 'isomerization-intermediates' associated with a (4α,6:4α,8)-bis-(-)-fisetinidol-(+)-catechin profisetinidin triflavanoid. Thus, base treatment of the selectively protected triflavanoid (197) gave complete conversion into a mixture comprising the hexahydrodipyrano[2,3-*f*:2′,3′-*h*]-chromene (199), the (-)-fisetinidol-(4α,10)-tetrahydropyrano[2,3-*f*]-chromene (200)*, and (-)-fisetinidol-(4α,6)-tetrahydropyrano[2,3-*h*]-chromene (201) (Scheme 7)[181]. These compounds presumably have a common origin in quinone-methide (198) by the stereochemical pathways indicated in Scheme 7. Identity of corresponding derivatives of analogues (199) and (200) with those of their natural counterparts was demonstrated by comparison of the physical data of phenolic methyl ether acetates as before. Investigation to the natural occurrence and

* *Non-systematic name/numbering to retain the heterocyclic oxygen of the pyrano substituted flavan-3-ol entity as position 1 consistently for all products*

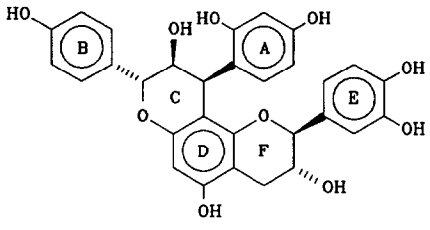


Scheme 7. Proposed route to the formation of hexahydropyranochromene (199) and isomerization-intermediates (200) and (201). Non-systematic numbering schemes are shown.

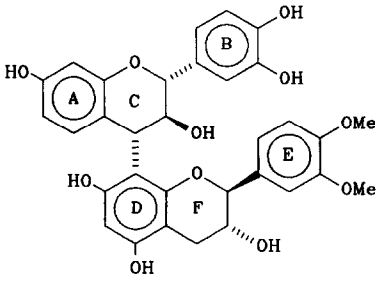
biomimetic synthesis of the remaining three diastereomers of triflavanoid (197) with 2*R* configuration are presently being undertaken.

7.2.5 Proguibourtinidin bi- and triflavanoids

Besides the presence of two 'isomerization-intermediates' related to the profisetinidin (200), but with constituent (+)-guibourtinidol units, from *Colophospermum mopane*[169,181], naturally occurring phlobatannins based on these 3,4',7-trihydroxyflavan-3-ol chain extender units are hitherto limited to a single example (202) from the heartwood of *Julbernardia globiflora*[182]. Its structure was confirmed by the well-established base-catalyzed pyran rearrangement of (+)-guibourtinidol-(4α,8)-(-)-catechin di-*O*-methyl ether (203), available *via* standard procedures[16,40,145,183].



(202)

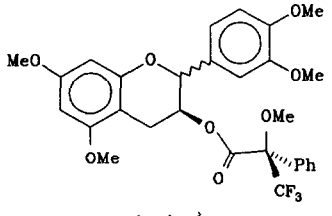


(203)

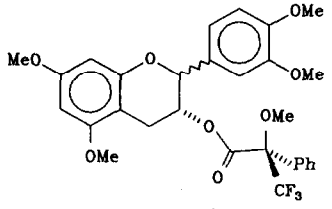
The proguibourtinidin-type phlobatannin (202) and the 3-OH(B) analogue from the heartwood of *Baikiaea plurijuga*[40] represent the first natural condensed tannins with (-)-catechin 'terminal' units. The co-existence of compound (202) and the (+)-guibourtinidol-(-)-epicatechins in *J. globiflora*[37] is significant since it strongly indicates that the same 'system' effecting the pyran rearrangement is also capable of inducing epimerization at C-2 of the (-)-epicatechin moiety hence converting it to a (-)-catechin unit *via* the very similar mechanisms for these processes outlined above.

Owing to the ubiquity of naturally occurring condensed tannins with (+)-catechin or (-)-epicatechin 'terminal' units, coupling constants of the heterocyclic protons of these moieties, *i.e.* $J_{2,3}$ *ca.* 7.0 and *ca.* 1.0 Hz, are often simply accepted as being indicative of the presence of these respective structural units. Demonstration of the presence of oligoflavanoids based on both (-)-catechin (*vide supra*) and (+)-epicatechin units[35,39,40,184,185] hence necessitated the development of a method capable of differentiating enantiomeric forms among the chain terminating units of these compounds. Preliminary results relevant to the absolute configurations of flavan-3-ols and 4-arylflavan-3-ols *via* a modified Mosher's method[186,187] were recently communicated[188].

Comparison of the chemical shifts of the B-ring protons in the *R*-(+)-α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA) esters of the methyl ethers of (+)- and (-)-catechin and of (-)- and (+)-epicatechin, indicated that these protons are conspicuously shielded in the diastereomeric esters (204) and (205) of the flavan-3-ols with 3*S* configuration [(+)-catechin and (+)-epicatechin] relative to their chemical shifts in the esters (206) and (207) of analogues having 3*R* configuration [(-)-epicatecin and (-)-catechin].

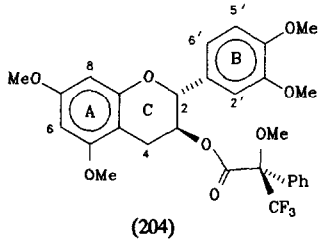


(204) ⦚ = ⦚
(205) ⦚ = ⦙



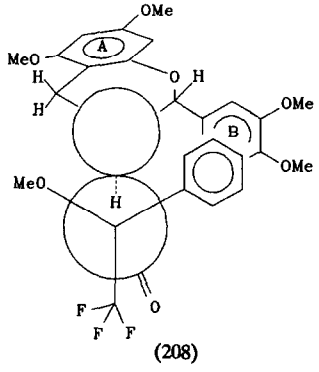
(206) ⦚ = ⦚
(207) ⦚ = ⦙

The consistency of these shielding effects in the *R*-(+)-MTPA esters is compatible with conformations in which the α-trifluoromethyl group, carbonyl, and carbinyl hydrogen are in the same plane and are approximately eclipsed. In conjunction with the proposals of Dale and Mosher[186], this subsequently permits the construction of configuration correlation models (208) and (209) for flavan-3-ols with respectively 3*S* and 3*R* configurations. These conformational models represent crucial arrangements in which the α-phenyl substituent of the *R*-(+)-

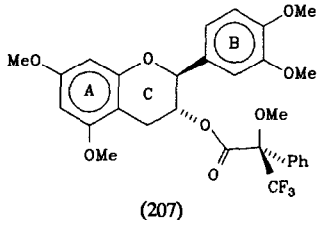


(204)

≡

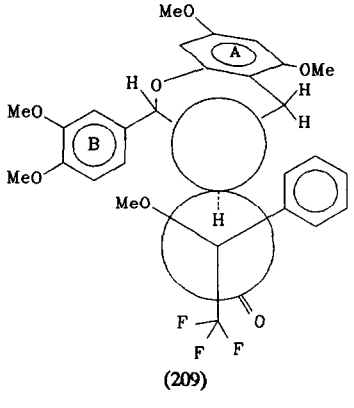


(208)



(207)

≡

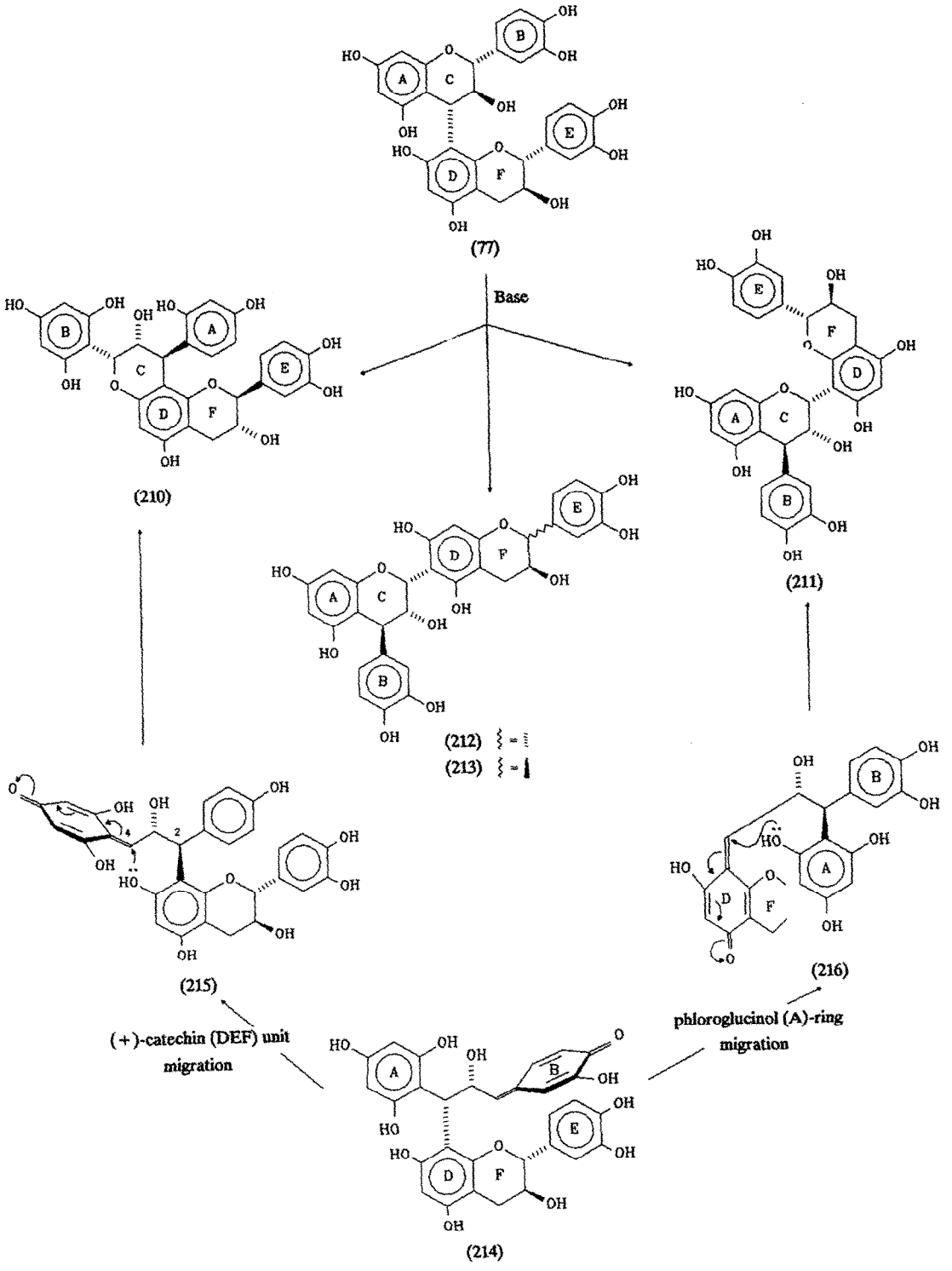


(209)

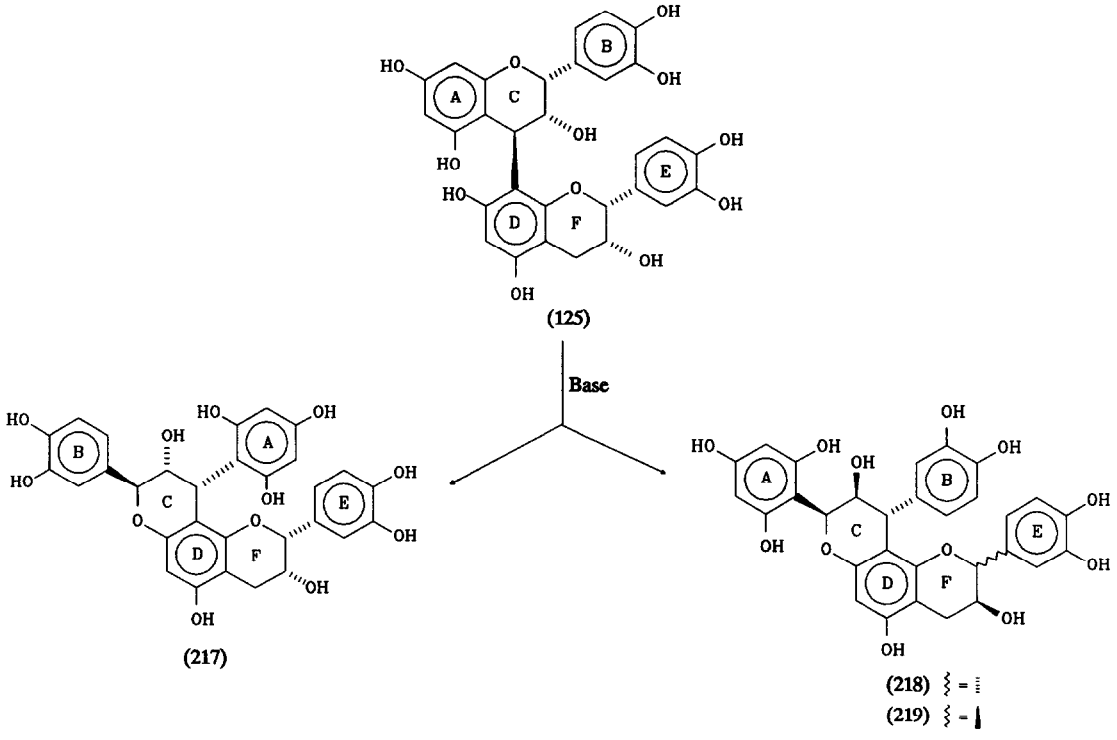
MTPA ester moiety is preferentially orientated towards the B-ring of flavan-3-ols with 3*S* absolute configuration. The protons of the aromatic ring which is juxtaposed with the α-phenyl substituent of the ester unit are then shielded by the mutual anisotropic effect. Similar arguments are also applicable to the (+)-MTPA esters of 4-arylflavan-3-ols[188].

7.2.6 Base-catalyzed Rearrangement of Procyanidins

Besides effecting a considerable degree of interflavanyl bond rupture (*cf.* refs. 44, 45) and the concomitant formation of polymeric compounds, the behaviour of procyanidin B-2 (125)[115] and B-3 (77)[189] under basic conditions conforms broadly to those of the profisetinidins and proguibourtinidins outlined above. Notable in the conversion of the 2,3-*trans*-3,4-*trans* procyanidin (77) is the exclusive formation of products (210) - (213) originating from 1,3-aryl migrations of predominantly the phloroglucinol moiety [(77) → (214) → (216) → (211)] and to a lesser extent of the (+)-catechin unit [(214) → (215) → (210)] in the intermediate quinone-methides; all these rearranged products, of course, possessing *R* absolute configuration at the equivalent of C-3 (C-ring) in procyanidin B-3 (77). Formation of analogues (212) and (213) are similarly explicable in terms of the phenomena of regio-isomerization/epimerization/1,3-aryl migration discussed in section 7.2.1 (*cf.* also ref. 189).


(77)
Base
(210)
(211)
(212)
(213)
(215)
(216)
(214)
(+)-catechin (DEF) unit migration
phloroglucinol (A)-ring migration

Procyanidin B-2 (125) is similarly subject to conversion to the tetrahydropyrano[2,3-*h*]chromenes (217) - (219), the formation of analogues (218) and (219) again requiring 1,3-migration of the (-)-epicatechin DEF moiety in the B-ring quinone-methide intermediate (*cf.* ref. 115). Phlobatannins (217) - (219) are accompanied by small quantities of procyanidin A-4 (127)[111] (section 3.5) which presumably forms by initial oxidative removal of hydride ion at C-2 (C-ring) in procyanidin B-2 (125).



Under the extremely mild conditions applied to procyanidin B-2 and B-3, no evidence could be found for rearrangements to catechinic acid-type products[160,163] reputed for either decreasing their reactivity towards aldehydes or enhancing acidity. These results hence indicate that with the proper selection of conditions, extraction of conifer barks[167,168] at alkaline pH may be performed without the adverse effects which have hitherto hampered the successful application of such an approach towards the economically important procyanidins.

7.2.7 Conclusion

Our recent demonstration of the diversity amongst the C-ring isomerized condensed tannins presumably indicates ubiquity similar to those of their 'conventional' bi- and tri-flavanoid precursors. The apparent conformational stability of the pyran-rearranged compounds and the relative planarity of the central 'core' after

dual isomerization of triflavanoids [*e.g.* the CDFI tetracyclic system of compound (199)] will possibly contribute to reduced solubility in aqueous media and thus enhancement of their affinity for collagen substrates. The 'liberation' of resorcinol- or phloroglucinol-type A-rings *via* facile ring isomerization in bi- and triflavanoid units present in commercially-available condensed tannins, may facilitate their activation for use in 'cold-set' adhesive applications.

ACKNOWLEDGEMENTS

None of the progress would have been possible without the enthusiastic support of Johann Burger, Desmond Young, Ben Bezuidenhoudt, Kobus Steenkamp, Petrie Steynberg, Annemarie Cronjé, Susan Bonnet, Hannes Malan, Esme Young, Johan Botha, Philip Viviers, and Jan van der Westhuizen. Financial support by the Foundation for Research Development, the Sentrale Navorsingsfonds of this University, and the Marketing Committee, Wattle Bark Industry of South Africa is gratefully acknowledged.

REFERENCES

1. Porter, L.J. In *The Flavanoids - Advances in Research since 1980* (ed. J.B. Harborne), Chapman and Hall, London, 1988, 21 and refs. cited therein.

2. Roux, D.G.; Ferreira, D. *Pure & Appl. Chem.*, 1982, 54, 2465.

3. Roux, D.G.; Ferreira, D. *Fortschr. Chem. Org. Naturst.*, 1982, 41, 47.

4. Drewes,, S.E.; Roux, D.G.; Eggers, S.H.; Feeney, J. *J. Chem. Soc. (C)*, 1967, 1217.

5. Drewes, S.E.;Roux, D.G.; Saayman, H.M.; Eggers, S.H.; Feeney, J. *J. Chem. Soc. (C)*, 1967, 1302.

6. Weinges, K.; Kaltenhäuser, W.; Marx, H.D.; Nader, E.; Nader, F.; Perner, J.; Seiler, D. *Liebigs Ann. Chem.*, 1968, 711, 184.

7. Weinges, K.; Göritz, K.; Nader, F. *Liebigs Ann. Chem*, 1968, 715, 164.

8. Geissman, T.A.; Yoshimura, N.N. *Tetrahedron Lett.*, 1966, 2669.

9. Weinges, K.; Perner, J.; Marx, H.D. *Chem. Ber.*, 1970, 103, 2344.

10. Thompson, R.S.; Jacques, D.; Haslam E.; Tanner, R.J.N. *J. Chem. Soc., Perkin Trans. 1*, 1972, 1387.

11. Jacques, D.; Haslam, E.; Bedford, G.R.; Greatbanks, D. *J. Chem. Soc., Perkin Trans. 1*, 1974, 2663.

12. Fletcher, A.C.; Porter, L.J.; Haslam, E.; Gupta, R.K. *J. Chem. Soc., Perkin Trans. 1*, 1977, 1628.

13. Pizzi, A. '*Wood Adhesives: Chemistry and Technology*', Marcel Dekker, New York, 1983.

14. Kreibich, R.E.; Hemingway, R.W. '*Proceedings of IUFRONTRI Symposium on Wood Adhesives*, 1985, 17, 1.

15. Creasy, L.L.; Swain, T. *Nature* (London), 1965, 208, 151.

16. Botha, J.J.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1978, 700; *J. Chem. Soc., Perkin Trans. 1*, 1981, 1235.

17. Botha, J.J.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Chem. Commun*, 1979, 510.

18. Botha, J.J.; Viviers, P.M., Young, D.A.; Du Preez, I.C.; Ferreira, D.; Roux, D.G.; Hull, W.E. *J. Chem. Soc., Perkin Trans. 1*, 1982, 527.

19. Young, D.A.; Ferreira, D.; Roux, D.G.; Hull, W.E. *J. Chem. Soc., Perkin Trans. 1*, 1985, 2529.

20. Young, D.A.; Kolodziej, H.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1985, 2537.

21. Brown, B.R.; Shaw, M.R. *J. Chem., Soc., Perkin Trans. 1*, 1974, 2036.

22. Steynberg, J.P.; Burger, J.F.W.; Young, D.A.; Brandt, E.V.; Steenkamp, J.A.; Ferreira, D. *J. Chem. Soc., Chem. Commun.*, 1988, 1055.

23. Steenkamp, J.A.; Malan, J.C.S.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1988, 2179.

24. Steynberg, J.P.; Burger, J.F.W.; Young, D.A.; Brandt, E.V.; Ferreira, D. *Heterocycles*, 1989, 28, 923.

25. Porter, L.J.; Wong, R.Y.; Benson, M.; Chan, B.G.; Vishwanadhan, V.N.; Gandour, R.D.; Mattice, W.L. *J. Chem. Res.*, 1986, (*S*), 86; (*M*), 830.

26. Viviers, P.M.; Botha, J.J.; Ferreira, D.; Roux, D.G.; Saayman, H.M. *J. Chem. Soc., Perkin Trans. 1*, 1983, 17.

27. Malan, J.C.S.; Steynberg, P.J.; Steynberg, J.P.; Young, D.A.; Bezuidenhoudt, B.C.B.; Ferreira, D. *Tetrahedron*, 1990, 46, 2883.

28. Van Heerden, F.R.; Brandt, E.V.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1981, 2483.

29. Malan, J.C.S.; Young, D.A.; Steynberg, J.P.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1990, 219.

30. Hemingway, R.W.; Foo, L.Y.; Porter, L.J. *J. Chem. Soc., Perkin Trans 1*, 1982, 1209.

31. Hemingway, R.W. In *Natural Products of Woody Plants 1, Chemicals Extraneous to the Lignocellulosic Cell Wall*, (ed. J.W. Rowe), Springer-Verlag, Berlin, 1989, 571.

32. Tindale, M.D.; Roux, D.G. *Phytochemistry*, 1974, 13, 829.

33. Steynberg, J.P.; Burger, J.F.W.; Malan, J.C.S.; Cronjé, A.; Young, D.A.; Ferreira, D. *Phytochemistry*, 1990, 29, 275.

34. Saayman, H.M.; Roux, D.G. *Biochem. J.*, 1965, 97, 794.

35. Ellis, C.J.; Foo, L.Y.; Porter, L.J. *Phytochemistry*, 1983, 22, 483.

36. Gupta, R.K.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1*, 1981, 1148.

37. Pelter, A.; Amenechi, P.I.; Warren, R.; Harper, S.H. *J. Chem. Soc. (C)*, 1969, 2572.

38. Foo, L.Y. *J. Chem. Soc., Chem. Commun*, 1986, 236.

39. Delle Monache, F.; Ferrari, F.; Marini-Bettolo, G.B. *Gazz. Chim. Ital.*, 1971, 101, 387.

40. Steynberg, J.P.; Burger, J.F.W.; Young, D.A.; Brandt, E.V.; Steenkamp, J.A.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1988, 3323.

41. Viviers, P.M.; Young, D.A.; Botha, J.J.; Ferreira, D.; Roux, D.G.; Hull, W.E. *J. Chem. Soc., Perkin Trans. 1*, 1982, 535.

42. Malan, J.C.S.; Young, D.A.; Steenkamp, J.A.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1988, 2567.

43. Steenkamp, J.A.; Ferreira, D.; Roux, D.G.; Hull, W.E. *J. Chem. Soc., Perkin Trans. 1*, 1983, 23.

44. Hemingway, R.W.; Foo, L.Y. *J. Chem. Soc., Chem. Commun.*, 1983, 1035.

45. Foo, L.Y.; Hemingway, R.W. *J. Chem. Soc., Chem. Commun.*, 1984, 85.

46. Pelter, A.; Amenechi, P.I. *J. Chem. Soc. (C)*, 1969, 887.

47. Hundt, H.K.L.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1978, 696; *J. Chem. Soc., Perkin Trans. 1*, 1981, 1227.

48. Engel, D.W.; Hattingh, M.; Hundt, H.K.L.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1978, 695.

49. Kolodziej, H.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1984, 343.

50. Young, E.; Brandt, E.V.; Young, D.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1986, 1737.

51. Malan, J.C.S.; Steenkamp, J.A.; Steynberg, J.P.; Young, D.A.; Brandt, E.V.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1990, 209.

52. Steenkamp, J.A.; Malan, J.C.S.; Roux, D.G.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1988, 1325.

53. Kolodziej, H. *J. Chem. Soc., Chem. Commun.*, 1987, 205; *J. Chem. Soc., Perkin Trans. 1*, 1988, 219.

54. Steynberg, J.P.; Ferreira, D.; Roux, D.G. *Tetrahedron Lett.*, 1983, 24, 4147; *J. Chem. Soc., Perkin Trans. 1*, 1987, 1705.

55. Botha, J.J.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1978, 698.

56. Botha, J.J.; Young, D.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1981, 1213.

57. Attwood, M.R.; Brown, B.R.; Lisseter, S.G.; Torrero, C.L.; Weaver, P.M. *J. Chem. Soc., Chem. Commun.*, 1984, 177.

58. Jacques, D.; Opie, C.T.; Porter, L.J.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1*, 1977, 1637.

59. Brown, B.R.; MacBride, J.A.H. *J. Chem. Soc.*, 1964, 3822.

60. Du Preez, I.C.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., (C)*, 1971, 336.

61. Hemingway, R.W.; Karchesy, J.J.; M^{c}Graw, G.W.; Wielesek, R.A. *Phytochemistry*, 1983, 22, 275.

62. Porter, L.J.; Foo, L.Y. *Phytochemistry*, 1982, 21, 2947.

63. Delcour, J.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1983, 1711.

64. Delcour, J.A.; Serneels, E.J.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1985, 669.

65. Kolodziej, H. *Phytochemistry*, 1985, 24, 2460.

66. Kolodziej, H. *Phytochemistry*, 1986, 25, 1209.

67. Schleep, S.; Friedrich, H.; Kolodziej, H. *J. Chem. Soc., Chem. Commun.*, 1986, 392.

68. Snatzke, G.; Katjar, M.; Snatzke, F. In *Fundamental Aspects and Recent Developments in Optical Rotatory Dispersion and Circular Diochroism* (eds. F. Ciardelli and P. Salvadori), Heyden, London, 1973, 148.

69. Barrett, M.W.; Klyne, W.; Scopes, P.M.; Fletcher, A.C.; Porter, L.J.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1*, 1979, 2375.

70. DeAngelis, G.G.; Wildman, W.C. *Tetrahedron*, 1969, 25, 5099.

71. Van der Westhuizen, J.H.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1981, 1220.

72. Haslam, E.; *J. Chem. Soc., Chem. Commun.*, 1974, 594.

73. Jacques, D.; Haslam, E. *J. Chem. Soc., Chem. Commun.*, 1974, 231.

74. Karchesy, J.J.; Hemingway, R.W. *J. Agric. Food. Chem.*, 1980, 28, 222.

75. Brown, A.G.; Eyton, W.B.; Holmes, A.; Ollis, W.D. *Phytochemistry*, 1969, 8, 2333, and refs. cited therein.

76. Hemingway, R.W.; M^{c}Graw, G.W.; Karchesy, J.J.; Foo, L.Y.; Porter, L.J. *J. Appl. Polym. Sci.: Appl. Polym. Symp.*, 1983, 37, 967.

77. Beart, J.E.; Lilley, T.H.; Haslam, E. *J. Chem. Soc., Perkin Trans. 2*, 1985, 1439.

78. Viviers, P.M.; Kolodziej, H.; Young, D.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1983, 2555.

79. Roux, D.G.; Ferreira, D. *Proceedings of the Phytochemical Society of Europe*, 1985, 25, 221.

80. Young, D.A.; Ferreira, D.; Roux, D.G. *J. Polym. Sci., Polym, Chem. Ed.*, 1986, 24, 835.

81. Weinges, K.; Marx, H.D.; Goritz, K. *Chem., Ber.*, 1970, 103, 2336.

82. Du Preez, I.C.; Rowan, A.C.; Roux, D.G. *Chem. Commun.*, 1971, 315.

83. Fletcher, A.C.; Porter, L.J., Haslam, E. *J. Chem. Soc., Chem. Commun.*, 1976, 627.

84. Young, D.A.; Cronjé, A.; Botes, A.L.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans 1*, 1985, 2521.

85. Ferreira, D.; Hundt, H.K.L.; Roux, D.G. *Chem. Commun.*, 1971, 1257.

86. Brandt, E.V.; Young, D.A.; Kolodziej, H.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1986, 913.

87. Brandt, E.V.; Young, D.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1987, 2353.

88. Drewes, S.E.; Ilsley, A.H. *J. Chem. Soc. (C)*, 1969, 897.

89. Young, D.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Perkin Trans. 1*, 1983, 2031.

90. Clark-Lewis, J.W. *Aust. J. Chem.*, 1968, 21, 2059; Clark-Lewis, J.W.; Williams, L.R. *ibid.*, 1967, 20, 2151.

91. Malan, J.C.S.; Steenkamp, J.A.; Young, D.A.; Ferreira, D. *Tetrahedron*, 1989, 45, 7859.

92. Saayman, H.M.; Roux, D.G. *Biochem. J.*, 1965, 96, 36.

93. Du Preez, I.C.; Rowan, A.C.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1970, 492.

94. Ferreira, D.; Du Preez, I.C.; Wijnmaalen, J.C.; Roux, D.G. *Phytochemistry*, 1985, 24, 2415.

95. Patil, A.D.; Desphande, V.H. *Indian J. Chem.*, 1982, 21B, 626.

96. Clark-Lewis, J.W.; Porter, L.J. *Aust. J. Chem.*, 1972, 25, 1943.

97. Fourie, T.G.; Du Preez, I.C.; Roux, D.G. *Phytochemistry*, 1972, 11, 1763; Malan, E.; Roux, D.G. *ibid.*, 1975, 14, 1835.

98. Foo, L.Y. *J. Chem. Soc., Chem. Commun.*, 1985, 1273.

99. Jacobs, E.; Ferreira, D.; Roux, D.G. *Tetrahedron Lett.*, 1983, 24, 4627.

100. Foo, L.Y. *J. Chem. Soc., Chem. Commun.*, 1989, 1505.

101. Young, D.A.; Young, E.; Roux, D.G.; Brandt, E.V.; Ferreira, D. *J. Chem. Soc., Perkin Trans 1*, 1987, 2345.

102. Brandt, E.V.; Young, D.A.; Young, E.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 11*, 1987, 1365.

103. Mayer, W.; Goll, L.; Von Arndt, E.M.; Mannshreck, A. *Tetrahedron Lett.*, 1966, 429.

104. Otsuka, H.; Fujioka, S.; Komiya, T.; Mizuta, E.; Takemoto, M. *Yakugaku Zasshi*, 1982, 102, 162.

105. Hikino, H.; Shimoyama, N.; Kasahara, Y.; Takahashi, M.; Konno, C. *Heterocycles*, 1982, 19, 1381.

106. Hikino, H.; Takahashi, M.; Konno, C. *Tetrahedron Lett.*, 1982, 23, 673.

107. Kasahara, Y.; Shimoyama, N.; Konno, C.; Hikino, H. *Heterocycles*, 1983, 20, 1741.

108. Kasahara, Y.; Hikino, H. *Heterocycles*, 1983, 20, 1953.

109. Ohigashi, H.; Minami, S.; Fukui, H.; Koshimizu, K.; Mizutani, F.; Sugiara, A.; Tomana, T. *Agric. Biol. Chem.*, 1982, 46, 2555.

110. Nonaka, G.; Morimoto, S.; Nishioka, I. *J. Chem. Soc., Perkin Trans. 1*, 1983, 2139.

111. Nonaka, G.; Morimoto, S.; Kinjo, J.; Nohara, T.; Nishioka, I. *Chem. Pharm. Bull.*, 1987, 35, 149.

112. Morimoto, S.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.*, 1985, 33, 4338; 1987, 35, 4717; 1988, 36, 33.

113. Hashimoto, F.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.*, 1989, 37, 3255.

114. Kashiwada, Y.; Morita, M.; Nonaka, G.; Nishioka, I.; *Chem. Pharm. Bull.*, 1990, 38, 856.

115. Burger, J.F.W.; Kolodziej, H.; Hemingway, R.W.; Steynberg, J.P.; Young, D.A.; Ferreira, D. *Tetrahedron*, 1990, 46, 5733.

116. Cronjé, A.; Burger, J.F.W.; Brandt, E.V.; Kolodziej, H.; Ferreira, D. *Tetrahedron Lett.*, 1990, 31, 3789.

117. Musso, H. In *Oxidative Coupling of Phenols* (eds. W.I. Taylor and A.R. Battersby), Marcel Dekker, Inc., New York, 1967, 75.

118. Geiger, H.; Quinn, C. In *The Flavonoids - Advances in Research since 1980*, (ed. J.B. Harborne), Chapman and Hall, London, 1988, 100 and refs. cited therein.

119. Weinges, K.; Bahr, W.; Ebert, W.; Goritz, K.; Marx, H-D. Fortschr, *Chem. Org. Naturst.*, 1969, 27, 158.

120. Weinges, K.; Ebert, W. *Phytochemistry*, 1968, 7, 153.

121. Weinges, K.; Ebert, W.; Huthwelker, D.; Mattauch, H.; Perner, J. *Liebigs Ann. Chem.*, 1969, 726, 114.

122. Weinges, K.; Mattauch, H.; Wilkins, C.; Frost, D. *Liebigs Ann. Chem.*, 1971, 754, 124.

123. Ahn, B-Z.; Stirner, F.G. *Arch. Pharm.* (Weinheim, Ger.), 1971, 304, 666.

124. Ahn, B-Z, *ibid.*, 1974, 307, 186.

125. Roberts, E.A.H.; Smith, R.F. *J. Sci. Food Agric.*, 1963, 14, 689.

126. Ollis, W.D.; Brown, A.G.; Eyton, W.B.; Holmes, A. *Nature*, 1969, 221, 742.

127. Ollis, W.D.; Coxon, D.T.; Holmes, A.; Cora, V.C.; Grant, M.S.; Tee, J.L. *Tetrahedron*, 1972, 28, 2819.

128. Collier, P.D.; Bryce, T.; Mallows, R.; Thomas, P.E.; Frost, D.J.; Korver, O.; Wilkins, C.K. *Tetrahedron*, 1973, 29, 125.

129.Coxon, D.T.; Holmes, A.; Ollis, W.D. *Tetrahedron Lett.*, 1970, 5247.

130. Haworth R.D.; Critchlow, A.; Haslam, E.; Tinker, P.B.; Waldron, N.M. *Tetrahedron*, 1967, 23, 2829.

131. Horner, L.; Weber, K.H.; Durckheimer, W. *Chem. Ber.*; 1961, 94, 2883.

132. Nonaka, G.; Kawahara, O.; Nishioka, I. *Chem. Pharm. Bull* , 1983, 31, 3906.

133. Coxon, D.T.; Ollis, W.D.; Holmes, A. *Tetrahedron Lett.*, 1970, 5241.

134. Haslam, E. Fortschr. *Org. Naturst.*, 1982, 41, 1.

135. Hashimoto, F.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.*, 1988, 1676.

136. Porter, L.J. In *Natural Products of Woody Plants 1, Chemicals Extrameous to the Lignocellulosic Cell Wall*, (ed. J.W. Rowe), Springer-Verlag, Berlin 1989, 651.

137. Hemingway, R.W. In *Chemistry and Significance of Condensed Tannins*, (eds. R.W. Hemingway, J.J. Karchesy), Plenum Press, New York, 1989, 83.

138. Haslam, E. In *The Flavonoids*, (eds. J.B. Harborne, T.J. Mabry, H. Mabry), Chapman and Hall, London,

1975, 505; *The Flavonoids. Advances in Research* (eds. J.B. Harborne, T.J. Mabry), Chapman and Hall, London, 1982, 417.

139. Ferreira, D.; Brandt, E.V. In *Chemistry and Significance of Condensed Tannins* (eds. R.W. Hemingway, J.J. Karchesy), Plenum Press, New, York, 1989, 153.

140. Czochanska, Z.; Foo, L.Y.; Newman, R.H.; Porter, L.J. *J. Chem. Soc., Perkin Trans. 1*, 1980, 2278.

141. Schilling, G.; Weinges, K.; Muller, O.; Mayer, W. *Liebigs Ann. Chem.*, 1973, 1471.

142. Markham, K.R.; Ternai, B. *Tetrahedron*, 1976, 32, 2607.

143. Schilling, G. *Liebigs Ann. Chem.*, 1975, 1828.

144. Porter, L.J.; Newman, R.H.; Foo, L.Y.; Wong, H.; Hemingway, R.W. *J. Chem. Soc., Perkin Trans. 1*, 1982, 1217.

145. Foo, L.Y.; Porter, L.J. *J. Chem. Soc., Perkin Trans.* 1, 1983, 1535.

146. Newman, R.H.; Porter, L.J.; Foo, L.Y.; Johns, S.R.; Willing, R.I. *Magn. Reson. in Chem.*, 1987, 25, 118.

147. Barofsky, D.F. In *Chemistry and Significance of Condensed Tannins* (eds. R.W. Hemingway, J.J. Karchesy), Plenum Press, New York, 1989, 175.

148. Clark-Lewis, J.W.; Jackman, L.M.; Spotswood, T.M. *Aust. J. Chem.*, 1964, 17, 632.

149. Fronczek, F.R.; Grannuch, G.; Tobiason, F.L.; Broeker, J.L.; Hemingway, R.W.; Mattice, W.L. *J. Chem. Soc., Perkin Trans. 11*, 1984, 1611.

150. Porter, L.J.; Wong, R.Y.; Chan., B.G. *J. Chem. Soc., Perkin Trans. 1*, 1985, 1413.

151. Baert, F.; Fouret, R.; Sliwa, M.; Sliwa, H. *Tetrahedron*, 1980, 36, 2765; Acta Cryst., 1983, B39, 444.

152. Bergmann, W.R.; Barkley, M.D.; Hemingway, R.W.; Mattice, W.L. *J. Am. Chem. Soc.*, 1987, 109, 6614.

153. Gaffield, W.; Foo, L.Y.; Porter, L.J. *J. Chem. Res. (S)*, 1989, 144.

154. Haslam, E. *Biochem. J.*, 1974, 139, 285.

155. Oh, H.I.; Hoff, J.E.; Armstrong, G.S.; Hoff, L.A. *J. Agric. Food Chem.*, 1980, 28, 395.

156. Hagerman, A.E.; Butler, L.G. *J. Biol. Chem.*, 1981, 256, 4494.

157. Bermann, W.R.; Mattice, W.L. *ACS Symp. Ser.*, 1987, no. 358, 162.

158. Tilstra, L.F.; Maeda, H.; Mattice, W.l. *J. Chem. Soc., Perkin Trans 11*, 1988, 1613.

159. Cho, D.; Tian, R.; Porter, L.J.; Hemingway, R.W.; Mattice, W.L. *J. Am. Chem. Soc.*, 1990, 112, 4273.

160. Sears, K.D.; Casebier, R.L.; Hergert, H.L.; Stout, G.H.; McCandlish, L.E. *J. Org. Chem.*, 1974, 39, 3244.

161. Herrick, F.H. *J. Agric. Food*, 1980, 28, 228.

162. Kiatgrajai, P.; Wellons, J.D.; Gollob, L.; White, J.D.; *J. Org. Chem.*, 1982, 47, 2910.

163. Courbat, P.; Weith, A.; Albert, A.; Pelter, A. *Helv. Chim. Acta*, 1977, 60, 1665.

164. Freudenberg, K.; Purrmann, L. *Chem. Ber.*, 1923, 56, 1185, *Liebigs Ann. Chem.*, 1924, 437, 274.

165. Kennedy, J.A.; Munro, M.H.G.; Powell, H.K.L.; Foo, L.Y. *Aust. J. Chem.*, 1984, 37, 885.

166. Mehta, P.P.; Whalley, W.B. *J. Chem. Soc.*, 1963, 5327.

167. Laks, P.; Hemingway, R.W. *J. Chem. Soc., Perkin Trans. 1*, 1987, 465.

168. Laks, P.; Hemingway, R.W.; Conner, A.H. *J. Chem. Soc., Perkin Trans. 1*, 1987, 1875.

169. Steenkamp, J.A.; Steynberg, J.P.; Brandt, E.V.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1985, 1678.

170. Steynberg, J.P.; Burger, J.F.W.; Young, D.A.; Brandt, E.V.; Steenkamp, J.A.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1988, 3331.

171. Steynberg, J.P.; Young, D.A.; Burger, J.F.W.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Chem. Commun.*, 1986, 1013.

172. Jensen, O.H.; Pederson, J.A. *Tetrahedron*, 1983, 39, 1609.

173. Sweeny, G.J.; Iacobucci, G.A. *J. Org. Chem.*, 1979, 44, 298.

174. Derdelinckx, G.; Jerumanis, J. *J. Chromatogr.*, 1984, 285, 231.

175. Ungaro, R.; Pochini, A.; Andreetti, G.D.; Domiano, P. *J. Chem. Soc., Perkin Trans. 11*, 1985, 197.

176. Bergman, W.R.; Viswanadhan, V.N.; Mattice, W.L. *J. Chem. Soc., Perkin Trans. 11*, 1988, 45.

177. Burger, J.F.W.; Steynberg, J.P.; Young, D.A.; Brandt, E.V.; Ferreira, D. *J. Chem. Soc., Perkin Trans 1*, 1989, 671.

178. Steynberg, J.P.; Burger, J.F.W.; Cronjé, A.; Bonnet, S.L.; Malan, J.C.S.; Young, D.A.; Ferreira, D. *Phytochemistry*, 1990, 29, 2979.

179. Slabbert, N.P. *Tetrahedron*, 1977, 33, 821.

180. Malan, J.C.S.; Young, D.A.; Steynberg, J.P.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1990, 227.

181. Steynberg, J.P.; Steenkamp, J.A.; Burger, J.F.W.; Young, D.A.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1990, 235.

182. Steynberg, P.J.; Burger, J.F.W.; Bezuidenhoudt, B.C.B.; Steynberg, J.P.; Van Dyk, M.S.; Ferreira, D. *Tetrahedron Lett.*, 1990, 31, 2059.

183. Van Dyk, M.S.; Steynberg, J.P.; Steynberg, P.J.; Ferreira, D. *Tetrahedron Lett.*, 1990, 31, 2643.

184. Delle Monache, F.; Ferrari, F.; Poce-Tucci, A.; Marini-Bettolo, G.B. *Phytochemistry*, 1972, 11, 2333.

185. Kashiwada, Y.; Iizuka, H.; Yoshioka, K.; Chen, R.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.*, 1990, 38, 888.

186. Dale, J.A.; Mosher, H.S. *J. Am. Chem. Soc.*, 1973, 95, 512.

187. Sullivan, G.R.; Dale, J.A.; Mosher, H.S. *J. Org. Chem.*, 1973, 38, 2143.

188. Hundt, A.F.; Burger, J.F.W.; Steynberg, J.P.; Steenkamp,J.A.; Ferreira, D. *Tetrahedron Lett.*, 1990, 31, 5073.

189. Steynberg, J.P.; Bezuidenhoudt, B.C.B.; Burger, J.F.W. Young, D.A.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1*, 1990, 203.