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Phytochemistry 64 (2003) 31–51
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Review

Phytochemistry of the mopane, *Colophospermum mopane*

Daneel Ferreira*, Jannie P.J. Marais, Desmond Slade

National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Received 17 December 2002; received in revised form 17 February 2003; accepted 26 February 2003

Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

The polyphenolic pool of the heartwood of the mopane, *Colophospermum mopane* Kirk ex J. Leonard, exhibits extreme diversity and complexity. It comprises a variety of monomeric flavonoids, e.g. flavan-3-ols, flavan-3,4-diols including the mopanols and peltogynols, flavonols, dimeric proanthocyanidins, e.g. proguibourtinidins, profisetinidins, promopanidins, propeltogynidins, and a variety of profisetinidin-type triflavanoids. The di- and tri-meric proanthocyanidins are accompanied by several functionalized tetrahydropyrano- and hexahydrodipyrano-chromenes (phlobatannins) that originate from the bi- and tri-flavanoids, respectively, via rearrangement of the pyran heterocycle(s). Owing to the predominance of the 5-deoxy (A-ring) flavan-3-ols, the chain terminating moieties in the biosynthesis of oligo- and poly-meric proanthocyanidins, the di- and tri-meric analogs also exhibit diversity as far as interflavanyl bonding positions are concerned. Such heterogeneity results from the reduced nucleophilicity of the A-rings of 5-deoxy flavan-3-ols, compared to the A-rings of the 5-oxy analogs (catechins), hence permitting alternative centers to participate in proanthocyanidin formation. Biomimetic-type syntheses were extensively utilized to unequivocally establish constitution and absolute stereochemistry of both the conventional and pyran ring rearranged-type di- and tri-meric compounds. Comprehension of the intricate mechanistic and stereochemical course of the pyran ring rearrangement reactions also contributed significantly to unambiguous structure elucidations. The aerial parts of the mopane are rich in essential oils that comprise mainly α-pinene and limonene, which are presumably responsible for the strong turpentine odor of the pods. The leaves also contain significant concentrations of β-sitosterol and stigmasterol which are apparently the source of sterols in various organs of the mopane moth, *Gonimbrasia belina*. Three diterpenes, dihydrogrindelic acid, labd-13*E*-en-15-oate and dihydrogrindelaldehyde are present in the bark and seeds, the latter compound exhibiting significant cytotoxicity against a human breast cancer cell line.

Keywords: *Colophospermum mopane*; Leguminosae; Proanthocyanidins; Proguibourtinidins; Profisetinidins; Promopanidins; Propeltogynidins; Trimeric profisetinidins; Tetrahydropyranochromenes; Hexahydrodipyranochromenes; Diterpenes; Essential oils

Contents

* Corresponding author. Tel.: +1-662-915-1572; fax: +1-662-915-7062.
E-mail address: dferreir@olemiss.edu (D. Ferreira).

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00152-3

1. Introduction

The mopane, *Colophospermum mopane* Kirk ex J. Leonard (previously *Copaifera mopane* Kirk ex Benth), a member of the Caesalpinioideae, is one of the principal trees in the dry regions of Southern Africa. It is one of the most distinctive vegetation groups, often forming pure stands. These have given rise to the now acceptable term 'mopane woodland' or '*Colophospermum* woodland' which has an atmosphere entirely of its own (Palgrave, 1983). The leaves and pods provide an important food source for many animals, while the roasted caterpillars of the mopane moth—commonly know as 'mopane worms'—are an important delicacy and protein source in the diet of local Africans. In addition, plant infusions are used in traditional medicine to treat syphilis, dysentery, diarrhea and inflammed eyes (Watt and Breyer-Brandwijk, 1962).

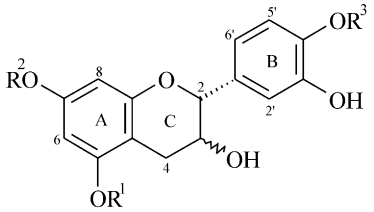
Initial chemical investigation of the heartwood extract of *C. mopane* was prompted by indications of 'an association of interrelated flavonoid compounds of potential interest in the study of the biogenesis of tannins, their stereochemistry, and their reddening on exposure to sunlight' (Drewes and Roux, 1965, 1966, 1967). These preliminary investigations focused mainly on the presence of di- and tri-meric flavanoids (Du Preez, 1971; Du Preez et al., 1971; Botha et al., 1982). Rapid advances in the development of chromatographic substrates/techniques gradually revealed a biosynthetic pool of considerable diversity and complexity (Steenkamp et al., 1985; Steenkamp, 1986). At this junction in time we assumed responsibility for the activities of the research group in Organic Chemistry at the University of the Orange Free State, Bloemfontein, South Africa, and became heavily involved in a phytochemical investigation of the polyphenols of *C. mopane*. This was also the time that we strengthened contact with Professor Jeffrey Harborne as Editor of *Phytochemistry* who was, like us, intrigued by the complexity of the problem and was strongly supportive of our efforts to unravel the polyphenolic pool of this source. This review is thus dedicated to Professor Harborne, honoring a great scholar of phytochemistry and recognizing his considerable influence and impact in this important area of natural products research.

2. Monomeric flavonoids

2.1. Flavan-3-ols and flavan-3,4-diols

The flavan-3-ols (catechins) and flavan-3,4-diols (leucoanthocyanidins) occupy key positions in proanthocyanidin biosynthesis (Ferreira et al., 1999). The most important features of the flavan-3-ols pertaining to the chemistry of the proanthocyanidins are the nucleophilicity of their A-rings, the aptitude of their heterocyclic rings to cleavage and subsequent rearrangement under mild basic conditions, the susceptibility of analogs with pyrocatechol- or pyrogallol-type B-rings to oxidative phenol coupling, and the conformational mobility of their pyran rings. Of similar importance is the role of flavan-3,4-diols as precursors to flavan-4-carbocations or A-ring quinomethane electrophiles.

The presence of (+)-catechin **1** and (−)-epicatechin **2** in the sapwood of the mopane has been qualitatively demonstrated by paper chromatography (Drewes and Roux, 1966). In addition, the sapwood also contains the



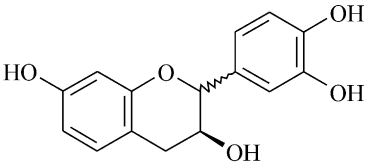
1 ≡ , $R^1 = R^2 = R^3 = H$

2 ≡ , $R^1 = R^2 = R^3 = H$

3 ≡ , R^1 or R^2 = xylofuranosyl, $R^3 = H$

4 ≡ , $R^1 = R^2 = H$, $R^3 = Me$

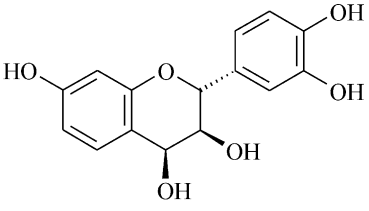
5- or 7-*O*-xylofuranoside **3** of (−)-epicatechin (Du Preez, 1971). (−)-Fisetinidol **5** and (+)-epifisetinidol **6**[1] occur in exceptionally high concentrations in the heartwood (Drewes and Roux, 1965), and may thus be anticipated to figure prominently as chain terminating units in the pool of proanthocyanidin-type metabolites.



5 ≡

6 ≡

The second participant in proanthocyanidin biosynthesis, the flavan-3,4-diols, also figure prominently in the heartwood. Among these are (+)-gleditsin (fisetinidol-4β-ol) **7**, (+)-peltogynol A **8**, (+)-peltogynol B **9**, (+)-mopanol A **10**, (+)-mopanol B **11** (Drewes and Roux, 1965, 1966), and epimopanol **12** (Malan et al., 1990a). Such diversity in structures of both flavan-3-ols and flavan-3,4-diols indicates the potential for similar variation in structural type among the condensed forms of these classes of compounds.



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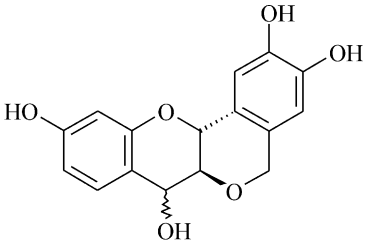
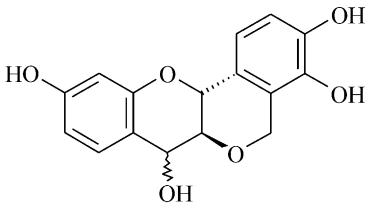


8 ≡

9 ≡

[1] According to nomenclature proposals (Hemingway et al., 1982), compound **6** should be named (+)-*ent*-epifisetinidol. This name will be used consistently, also in naming of the dimeric analogs.



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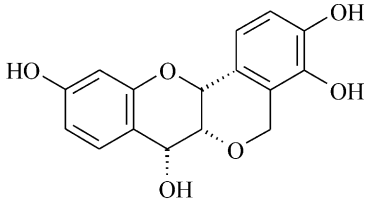
11 ≡



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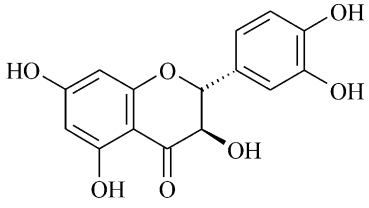
2.2. Dihydroflavonols and flavonols

(+)-Dihydroquercetin **13** was isolated in 7% yield (on extract weight) from the sapwood of the mopane (Drewes and Roux, 1966). The flavonols fisetin **14**, mopanin **15** and tentatively peltogynin **16** were obtained from the heartwood (Drewes and Roux, 1967).



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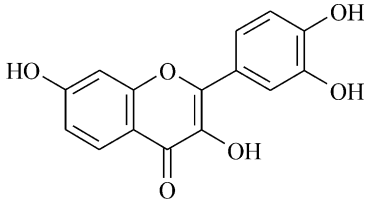

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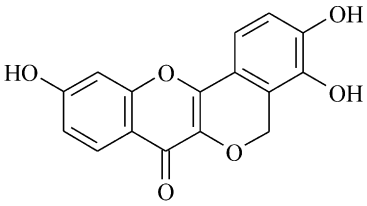

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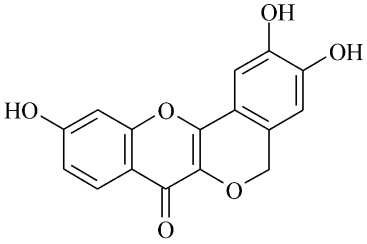

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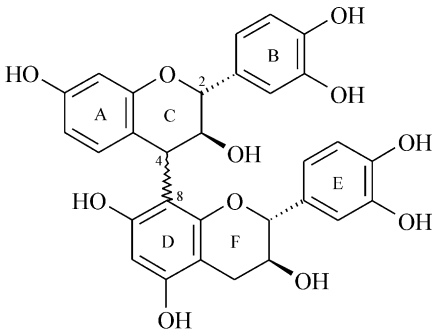
3. Dimeric flavanoids

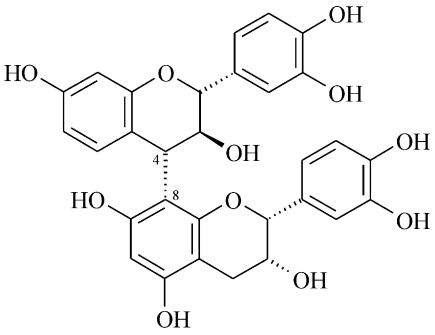
3.1. Profisetinidins (3,7,3′,4′-tetrahydroxylation)

The profisetinidins are the most important polyflavanoids of commerce, making up the major constituent of wattle (*Acacia mearnsii*) and quebracho (*Schinopsis* species) (Ferreira et al., 1999). No less than sixteen profisetinidin-type biflavanoids **17**–**32** have been identified in the heartwood extract of *C. mopane*. These compounds are based on (+)-catechin **1** (Steenkamp et al., 1988), (−)-epicatechin **2** (Steynberg et al., 1990a), (+)-afzelechin (the 3′-deoxy analog of **1**) (Steenkamp et al., 1988), (−)-fisetinidol **5** and (+)-*ent*-epifisetinidol **6** (Malan et al., 1990b, 1990c) as chain terminating DEF moieties, *i.e.* the major complement of nucleophilic flavan-3-ols present in the sap- and heart-woods.



17 ≡

18 ≡



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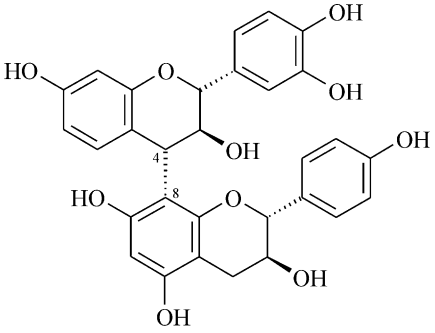

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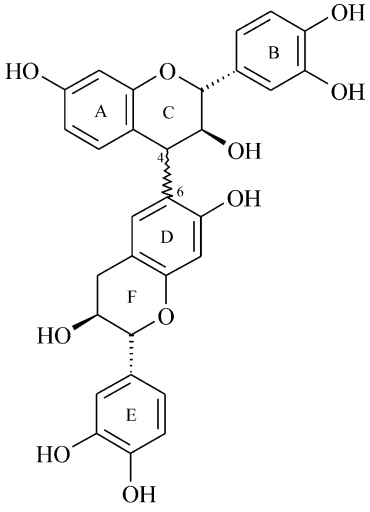
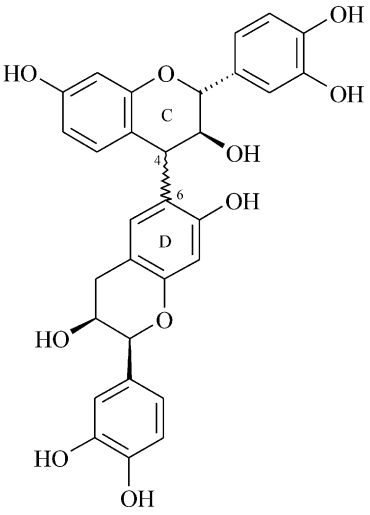


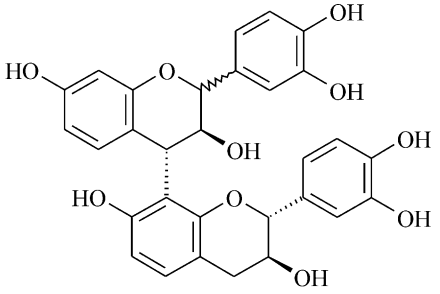
21 ≡

22 ≡



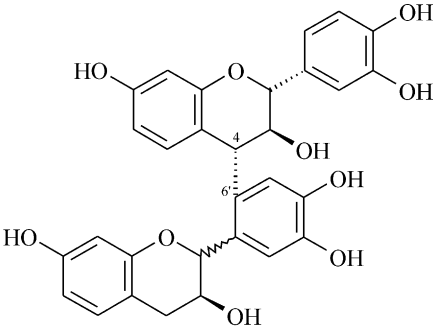
23 ≡

24 ≡



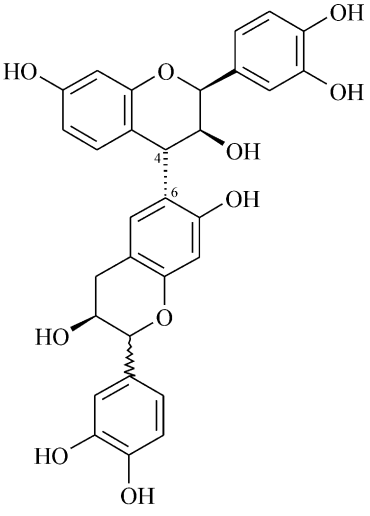
27 ≡

28 ≡



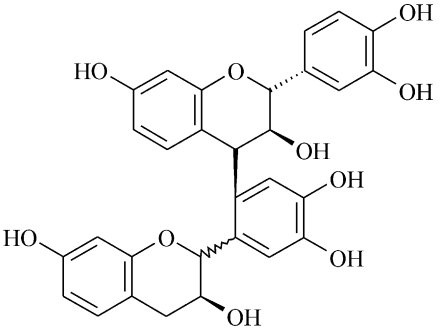
29 ≡

30 ≡



25 ≡

26 ≡

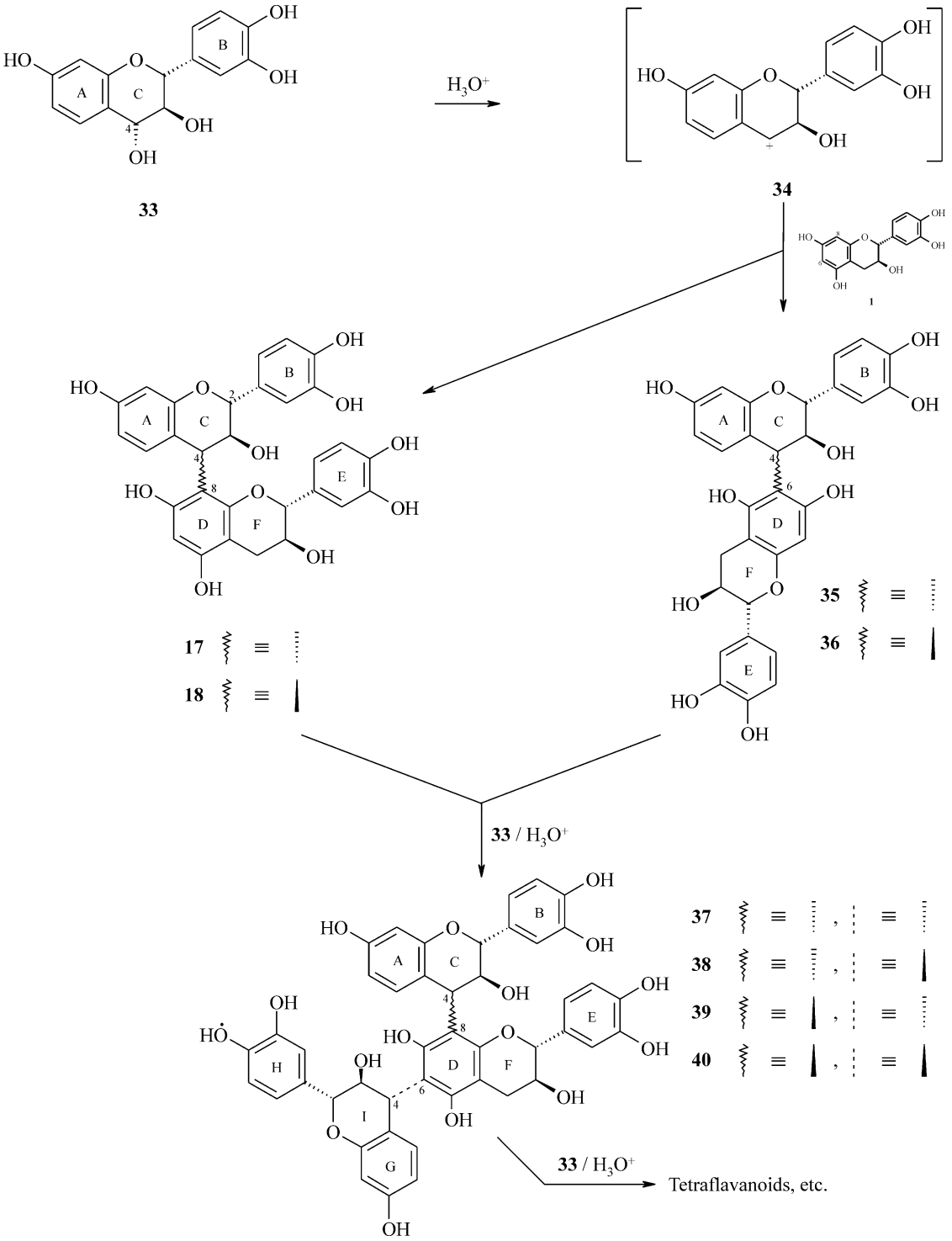


31 ≡

32 ≡

Biomimetic-type syntheses played a crucial role in the structure elucidation of the majority of the dimeric profisetinidins **17**–**32**. The protocol is demonstrated in Scheme 1 for the synthesis of the fisetinidol-(4α→8)-catechin **17** and fisetinidol-(4β→8)-catechin **18**, as well as the (4→6)-linked isomers **35** and **36**, and the analogous angular triflavanoids **37**–**40**. The flavan-3,4-diol, (+)-fisetinidol-4α-ol **33**, possessing a *p*-hydroxybenzyl alcohol A-ring functionality, is capable of generating a

C-4 carbocation **34** (or its A-ring quinomethane equivalent) under mild acidic conditions (Ferreira et al., 1999). It may subsequently be captured via interaction with the potent nucleophilic centers of the A-ring of (+)-catechin **1** to afford predominantly the fisetinidol-(4α→8)- and-(4β→8)-catechin biflavanoids **17** and **18** (Botha et al., 1981a), and to a lesser extent also the (4→6)-regioisomers **35** and **36**. Substitution at the remaining and more potent nucleophilic site of the D-ring compared with that of the A-ring of these biflavanoids by carbocation **34** would then lead to the angular triflavanoids **37**–**40**.



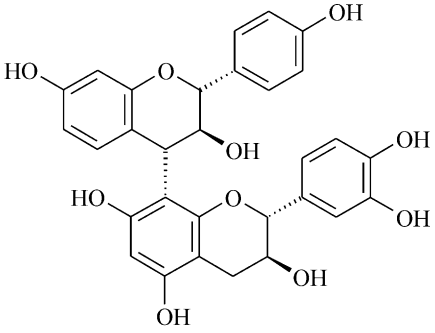
Scheme 1. Biomimetic-type synthesis of di- and tri-meric profisetinidins.

The fisetinidol-(4α→8)-epicatechin **19** was accessible *via* condensation of (+)-fisetinidol-4α-ol **33** and (−)-epicatechin **2** (Botha et al., 1981a). When using the 5-deoxy (A-ring) flavan-3-ols, (−)-fisetinidol **5** and (+)-*ent*-epifisetinidol **6**, coupling of the carbocation **34** is regiospecific and leads to the formation of the (4α→6)- and (4β→6)-bis-fisetinidols **21** and **22**, and fisetinidol-(4α→6)- and (4β→6)-*ent*-epifisetinidols **23** and **24**, respectively (Botha et al., 1981a). We will address the formation of the *ent*-epifisetinidol-(4α→6)-fisetinidol **25** and the (4α→6)-bis-*ent*-epifisetinidol **26** in Section 5.1. It should also be pointed out that 4′-*O*-methyl-(+)-catechin **4** may be used to synthesize di- and tri-meric analogs of e.g. types **17** and **37** which are selectively protected at 4′-OH of their E-rings. These protected compounds played a key role in the structure elucidation and synthesis of analogs with rearranged pyran heterocyclic rings (see Section 5).

Unique among the dimeric profisetinidins from mopane is the occurrence of linkages to the C-8 position of fisetinidol units, e.g. in (4α→8)-bis-fisetinidol **27** and *ent*-epifisetinidol-(4α→8)-fisetinidol **28** (Malan et al., 1990b) as well as to C-6′ (B-ring) with formation of the fisetinidol-(4α→6′)-fisetinidol and-*ent*-epifisetinidols **29** and **30**, and fisetinidol-(4β→6′)-fisetinidol and-*ent*-epifisetinidols **31** and **32** (Steenkamp et al., 1988; Malan et al., 1990b, 1990c). In the absence of structure confirmation of the (4α→8)- and (4β→6′)-isomers via synthesis we relied heavily on chiroptical data to establish the absolute configuration at C-4 (C-ring). Application of the CD aromatic quadrant rule (De Angelis and Wildman, 1969; Van der Westhuizen et al., 1981) to the biarylmethylidene C-4 stereocenter indicates that 4α- and 4β-substituted analogs should give negative and positive Cotton effects, respectively, in the 225–245 nm region of their CD spectra. When taken in conjunction with ^{1}H NMR coupling constants this information then permits definition of the absolute configuration at C-2 and C-3 of the C-ring. Comparison of CD data in the longer wavelength region (270–300 nm) with those of appropriate reference compounds facilitated tentative assignment of the absolute configuration of the stereocenters of the DEF-flavanyl moieties.

3.2. Proguibourtinidins (3,7,4′-trihydroxylation)

Proguibourtinidins with their 4′,7-dihydroxy phenolic functionality represent a relatively rare group of proanthocyanidins which, while occurring as minor components in the heartwoods of Australian *Acacia* spp. (Tindale and Roux, 1974), predominate in the South African species *Guibourtia coleosperma* (Saayman and Roux, 1965; Steynberg et al., 1987), *Julbernardia globiflora* (Pelter et al., 1969), and *Acacia luederitzii* (Du Preez et al., 1970; Ferreira et al., 1985). Seven members **41**–**47** of this group of proanthocyanidins were identified in the heartwood of the mopane, displaying a similar heterogeneity of coupling positions to the lower unit compared to the profisetinidins from the same source. These compounds include guibourtinidol-(4α→8)-catechin **41** (Steenkamp et al., 1988), the guibourtinidol-(4α→6)- and-(4β→6)-fisetinidols **42** and **43** (Steenkamp et al., 1988; Malan et al., 1990d), the guibourtinidol-(4α→6)- and-(4β→6)-*ent*-epifisetinidols **44** and **45** (Malan et al., 1990d) and the guibourtinidol-(4α→8)- and-(4α→6′)-fisetinidols **46** and **47**, respectively (Malan et al., 1990b).



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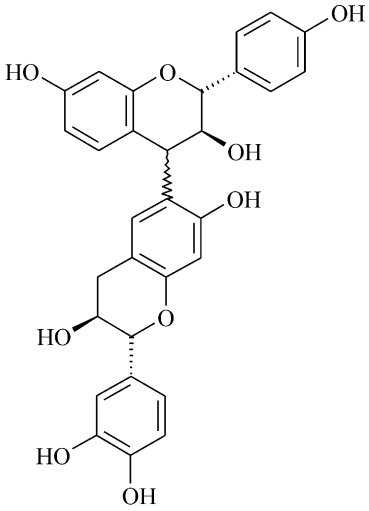
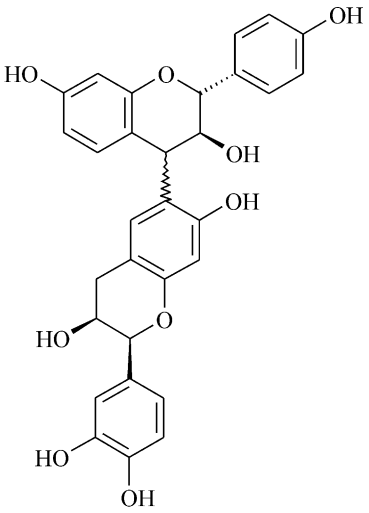


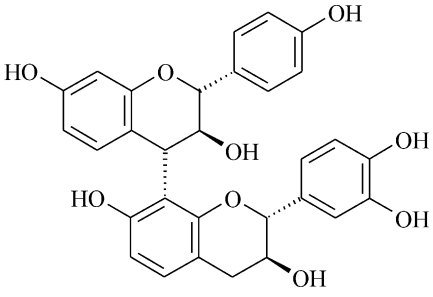
42 ≡

43 ≡



44 ≡ ⋮

45 ≡ |



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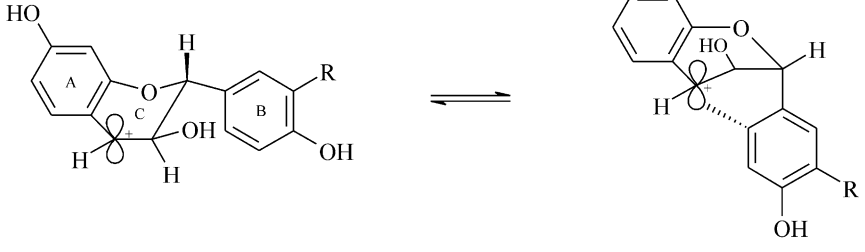
In order to gain insight into the chemistry of the formation of the guibourtinidol-(4→6)-fisetinidols/*ent*-epifisetinidols **42**–**45**, we studied their synthesis *via* the biomimetic-type principles advanced in Scheme 1. Thus, condensation of (+)-guibourtacacidin [(+)-guibourtinidol-4α-ol] **48** and (−)-fisetinidol **5** in 0.1 M HCl for 140 h at 45 °C afforded a mixture comprising considerable quantities of (−)-fisetinidol **5** and (+)-*ent*-epifisetinidol **6** (ca. 4:1) and the proguibourtinidins **42**–**45** in 19% overall yield (Scheme 2).

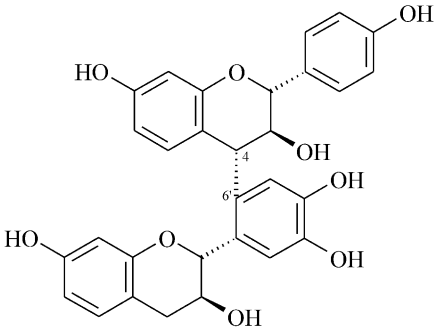
Notable in this conversion are the prolonged reaction time, conspicuously low yields, and more severe conditions compared with those for the condensation of (+)-fisetinidol-4α-ol **33** with (+)-catechin **1** (20 °C, 5 h, 45% yield) (Botha et al., 1981a) and (−)-fisetinidol **5** (40 °C, 24 h, 36% yield) (Steenkamp et al., 1983), and the predominant formation of the (4β→6)-analogs **43** and **45** relative to that of the (4α→6)-isomers **42** and **44**. Since the prevailing conditions would not permit interconversion of the (4β→6)- and (4α→6)-analogs, the observed product ratio presumably reflects kinetic control (Botha et al., 1981b) of coupling of the flavan-3-ol moiety to the carbocation intermediates **49** and **50**. Whereas the enhanced rate of the reaction of (+)-fisetinidol-4α-ol **33** with (+)-catechin **1** compared with that with (−)-fisetinidol **5** is attributable to the superior nucleophilicity of the phloroglucinol-type A-ring of (+)-catechin vs. the resorcinol-type A-ring of (−)-fisetinidol, differences in the relative rates of condensation of (+)-fisetinidol-4α-ol **33** and (+)-guibourtacacidin **48** with (−)-fisetinidol **5** must be sought in the rates of formation of the C-4 carbocationic intermediates **49** and **50** derived from the flavan-3,4-diols. The significant differences may be rationalized on the assumption that the A-ring delocalized C-4 carbocations are additionally stabilized by charge donation from the B-ring via an A-conformation **51** and **52** (Porter et al., 1986). The more electron-rich pyrocatechol function in **52** is more effective than the mono-oxygenated moiety in **51** thus leading to higher condensation rates for (+)-fisetinidol-4α-ol **33**.

The relatively drastic acidic conditions required for inducing formation of the C-4 carbocation **49** presumably also initiates conversion of (−)-fisetinidol **5** into its C-2 diastereoisomer, (+)-*ent*-epifisetinidol **6**, by recyclization via 2-OH(A) and the *si*-face at C-2 in a presumed B-ring quinomethane intermediate of type **53**. Replacement of (−)-fisetinidol by (+)-*ent*-epifisetinidol as nucleophile in stereoselective coupling with the benzylic carbocation **49** may then explain genesis of the guibourtinidol-(4α→6) and (4β→6)-*ent*-epifisetinidols **44** and **45**. Such racemization at C-2 of the fisetinidol moiety may, however, also occur at the 'dimeric' level, e.g. **42**→**44** and **43**→**45**, by a similar mechanism. A

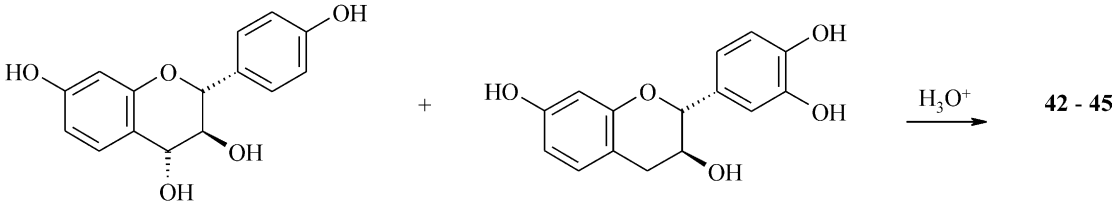


49 R = H
50 R = OH

51 R = H
52 R = OH



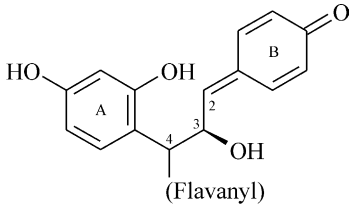
47



48 5

Scheme 2. Synthesis of guibourtinidol-(4→6)-fisetinidols and *ent*-epifisetinidols **42–45**.

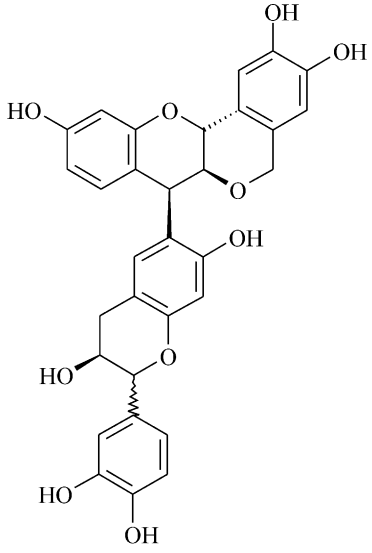
notable feature regarding the acid-catalyzed racemizations is the apparent absence of a similar phenomenon at C-2 of the 'upper' guibourtinidol moiety. Such an observation may indicate an increased susceptibility to B-ring quinomethane formation at the pyrocatechol E-ring under acid-catalysis compared to the phenol B-ring in proguibourtinidins **42–45**.



53

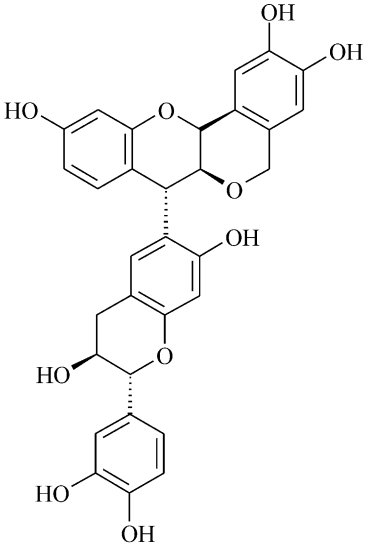
3.3. Propeltogynidins (7,4′,5′-trihydroxylation) and promopanidins (7,3′,4′-trihydroxylation)

The peltogynols **8** and **9**, and mopanols **10** and **11** feature as incipient electrophiles in the formation of a novel series of propeltogynidins **54–56** and promopanidins **58–62** (Malan et al., 1990a). We are also including the related fisetinidol-(4α→6′)-peltogynol **57**, both a profisetinidin- and leucopeltogynidin-type biflavanoid, and the unique oxidatively coupled guibourtinidol-(3′→4′)-*ent*-epimopanone **63**, a non-proanthocyanidin, in this section. The latter compound apparently arises *via* phenol oxidative coupling, initiated by generation of an oxygen radical at 4-OH(B) of *ent*-epimopanone. Its identification thus represents a rare exception in a metabolic pool where $C(sp^3)\rightarrow C(sp^2)$ coupled analogs, originating from a two-electron coupling process, predominate.

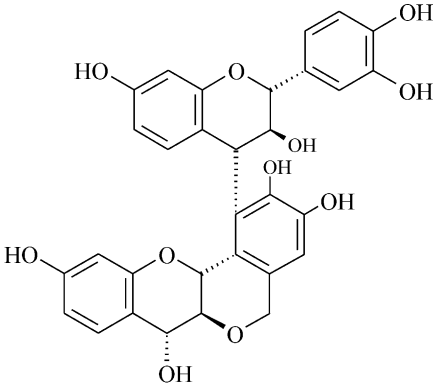


54 ≡

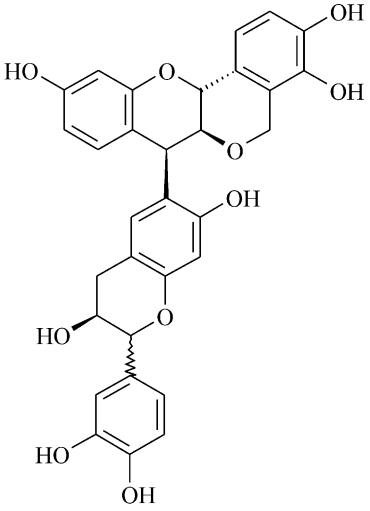
55 ≡



56

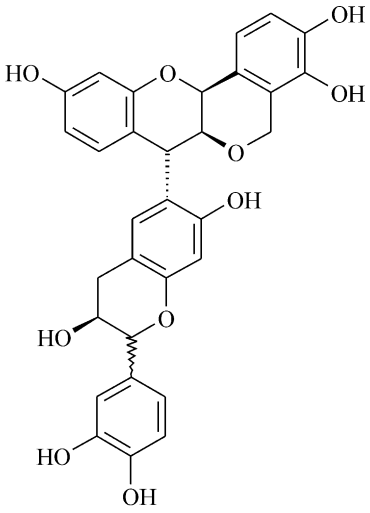


57



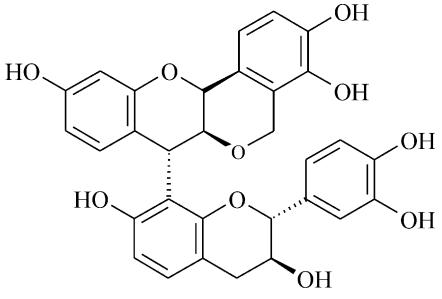
58 ≡

59 ≡

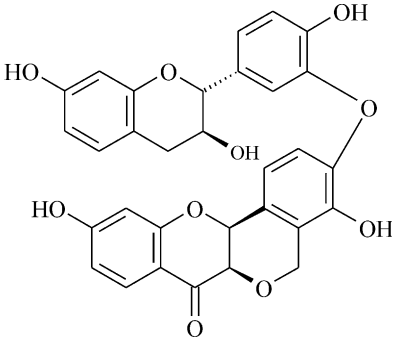


60 ≡

61 ≡



62



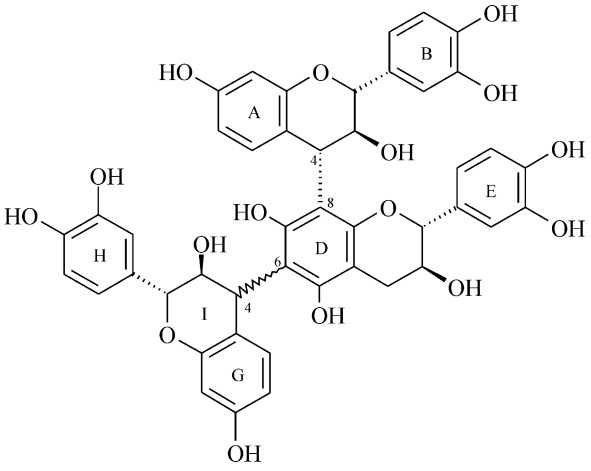
63

Acid catalyzed condensations involving (+)-peltogynol A **8** and (+)-mopanol A **10**, as source of peltogynane/mopanane C-4 carbocations, and (−)-fisetinidol **5** require conditions which are considerably more severe than those which lead to facile reaction of (+)-fisetinidol-4α-ol **33** with the same flavan-3-ol (Van Heerden et al., 1981; Malan et al., 1990a). This implies that generation of 4-carbocations occurs with great difficulty in the case of peltogynol A **8** and mopanol A **10** compared

with the 4-carbocation of fisetinidol-4α-ol **33**. These differences in the activation energy leading to the intermediate C-4 carbocations are presumably explicable in terms of the conformational rigidity of the C-ring of the peltogynols and mopanols. Such rigidity implies that the C-ring of these compounds is restricted to an (E)C-3 sofa conformation hence eliminating contributions by an A-conformer whereby charge donation from the B-ring may contribute towards a decrease in the activation energy.

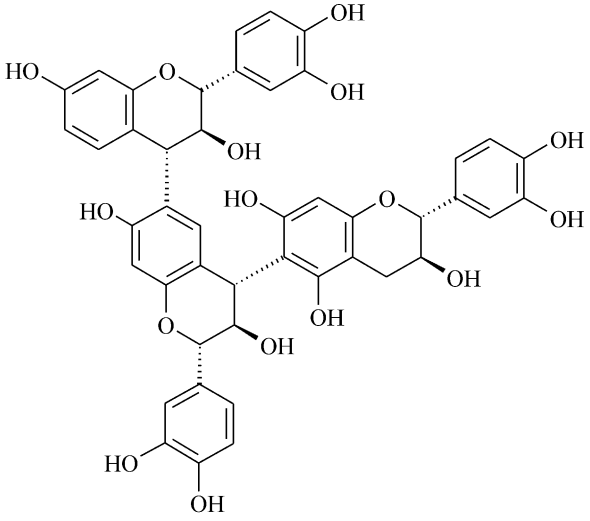
4. Trimeric profisetinidins

In contrast to the diverse group of dimeric flavanoids in the heartwood of the mopane, only two profisetinidin-type triflavanoids, fisetinidol-(4α→6)-catechin-(8→4α)-fisetinidol **64** and its (4β→6)-isomer **65** have thus far been identified (Du Preez et al., 1971; Botha et al., 1982). The structures of these trimers have been comprehensively elucidated via synthesis (Botha et al., 1982). It is interesting to note that the structure of the all-*trans* isomer **64** was initially claimed as **66** (Du Preez et al., 1971). It was only after our synthetic studies, which unequivocally established that the second site of interflavanyl coupling in fisetinidol-(4→8)-catechins of type **17** is indeed the vacant nucleophilic position of the phloroglucinol D-ring, that the 'angular' nature of bis-fisetinidol-catechin triflavanoid structures, e.g. **64**, was generally accepted (Botha et al., 1979, 1982). It was also the recognition of line broading and duplication of signals in the ^{1}H NMR spectra of the permethyl aryl ether acetates of the fisetinidol-(4α→6)-catechin **35** and bis-fisetinidol-catechin triflavanoid **64** that led to one of the pioneering papers (Du Preez et al., 1971) as far as the phenomenon of restricted rotation about the interflavanyl bond(s) is concerned (see also Weinges et al., 1970; Fletcher et al., 1976).



64 ≡

65 ≡

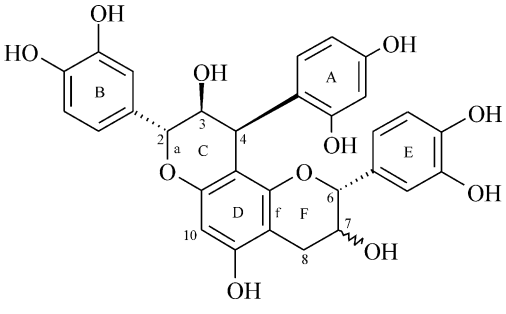


66

5. Tetrahydropyrano- and hexahydrodipyranochromenes

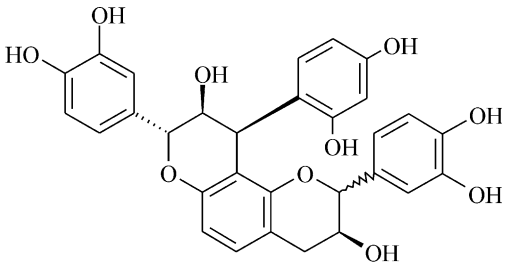
5.1. Tetrahydropyranochromenes

The natural occurrence of a novel class of C-ring isomerized condensed tannins termed phlobatannins in the heartwood of *C. mopane* has been demonstrated (Steenkamp et al., 1985). This initiated a considerable research effort aimed at unraveling the complex structural and chemical issues of the unique class of oligomeric flavanoids. These compounds comprise the functionalized 3,4,7,8-tetrahydro-2*H*,6*H*-pyrano[2,3-*f*]-chromenes **67**–**73**, the 3,4,6,7-tetrahydro-2*H*,8*H*-pyrano[3,2-*g*]chromenes **74**–**77**, and the 6-(benzopyran-2-yl)-4-arylflavan-3-ol **78**.



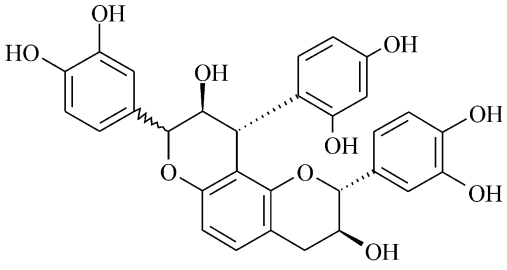
67 ≡

68 ≡



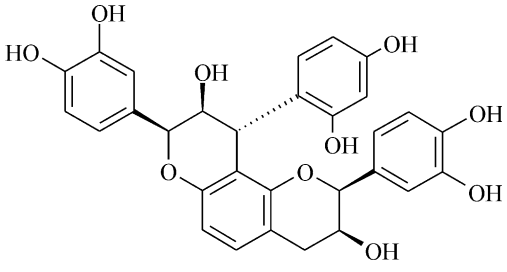
69 ≡

70 ≡

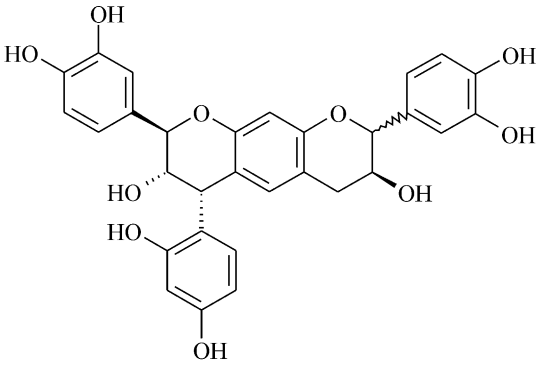


71 ≡

72 ≡

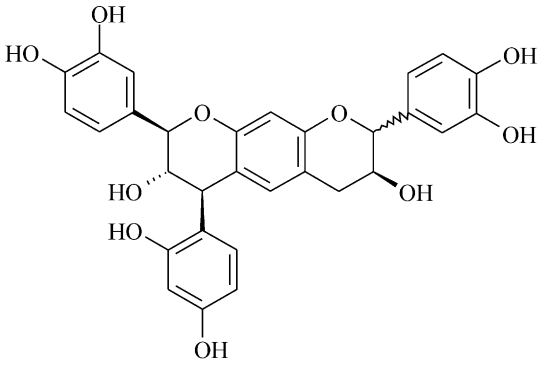


73



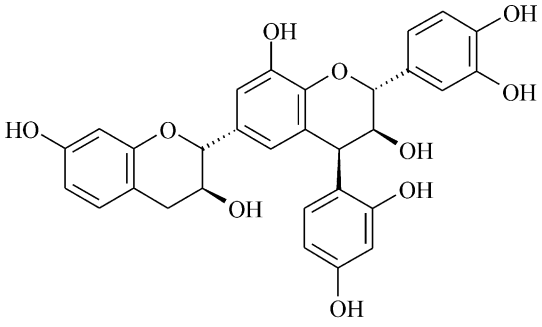
74 ≡

75 ≡



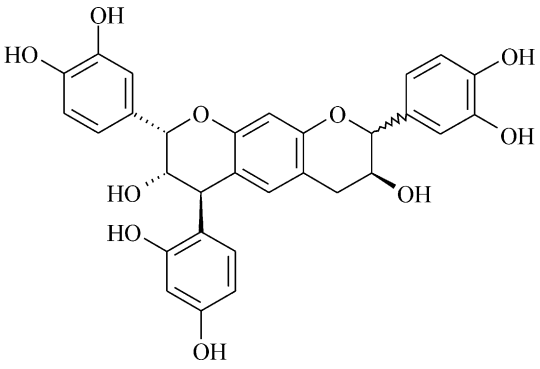
76 ≡

77 ≡



78

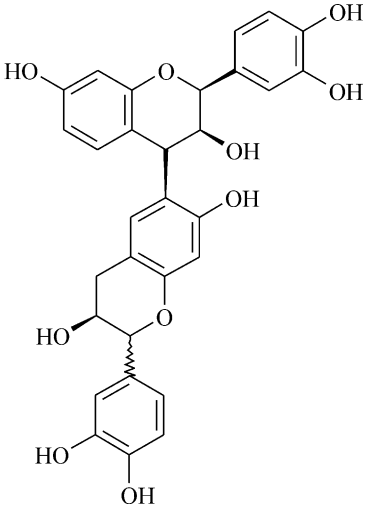
The structures of the tetrahydropyrano[2,3-*f*]chromenes **67** and **68**, and the tetrahydropyrano[3,2-*g*]chromenes **74–77** were confirmed by the synthetic protocol applicable to fisetinidol-(4→6)- and (4→8)-catechin analogs (Steynberg et al., 1988a,b). It is demonstrated in Scheme 3 for the tetrahydropyrano[3,2-*g*]chromenes **74–77** *via* the (4→6)-bis-fisetinidols **21** and **22** (Malan et al., 1990c). Thus, treatment of the (4α→6)-bis-fisetinidol **21** at pH 10 (0.025 M Na_2CO_3–0.25 M $NaHCO_3$ buffer) for 7 h at 50 °C gave conversion into a mixture of starting material, the (4α→6)-bis-fisetinidols **25** and **26**, and the functionalized tetrahydropyrano[3,2-*g*]chromenes **74** and **75**, and **81** and **82**. Similar treatment of the (4β→6)-bis-fisetinidol **22** afforded a mixture comprising the (4β→6)-bis-fisetinidols **83** and **84**, and the tetrahydropyrano[3,2-*g*]chromenes **76** and **77**, and **79** and **80**.



79 ≡

80 ≡

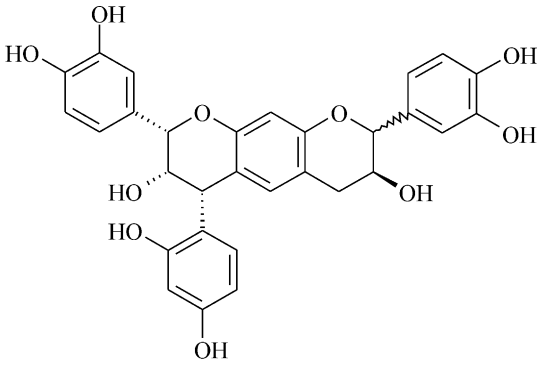
Under mild basic conditions the (4→6)-bis-fisetinidols **21** and **22** are presumably converted into quinomethanes **85** and **86**, respectively, involving both the B- and E-rings (Scheme 3). Reversal of this process by stereoselective recyclization via 2-OH of both the A-and D-ring and the quinomethane faces at C-2 and C-2′ as indicated may feasibly explain the genesis of the natural *ent*-epifisetinidol-(4α→6)-fisetinidol **25**, (4α→6)-bis-*ent*-epifisetinidol **26**, the *ent*-epifisetinidol-(4β→6)-fisetinidol **83**, and of the (4β→6)-bis-*ent*-epifisetinidol **84**. These observations, when taken in conjunction with the readily occurring epimerization of (+)-catechin **1** and (−)-epicatechin **2** under mild basic conditions (Foo and Porter, 1983), may well indicate that the natural *ent*-epifisetinidol-(4α→6)-fisetinidol **25** and (4α→6)-bis-*ent*-epifisetinidol **26** are 'biosynthetic artifacts', hence eliminating the need to invoke the occurrence of flavan-3-ol and/or flavan-3,4-diol precursors with 'abnormal' C-ring configurations in *C. mopane*.



83 ≡

84 ≡

Quinomethanes **85** and **86** presumably also serve as precursors to the tetrahydropyrano[3,2-*g*]chromenes i.e. **85**→**74**, **75**, **81** and **82**, and **86**→**76**, **77**, **79** and **80** via the stereochemical pathways indicated in Scheme 3 (cf. Steynberg et al., 1988a; Malan et al., 1990c). The stereoselectivity of the pyran recyclization involving the (4α→6)-quinomethane **85** contrasts with the observed stereospecificity for similar conversions of quinomethanes derived from fisetinidol-(4α→6 and 8)-catechins (Steynberg et al., 1988a). It may be attributed to reduced nucleophilicity of the resorcinol-type D-ring in intermediate **85** compared with that of the phloroglucinol moiety in the corresponding intermediate of the fisetinidol-catechins, hence permitting sufficient time for rotation about the C_2–C_3 bond and attack of 7-OH(D) to both faces at C-2.



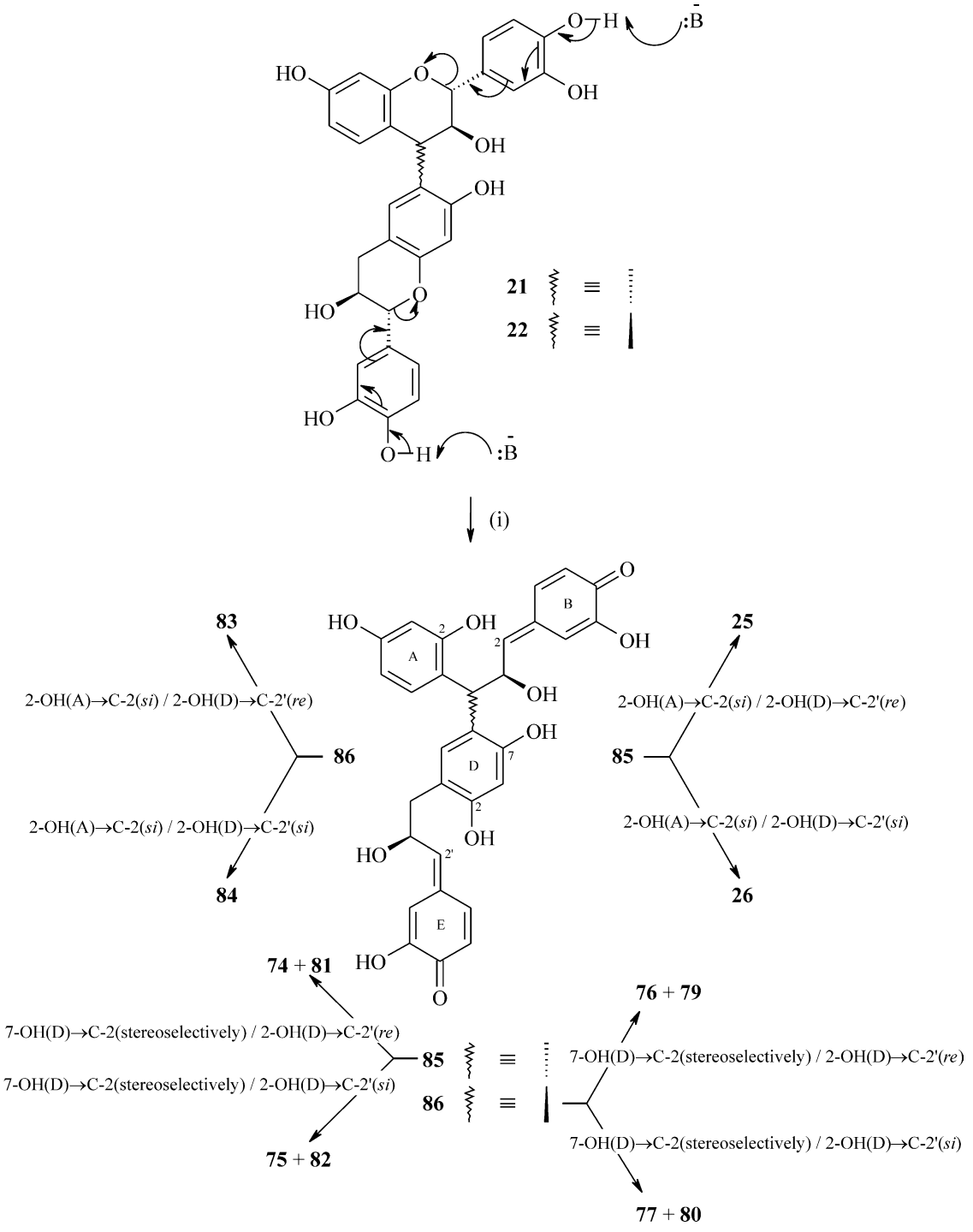
81 ≡

82 ≡

Despite the fact that the tetrahydropyrano[3,2-*g*]chromenes **79**–**82** have hitherto not been found in the mopane, the conspicuous similarities between the in vivo and in vitro processes for their formation are clear. The mild basic conditions effecting the transformations in Scheme 3 thus presumably closely match those that prevail in nature. These conditions may then also explain the vast number of compounds in the metabolic pool of *C. mopane* exhibiting a C-2 epimeric relationship to the predominant (−)-fisetinidol monomer **5**.

Identification of the 6-(benzopyran-2-yl)-4-arylflavan-3-ol **78** indicates that the B→E-ring linked profisetinidins, e.g. **29**, are also susceptible to rearrangement of their pyran heterocyclic rings. Compound **78** may thus plausibly be derived from (4α→5′)-bis-fisetinidol **87** via an intermediate B-ring quinomethane **88** (Scheme 4).

The majority of ring-isomerized metabolites in the mopane thus originate from precursors in which the nucleophilicity of the phenolic rings effecting isomerization is of comparable (for **27**→**67**) or lower (for **87**→**78**) magnitude than those of the rings acting as leaving groups. Rearrangement of the pyran C-ring presumably leads to a decrease in conformational energy by partial



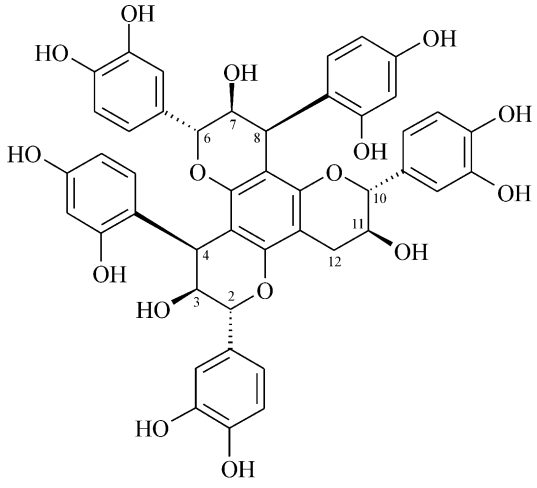
Scheme 3. Proposed route to the formation of tetrahydropyrano[3,2-*g*]chromenes **74**–**77** and **79**–**82**, the (4→6)-bis-*ent*-epifisetinidols **26** and **84**, and *ent*-epifisetinidol-(4→6)-fisetinidols **25** and **83** with unusual heterocyclic configurations; Reagents and conditions: (i) $NaHCO_3$–Na_2CO_3, 50 °C, 7 h, N_2.

removal of steric strain caused by mutual rotation of bulky groups about the interflavanyl bond of the biflavanoid precursors. Generation of the conformationally more stable product, e.g. **67**, presumably provides the main impetus for these pyran rearrangements rather than the effect of different nucleophilicities of the participating phenolic rings as was initially postulated (Steenkamp et al., 1985).

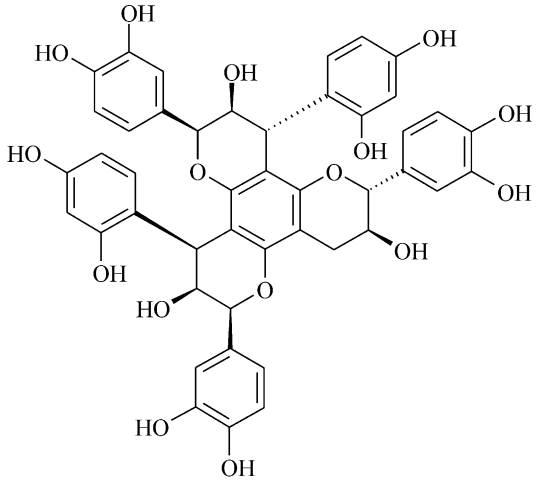
5.2. Hexahydrodipyranochromenes

Several members of the hexahydrodipyranochromene class of compounds, representing the products of pyran rearrangement of the flavan-3-ol constituent units in bis-fisetinidol-catechin and-epicatechin triflavanoids of types **64** and **65**, have been characterized in the heartwood extract of the mopane. These compounds com-

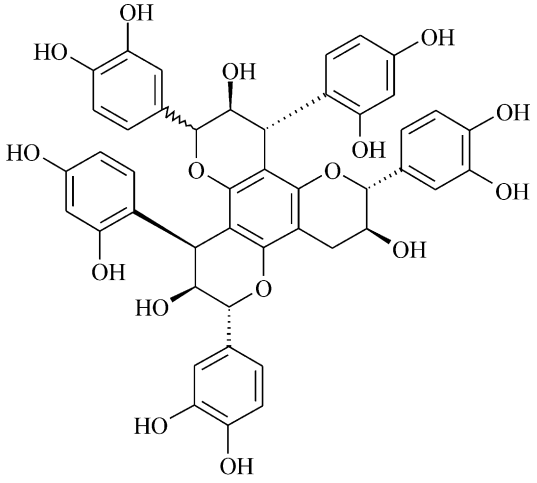
prise the functionalized 3,4,7,8,11,12-hexahydro-2*H*,6*H*,10*H*-dipyranochromenes **89–92**, the 6-flavanyltetrahydropyrano[2,3-*h*]chromenes **93–97**, and the 10-flavanyltetrahydropyrano[2,3-*f*]chromene **98**, the latter six compounds representing 'isomerization-intermediates'.



89

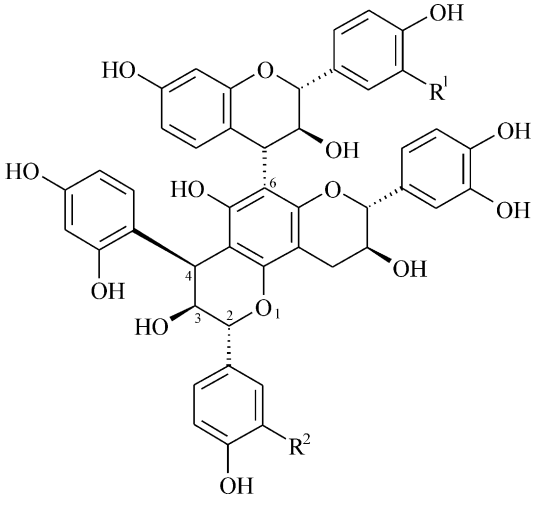


90



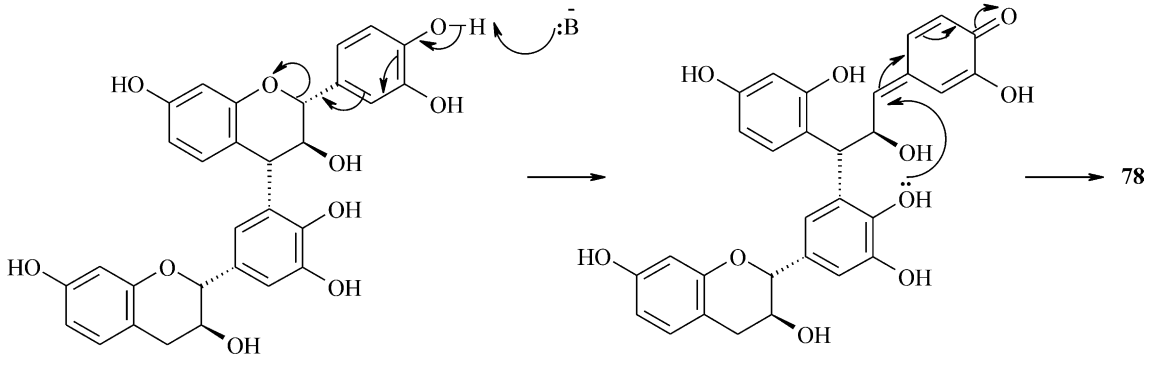
91 ≡

92 ≡



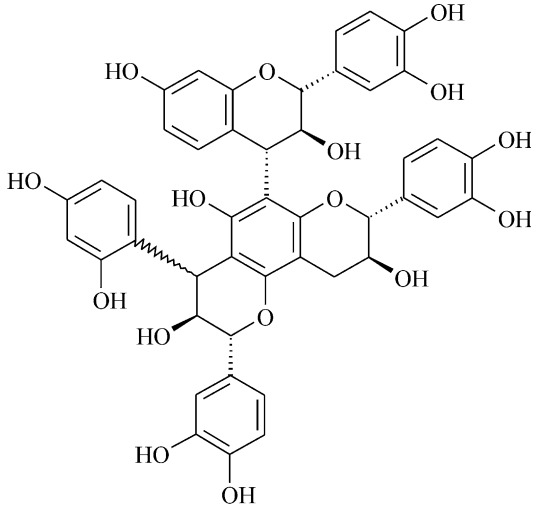
93 $R^1 = H$, $R^2 = OH$

94 $R^1 = OH$, $R^2 = H$



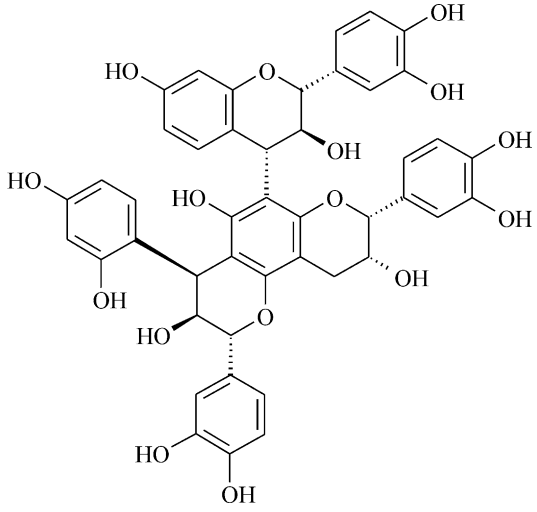
87 **88** **78**

Scheme 4. Proposed route to the formation of the 6-(benzopyran-2-yl)-4-arylflavan-3-ol **78**.

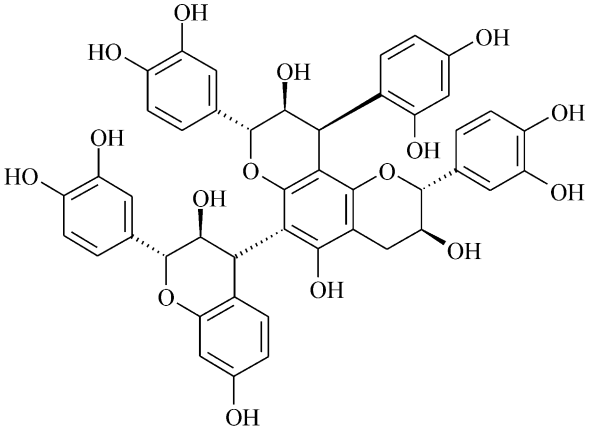


95 ≡

96 ≡



97



98

The protocol to establish the structure of these complex polyphenols via semi-synthesis is demonstrated in Scheme 5 for the (4α→6;4α→8)-bis-fisetinidol-catechin triflavanoid **37**. In view of the susceptibility of constituent units in oligoflavanoids to epimerization at C-2 and also subsequent unwanted rearrangements (Steynberg et al., 1988a, 1988b), we used the (4α→6;4α→8)-bis-fisetinidol-catechin 4-*O*(E)-methyl ether **99** (Steynberg et al., 1990b). Since the naturally occurring trimeric analogs were identified as the permethylaryl ether triacetates, the 4-*O*(E)-methyl group in the synthetic derivatives did not interfere with the structure elucidations.

Thus, under mild basic conditions the bis-fisetinidol-catechin **99** is presumably transformed into the quinomethane **100** involving both the B- and H-rings (Scheme 5). The stereospecific pyran recyclization via 7-OH(D) and 5-OH(D) and the *re*-faces at C-2 and C-2′, respectively, encountered for biflavanoids with 2,3-*trans*-3,4-*trans* constituent units (Steynberg et al., 1990b), may feasibly rationalize the genesis of the dipyranochromene **89** as a product of dual isomerization. The 'isomerization-intermediates' **95** and **98** presumably also have a common origin in quinomethane **100** by the stereochemical pathways indicated in Scheme 5. Notable in Scheme 5 is the conspicuous absence of a tetrahydropyranochromene originating from cyclization involving 7-OH(D) and C-2′. This may reflect preferred conformations about the interflavanyl bonds in quinomethane **100** favoring attack of 7-OH(D) at C-2 and of 5-OH(D) at C-2′.

A similar approach also facilitated the structure elucidation of the products of base-catalyzed pyran ring rearrangement of the (4β→6;4α→8)-(Bonnet et al., 1996a), (4β→6;4β→8)-(Bonnet et al., 1996b), and (4α→6;4β→8)-(Bonnet et al., 1996c) bis-fisetinidol-catechin profisetinidin triflavanoids, and of related bis-fisetinidol-epicatechin profisetinidin triflavanoids (Bonnet et al., 1996d).

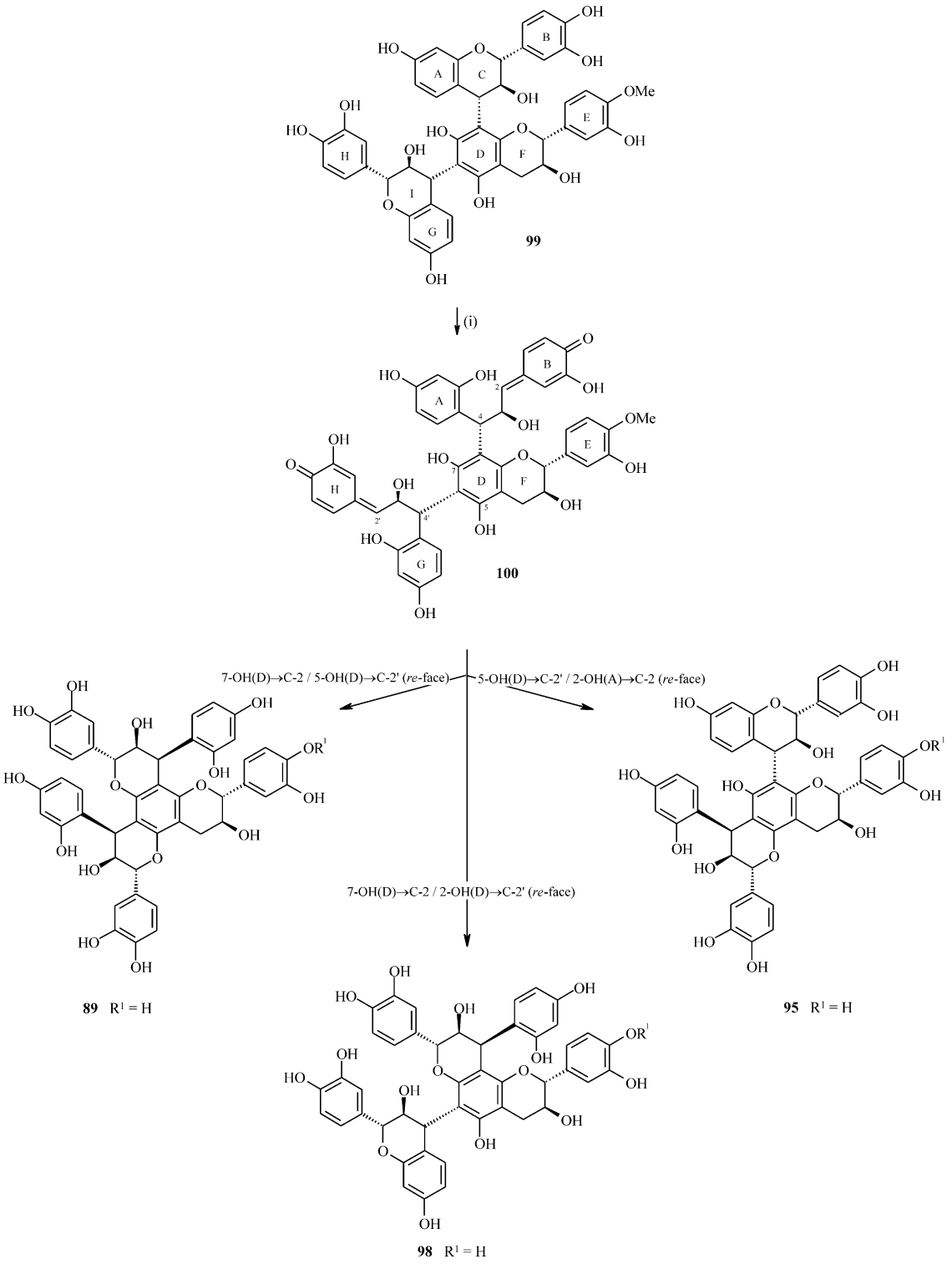
These results demonstrated a remarkable structural diversity among the C-ring isomerized analogs and presumably indicate ubiquity in nature similar to that of their apparent precursors at the triflavanoid level. It furthermore indicates that the phenomenon of pyran ring rearrangement with concomitant 'release' of the resorcinol-type functionality and, hence, the availability of potent nucleophilic centers, especially at the triflavanoid and higher oligomeric levels, may significantly contribute towards the utility of the polymeric proanthocyanidins in cold setting adhesives and leather tanning applications.

6. Miscellaneous

The aerial parts of the mopane are rich in essential oils that comprise mainly α-pinene and limonene, as

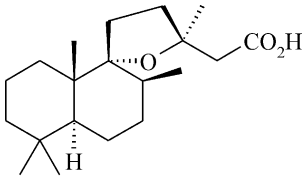
well as at least 36 compounds in lesser quantities according to GC and GC/MS analyses (Chagonda et al., 1999; Brophy et al., 1992). These compounds are presumably responsible for the strong turpentine odor of the pods. The leaves also contain significant concentrations of β-sitosterol and stigmasterol which are apparently the source of sterols in various organs of the mopane moth, *Gonimbrasia belina* (Cmelik, 1970).

Mopaneol A **104** and mopaneol B **105** were identified in hexanes extracts from mopane leaves and seed husks,



Scheme 5. Proposed route to the formation of the hexahydrodipyranochromene **89** and 'isomerization-intermediates' **95** and **98**; reagents and conditions: (i) $NaHCO_3$–Na_2CO_3, 50 °C, 5 h, N_2.

respectively (Reiter et al., 2003). The corresponding aldehydes **106** and **107** were obtained as an inseparable mixture from the hexanes extract of mopane roots. These compounds represent primitive diterpenes that are regarded as the 'missing links' in the biosynthesis of the 9,13-epoxylabdanes. The proposed genesis from geraniol pyrophosphate also attempted to explain the unusual C-3-α hydroxyl and C-8-β methyl groups in compounds **101**–**107**, as well as the highly variable configurations at C-9 and C-13.



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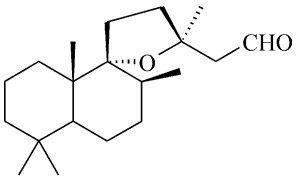

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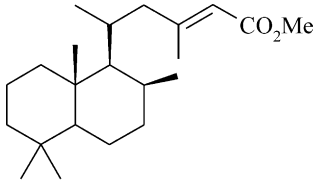

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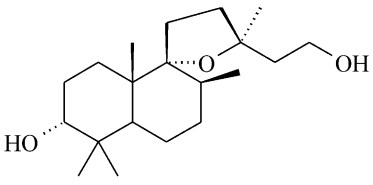

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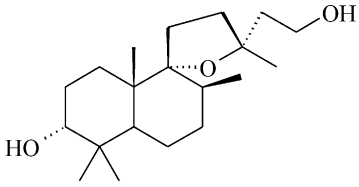

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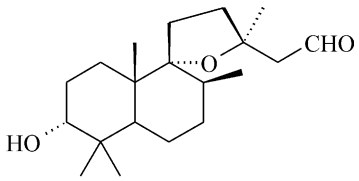

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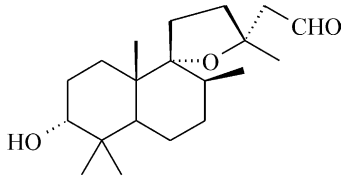

107

Three diterpenes, dihydrogrindelic acid **101**, dihydrogrindelaldehyde **102** and methyl labd-13*E*-en-15-oate **103** are present in the bark and seeds. Dihydrogrindelaldehyde exhibits significant cytotoxicity against a human breast cancer cell line (Mebe, 2001).

There is also a considerable collection of papers dealing with mopane issues other than its phytochemistry, e.g. its importance as a fodder tree. These papers are readily accessible via electronic data bases and need not be discussed here.

7. Concluding remarks

This comprehensive analysis summarizes our knowledge regarding the phytochemical composition of the aerial parts and the heartwood of the mopane. Initiated by an examination of the heartwood that 'indicated an association of interrelated flavonoid compounds of potential interest in the study of the biogenesis of tannins, their stereochemistry, and their reddening on exposure to sunlight' (Drewes and Roux, 1966), these modest efforts were gradually accelerated and eventually culminated in gathering a considerable amount of information relevant to the chemistry of the C_6–C_3–C_6-type polyphenols. In addition to the first contribution focusing on the adverse effects of restricted rotation about the interflavanyl bond(s) on NMR spectra, this research also led to the first comprehension of the mode of successive coupling of flavan-4-yl carbocations to (+)-catechin and hence of the 'angular' nature of bis-fisetinidol-catechin profisetinidin triflavanoids. The first demonstration of the natural occurrence of a novel class of a proanthocyanidin-related group of compounds with rearranged pyran heterocycles, the functionalized tetrahydropyrano- and hexahydrodipyranochromene classes of phlobatannins, initiated a comprehensive

study aimed at unraveling the complex physical and chemical properties of this complex group of natural products. With the tremendous advances that have been made in the field of chromatography and spectroscopy/spectrometry, the scene is now set for a next generation of phytochemists to indulge in the intricacies of the mopane metabolic pool at the tetraflavanoid level.

Acknowledgements

We thank all our collaborators in the Chemistry Department at the University of the Free State, Bloemfontein, cited in the reference for their diligent efforts, the 'Sentrale Navorsingsfonds' of Free State University, and the Foundation for Research Development (now the National Science Foundation), Pretoria, for generous financial support. This work was supported in part by the United States Department of Agriculture, Agricultural Research Service Specific Cooperative Agreement, No. 58-6408-2-0009.

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Desmond Slade graduated from the University of Stellenbosch, South Africa in 2000, where he obtained his PhD (Chemistry) on the chemical characterization of the interdigital secretion of the black wildebeest under the supervision of Professor Ben V. Burger. He started as a Postdoctoral Research Associate at the National Center for Natural Products Research, University of Mississippi, at the end of 2000, working on the synthesis of antimalarial 8-aminoquinolines and the synthesis of buprenorphine-3-β-D-glucuronide and norbuprenorphine-3-β-D-glucuronide and their deuterated analogs under the supervision of Dr. Daneel Ferreira.