STRUCTURE AND SYNTHESIS OF A NOVEL HOMOISOFLAVANONE FROM *SCILLA NATALENSIS* AND SYNTHESIS OF SELECTED PROCYANIDINS THROUGH THE C-4 FUNCTIONALIZATION OF FLAVAN-3-OLS

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TABLE OF CONTENTS

DISCUSSION

EXPERIMENTAL

SUMMARY

Scilla natalensis planch (Hyacinthaceae), commonly known as Wild squill, Blue squill, Blue hyacinth, Blouberglelie, Blouslangkop, Inguduza, is one of the plants that are widely used in traditional medicines and it grows naturally over large parts of Southern Africa. While the plant is widely used in traditional medicine by indigenous African people, phytochemical investigations have revealed this plant to contain a variety of biologically active compounds that show anti-inflammatory, antibacterial, antischistosomic, anthelmintic and cytotoxicity activity. In order to determine whether its traditional use is supported by actual pharmacological effects, it was decided to re-investigate the chemical composition of *Scilla natalensis*. Repeatetive column - and preparative thin layer chromatography together with acetylation of the methanol extract of the bulbs of the plant led to the isolation of five known compounds, 3',4'-Di-*O*-acetylchavicol, 4',4''-Di-*O*-acetyl-3"-methoxynyasol, 5,7-Diacetoxy-3-(3'-acetoxy-4'-methoxybenzyl)chroman-4-one, 4'-*O*-acetyl-5,7-di-*O*-methyl-naringenin, and 2",3",4",5,6"-Penta-*O*-acetyl-4'-*O*-methyl-apigenin-7-*O*-*β*-D-glucopyranoside as well as the novel homoisoflavanone, 5,6,7-triacetoxy-3-(3',4'-dimethoxybenzyl)-chroman-4-one. While the isolated metabolites were all identified and characterised by spectroscopic means involving 1- and 2D-NMR experiments, all of the known compounds were isolated from *Scilla natalensis* for the first time.

Since the homoisoflavonoids have been found to possess widespread physiological activity and to give final proof of the structure of the isolated novel homoisoflavanone, in particular the position of the third OH on the A-ring, the synthesis of this compound was attempted. While several synthetic routes towards homoisoflavanones have been reported in literature, it was decided to follow the dihydrochalcone approach for the synthesis of this new homoisoflavanone. In this methodology the dihydrochalcone is subjected to α-alkylation with a C-1 fragment containing another leaving group that can be displaced in the final cyclization process for formation of the heterocyclic C-ring. The desired dihydrochalcone would become available by reduction of the

chalcone which can be formed by aldol condensation of the appropriate acetophenone and benzaldehyde. In this instance, however, this synthetic approach was hampered by the unavailability of the required 2,3,4,6-hydroxyacetophenone. It was therefore decided to test the synthesis on a model compound, 2-hydroxyacetophenone, and to investigate the appropriate C-1 fragment to use, before attempting the challenging synthesis of the required acetophenone.

Thus standard Claisen-Schmidt aldol condensation between 2-hydroxyacetophenone and 3,4-dimethoxybenzaldehyde afforded the required chalcone (68 % yield), which was subjected to hydrogenation over 5 % Pd/C to give the dihydro equivalent in quantitative yield. To introduce the C-1 fragment it was decided to utilise a modified Baker-Venkataraman rearrangement strategy followed by reduction of the ester functionality and subsequent Mitsunobu cyclization. While ester formation between ethylchloroformate and 2'-hydroxy-3,4-dimethoxydihydrochalcone proceeded well, the rearrangement part of the reaction led to the unexpected formation of 3-(3',4'-dimethoxybenzyl)-4-hydroxycoumarin. Although this product could be transformed into the desired homoisoflavanone, it would take three more steps and it was therefore decided to evaluate a Vilsmeier- Haack type α-formylation for introducing the additional carbon atom into the dihydrochalcone moiety. While treatment of the 2'-hydroxydihydrochalcone with N,N-dimethylformamide (DMF), PCl_5 and BF_3etherate afforded only the 3-(3',4'-dimethoxybenzyl)-isoflavone in 40 % yield, subsequent hydrogenation over 10 % Pd/C led to the isolation of three products, i.e. 3-(3',4'-dimethoxybenzyl)chromane (43%), 3-(3',4'-dimethoxybenzyl)chromanone (the desired homoisoflavanone) (3%), and the 3,4-*cis*- and *trans*-3-(3',4'-dimethoxybenzyl)chroman-4ols (3% each). Although the desired product was obtained in only 3% yield due to over-hydrogenation, the reaction was not repeated on larger scale as it was already established that the homoisoflavanone could indeed be formed in this way. In the second part of this dissertation the issue of determining the absolute configuration at the different chiral centres of flavonoids was to be addressed. Although this has up to now been done by circular dichroism (CD) measurement, this method has led to ambiguities and is plagued with a

host of empirical rules that has to be applied. It was therefore decided to investigate the application of vibrational circular dichroism (VCD) to the determination of the absolute conformation of flavonoids. In order to generate a data base and eventually apply the technique of VCD to the stereochemistry of proanthocyanidins, it was decided that the investigation should be started form a flavonoid with only one chiral centre and systematically increase the number of stereo centres until the level of oligomeric compounds is reached. Since (+)-catechin [(2*R*,3*S*)-(+)-3,3',4',5,7-penta-hydroxyflavan, and (-)-epicatechin [(2*R*,3*R*)-(-)-3,3',4',5,7-penta-hydroxy-flavan] are freely available in optical active form and can be transformed into their respective enantiomers, the whole synthetic endeavour was based on these compounds. In this dissertation the aim therefore was to functionalize (+)-catechin and (-)-epicatechin in the 4-position followed by the synthesis of 4-arylflavan-3-ols and ultimately proanthocyanidins B1 to B4.

Thus DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) oxidation of tetra-O-methyl-(+)-catechin, tetra-O-benzyl-(+)-catechin, and tetra-O-benzyl-(-)-epicatechin in the presence of ethylene glycol, gave the 4-hydroxyethoxy derivatives in 46, 60, and 50 % yields respectively. Treatment of the latter two compounds with perbenzylated fluoroglucinol under $TiCl_4$ catalysis led to only the corresponding 2,4-*cis*-4-arylflavan-3-ols in 65 and 55 % yields. The formation of proanthocyanidins B1, B2, B3, and B4 were successfully achieved through similar coupling of perbenzyled catechin and - epicatechin with their respective 4-hydroxyethoxy analogues. It has, however, to be mentioned that the characterization of the perbenzylated B1 to B4 products by NMR were virtually impossible, since the spectra of these compounds were very complicated because of severe duplication of signals due to restricted rotation. In order to have all possible isomers available in free phenolic form for VCD studies, debenzylation of the synthesised 4-arylflavan-3-ols and procyanidins B1 to B4 as well as the synthesis of B5 to B8 will be attended to during the candidate's PhD studies.

OPSOMMING

Scilla natalensis planch (Hyacinthaceae), ook bekend as Wild squill, Blue squill, Blue hyacinth, Blouberglelie, Blouslangkop, Inguduza, wat wydverspeid oor die hele suidelike Afrika aangetraf word, is een van die plante wat baie algemeen as tradisionele geneesmiddel deur Afrikane oor die hele Suider-Afrika gebruik word. Fitochemiese ondersoeke na die bestanddele van hierdie plant, het die teenwoordigheid van ‘n aantal fisiologies aktiewe verbindings wat oa anti-inflammatoriese -, antibakteriese -, antiskisontiese -, antihelmintiese – en sitotoksiese aktiwiteit vertoon, aangedui. Ten einde te probeer vasstel of ‘n verwantskap tussen die tradisionele gebruike van die plant ekstrak en werklike farmakologiese werking daarvan wel bestaan, is die huidige her-ondersoek na die chemiese bestanddele van *Scilla natalensis,* aangepak. Herhaaldelike kolom- en preparatiewe dunlaag chromatografiese skeidings tesame met asetilering van die metanol ekstrak van die bolle van die plant, het daartoe gelei dat vyf bekende verbindings nl. 3’,4’-Di-*O*-asetielchavicol, 4’,4’’-Di-*O*-asetiel-3”-metoksinyasol, 5,7-Diasetoksi-3-(3’-asetoksi-4’-metoksibensiel)chroman-4-oon, 4’-*O*-asetiel-5,7-di-*O*-metiel-naringenien, en 2”,3”,4”,5,6”-penta-*O*-asetiel-4’-*O*-metielapigenien-7-*O*-glukosied, vir die eerste keer uit hierdie plant geïsoleer is. Genoemde verbindings is in die ekstrak vergesel van die nuwe homoisoflavanoon, 5,6,7-triasetoksi-3-(3’,4’-dimetoksibensiel)-chroman-4-oon, wat soos die bekende verbindings mbv 1- en 2-dimensionele KMR spektroskopie volledig gekarakteriseer is.

Aangesien die fisiologiese aktiwiteit van homoisoflavonoïede bekend is en ten einde finale struktuurbewys, veral tov die posisie van die derde OH groep op die A-ring, van die unieke homoisoflavanoon te lewer, is die sintese van hierdie nuwe metaboliet aangepak. Hoewel verskeie sintetiese roetes vir die bereiding van homoisoflavonoïede is in die literatuur beskryf is, is besluit om van die roete *via* die dihidrochalkoon tydens hierdie ondersoek te volg. Hiervolgens word α-alkilering van die dihidrochalkoon met ‘n C-1 fragment wat ‘n verlatende groep bevat wat weer tydens die sikliseringstap verplaas kan word, uitgevoer, terwyl die dihidrochalkoon op sy

beurt dmv ‘n aldolkondensasie tussen die korrek gesubstitueerde asetofenoon en bensaldehied *via* die chalkoon, verkry word. Aangesien die 2,3,4,6-tetra- hidroksi-asetofenoon wat vir die sintese benodig word, nie kommersiëel beskikbaar is nie, is besluit om die sintetiese roete mbv ‘n modelverbinding te toets en terselfdertyd ook vas te stel watter C-1 fragment die geskikste vir die alkilering en ringsluiting, sou wees.

Volgens bogenoemde strategie is die modelchalkoon in 68 % opbrengs dmv die standaard Claisen-Schmidt aldolkondensasie tussen 2-hidroksi-asetofenoon en 3,4-dimetoksibens-aldehied verkry, waarna dit mbv hidrogenering oor 5 % Pd/C in kwantitatiewe opbrengs na die dihidro-ekwivalent omgeskakel is. Ten einde die konstruksie van die C-ring te bewerkstellig is besluit om van ‘n gemodifiseerde Baker-Ventkataraman herrangskikking gebruik te maak, waarna reduksie van die ester gevolg deur ‘n Mitsunobu ringsluiting die verlangde homo- isoflavonoïed sou lewer. Hoewel verestering van 2’-hidroksi-3,4-dimetoksidihydrochalkoon met etielchloroformaat uitstekend verloop het, het die herrangskikkingstap tot die onverwagse vorming van 3-(3’,4’-dimetoksibensiel)-4-hidroksikumarien gelei. Alhoewel die gevormde kumarien wel na die verlangde homoisoflavanoon omgeskakel kon word, sou hierdie proses drie addisionele stappe behels, sodat die moontlikheid van ‘n Vilsmeier-Haack tipe α-formielering as eenstap metode eerder ondersoek is. Behandeling van die 2’-hidroksi- dihidrochalkoon met N,N-dimetielformamied (DMF), PCl_5 en BF_3eteraat was geslaagd en het slegs die 3-(3’,4’-dimetoksibensiel)-isoflavoon in 40 % opbrengs gelewer. Hierteenoor het die hidrogeneringsreaksie (10 % Pd/C in asetoon) om die homoisoflavoon na die homo- isoflavanoon om te skakel, tot die vorming van drie produkte, te wete 3-(3’,4’-dimetoksi- bensiel)-chromaan(43 %), 3-(3’,4’-dimetoksibensiel)-chromanoon (die verlangde produk)(3%) en die 3,4-*cis*- en 3,4-*trans*-3-(3’,4’-dimetoksibensiel)-chroman-4-ole (3% elk), gelei. Hoewel die verlangde produk, weens oor-hidrogenering, in slegs 3 % opbrengs verkry is, is die proses nie herhaal ten einde beter opgrengste te verkry nie, aangesien dit reeds bewys is dat die homoisoflavanoon mbv hierdie metodiek suksesvol berei kan word.

In die tweede gedeelte van hierdie verhandeling is ‘n poging aangewend om die probleem van die bepaling van die absolute configurasie by al die chirale sentrums van flavanoïede, aan te spreek. Hoewel sirkulêre dichroïsme (SD) tans algemeen vir hierdie doel gebruik word, is talle voorbeelde van teenstrydige resulate in die literatuur gerapporteer en moet ook gebruik gemaak word van ‘n hele aantal empiriese reels om die absolute configurasie van ‘n molekuul af te lei. Ten einde hierdie probleem te probeer aanspreek is besluit om die toepassing van die moderne tegniek van vibrasionele sirkulêre dichroïsme (VSD) op die bepaling van die absolute konfigurasie van flavanoïede en proantosianidiene te ondersoek. Aangesien ‘n volledige databasis van die VSD spektra van ‘n reeks flavanoïede hiervoor nodig sou wees, is die sintese van ‘n aantal flavanoïede met toenemede aantal chirale sentra tydens hierdie studie onderneem. Weens die algemene beskikbaarheid van (+)-katesjien [(2*R*,3*S*)-(+)-3,3’,4’,5,7-penta-hidroksiflavaan] en (-)-epikatesjien [(2*R*,3*R*)-(-)-3,3’,4’,5,7-pentahidroksiflavaan] en die feit dat hierdie twee verbindings relatief maklik na hulle onderskeie enantiomere omgeskakel kan word, is besluit om die hele ondersoek op hiedie twee verbindings te basseer.

Ten einde die 4-posisie van (+)-katesjien en (-)-epikatesjien te funksionaliseer sodat die verlangde koppelings bewerkstellig kan word, is tetra-O-metiel-(+)-katesjien, tetra-O-bensiel-(+)-katesjien, en tetra-O-bensiel-(-)-epikatesjien met DDQ (2,3-dichloro-5,6-disiano-1,4-bensokinoon) in die teenwoordigheid van etileenglikol behandel en die 4-hidroksi-etoksi derivate in onderskeidelik 46, 60 en 50 % opbrengs verkry. Titaantetrachloried ($TiCl_4$) gekataliseerde koppeling van laasgenoemde twee uitgangstowwe met gebensileerde floroglusinol het slegs die ooreenstemmende 2,4-*cis*-4-arielflavan-3-ole in onderskeidelik 65 en 55 % opbrengs gelewer. Gebensileerde prosianidiene B1 tot B4 is eweneens suksesvol dmv reaksie van gebensileerde katesjien en – epikatesjien respektiewelik met hulle 4-hidroki-etoksi analoë daargestel. Aangesien die KMR-spektra van die gebensileerde B1 tot B4 weens rotasie beperking geweldige verdubbeling en verbreding van seine vertoon, kon die suiwerheid van hierdie produkte nie bevestig word nie, en sal volledige karakterisering van hierdie produkte later wanneer die

bensielgroepe verwyder is dmv die metieleter asetate, indien nodig, gedoen word. Ten einde die VSD spekta van die vry-fenoliese prosianidiene beskikbaar te hê vir vergelyking met die natuurlike vorms, moet die beskermende groepe in elk geval verwyder word en sal hierdie aspek van die program asook die sintese van die oorblywende isomere, B5 tot B8, tydens die kandidaat se PhD studies aandag geniet.

CHAPTER 1

1. Introduction to Flavonoids

Polyphenolic compounds are secondary metabolites with structures characterized by the presence of one or more aromatic rings bearing hydroxyl substituent(s)[1,2]. Typical flavonoids, with carbon structure C_6-C_3-C_6 **(Figure 1)** are the most studied compounds in this class.

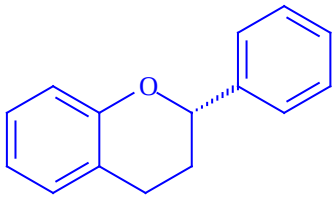

Figure 1: Basic skeleton of monomeric flavonoids.

The flavonoid pigments, one of the most numerous and widespread groups of natural constituents, are of importance and interest not only because of their significant natural functions in the economy of the plant, but also because certain members of the group are physiologically active in humans[3]. Many flavonoids are found in vascular plants and contribute to biological activities such as anti-inflammatory, antiallergic, antischemic, antiplatelet, immunomodulatory, and antitumoral activities[4,5,6].

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[1] Parr, A.J.; Bolwell, G.P. *J. Sci. Food Agric*. **2000**, *80*, 985.
[2] Robards, K.; Prenzler, P.D.; Tucker, G.; Swatsitang, P.; Glover, W. *Food Chem*. **1999**, *66*, 401.
[3] Harborne, J.B. *In Natural products of woody plants I*, Springer-Verlag Berlin Heidelberg New York, **1989**, 533.
[4] Prior, R.L.; Cao, G. *Nutr. Clin. Care* **2000**, *3*, 279.
[5] Ielpo, M.T.L.; Basile, A.; Mirando, R.; Moscatello, V.; Nappo, C.; Sorbo, S.; Laghi, E.; Ricciardi, M.M.; Ricciardi, L.; Vuotto, M.L. *Fitoterapia* **2000**, *71*, S101.
[6] Craig, W.J. *Am. J. Clin. Nutr.* **1999**, *70*, 491S.

Anthocyanins **(1)** is one class of flavonoids which are intensely coloured giving the red and blue colours in flowers, fruits, and other coloured plant tissues[3], hence very rare in woody tissue. What is however predominant in wood, is the less intense coloured flavonoids, namely, flavanones **(2)**, flavones **(3)**, flavonols **(4)**, and dihydroflavonols **(5) (Figure 2)**.

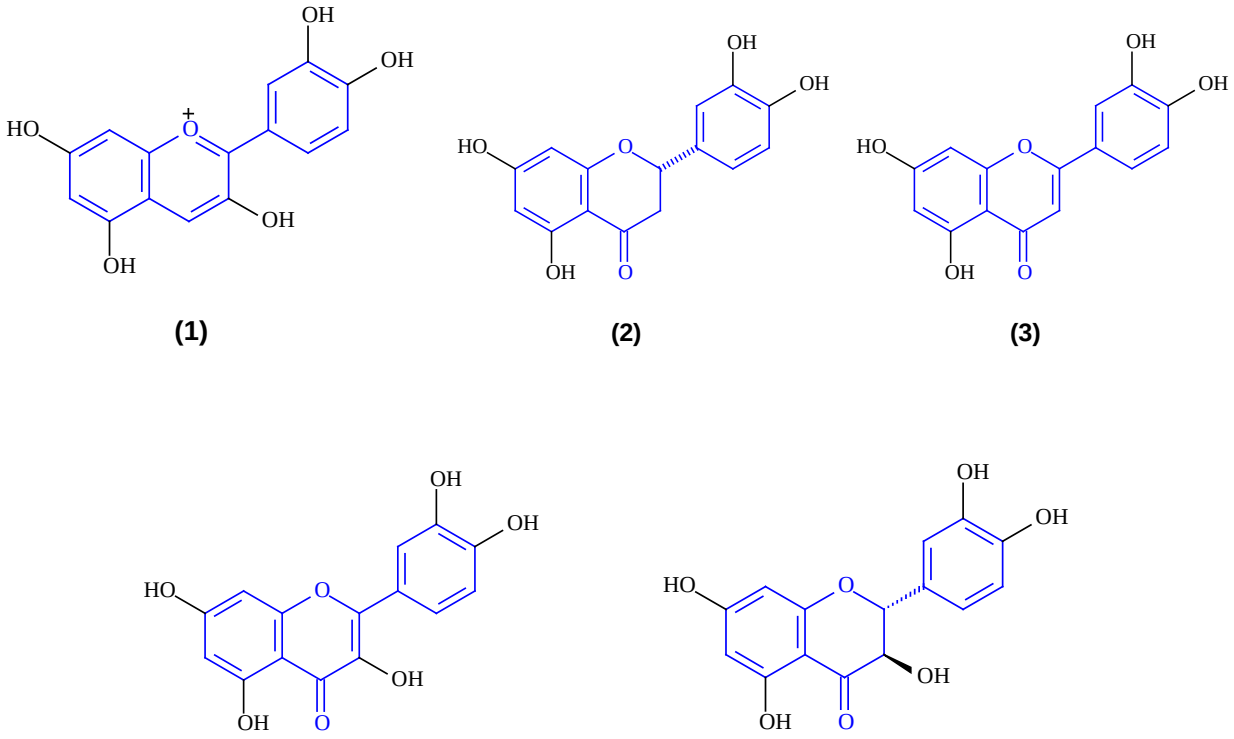

Figure 2: Five classes of monomeric flavonoids.

Flavonoids are also components in the diet of numerous herbivores and omnivores, including humans[7]. They are mainly found in fruits, vegetables, and beverage such as red wine, tea, beer and their intake may reach 1g/day[8]. Various herbs also contain flavonoids[9]. Almost all the flavonoid classes are present in herbs with proven therapeutic activity, including **(3)**, **(4)**, dihydrochalcones **(6)** (directly related to the chalcone **(10)** and derived from them by reduction of the α,β-double bond), isoflavones **(7)**, flavanols **(8)**, flavonolignans **(9)**, and **(5) (Figure 3)**[10].

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[7] Karakaya, S.; Nehir, S.E.L. *Food Chem*. **1999**, *66*, 289.
[8] Petersen, J.; Dwyer, J. *Nutr. Res*. **1998**, *18*, 1995.
[9] Pietta, P.G. *Flavonoids in medicinal plants*. New York: Marcel Dekker, **1998**, 61.
[10] Rice-Evans, C.A.; Packer, L. *Flavonoids in health and disease.* New York: Marcel Dekker, **2003**, 43.

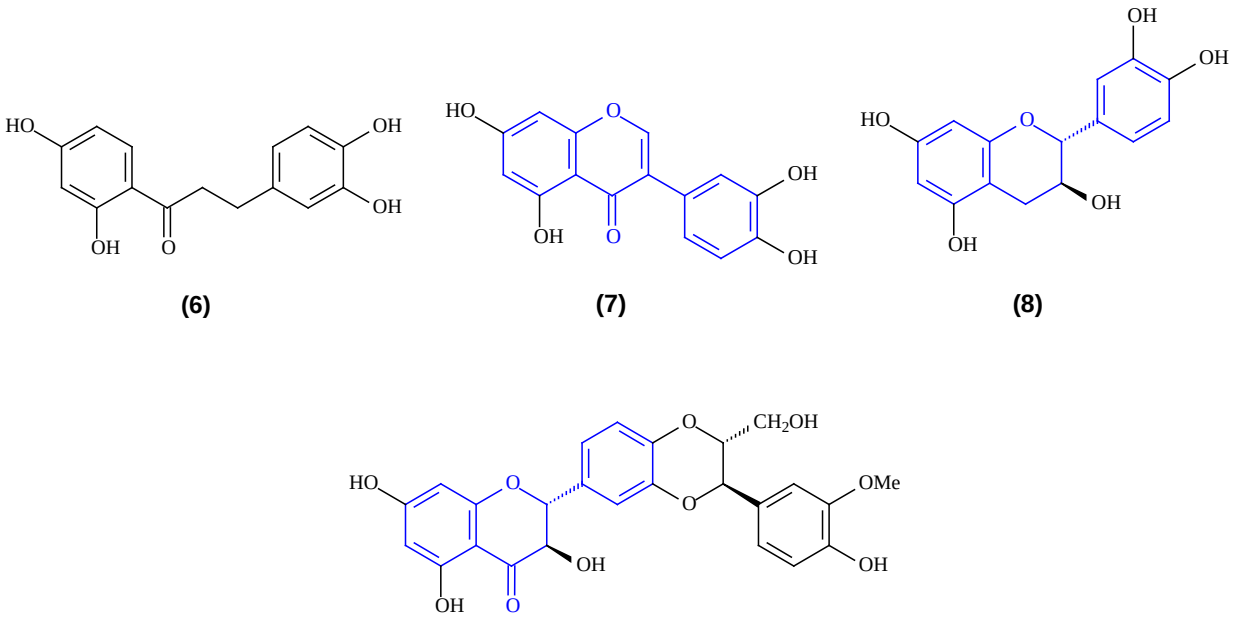


Figure 3: Other four classes of flavonoids.

The other two classes of yellow phenolic anthochlor pigments – the chalcones **(10)**, acyclic C_6-C_3-C_6 compounds and aurones **(11)** – are of restricted distribution in the plant kingdom[11,12] **(Figure 4)**. These two classes are related in that **(11)** are formed from **(10)** by dehydrogenation process and related chalcone-aurone pairs tend to be found together in the same plant source. The main occurrence of chalcones and aurones are in the floral tissues of members of the Compositae, where they are responsible for the yellow colour in certain families and genera – e.g. in *Coreopsis*.

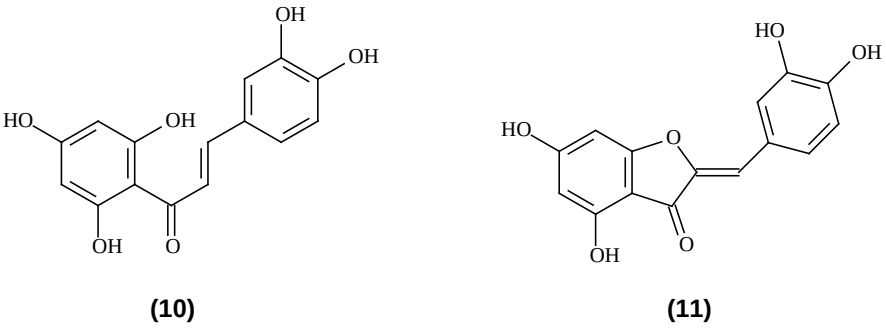


Figure 4: Chalcone and aurone.

[11] Harborne, J.B.; Mabry, T.J.(eds) *The flavonoids: Advances in research*. Chapman & Hall London, **1982**, 744.

[12] Harborne, J.B.; Mabry, T.J.; Mabry, H.(eds) *The flavonoids: Advances in research*. Chapman & Hall London, **1975**, 1204.

CHAPTER 2

2. *Scilla natalensis* (Hyacinthaceae)

2.1 Introduction

Traditional medicine is widely used by many indigenous people in Africa who still incorporate herbal medicine in their daily existence. The plants that are used in traditional medicines are likely, and in some cases already known, to contain pharmacologically active compounds. For this reason, medicinal plants have become the focus of intense study in recent years to determine whether their traditional uses are supported by actual pharmacological effects or are merely based on folklore. With the increasing acceptance by Western health-systems of traditional medicine as an alternative form of health care, there is an urgent need for an evaluation of traditional methods of treatment. Considerable importance has, therefore, been placed on the screening of medicinal plants for active compounds[13].

In South Africa the essentially Eurasian genus *Scilla L*. (Hyacinthaceae) is represented by at least six species, including *Scilla natalensis planch*, *Scilla kraussii bak* and *Scilla dracomontana hilliard* and *burtt*[14]. *Scilla natalensis*, also known as Wild squill, Blue squill, Blue hyacinth, Blouberglelie, Blouslangkop, Inguduza, is characterized by large bulbs, basal strap-like leaves and simple racemes of small blue flowers **(Figure 5)**. This species is widely distributed in Southern Africa, occurring in Lesotho, Kwazulu-Natal, Swaziland, eastern Free State and Gauteng[15]. Large quantities of these bulbs are harvested, processed and sold by traditional healers. *Scilla natalensis planch* is also one of the most popular plant species sold at many of the medicinal

[13] Sparg, S.G.; Van Staden, J.; Jäger, A.K. *Journal of Ethnopharmacology* **2002**, *80*, 95.
[14] Crouch, N.R.; Bangani, V.; Mulholland, D.A. *Phytochemistry* **1999**, *51*, 943.
[15] Du Plessis, N.; Duncan, G. *Bulbous Plants of Southern Africa – A Guide to Their Cultivation and Propagation*. Cape Town: Tafelberg Publishers Ltd. **1988**.

markets in South Africa. The plant is used for the treatment of various ailments such as fractures, gastro-intestinal ailments like stomachache, constipation and diarrhea, and pain-producing ailments such as paralysis, rheumatism, and sprains. These ailments are usually treated by the administration of infusions, decoctions, emetics and enemas[16]. Amongst indigeneous people parts of *S. natalensis* have been used by the Zulus as a purgative[17] and to facilitate labour at term[18], although the plant has been reported to be toxic to sheep[19]. The Sotho eat cooked bulbs as an aperient, use bulb decoctions in enemas for the treatment of internal tumours, and treat lung sickness in cattle[20]. The powdered bulbs are also rubbed over sprains and fractures by the Southern Sotho, while the Tswana rub powdered bulbs onto their backs, joints and other body parts with the belief that it makes them strong and resilient to witchcraft[21].

Figure 5: *Scilla natalensis planch*.

[16] Hutchings, A. *Bothalia* **1989**, *19,* 111.
[17] Gerstner, J. *Bantu Studies* **1939**, *13*, 49.
[18] Gerstner, J. *Bantu Studies* **1941**, *15*, 369.
[19] Kellermann, T.S.; Coetzer, J.A.W.; Naude, T.W. *In Plant poisonings and mycotoxicoses of livestock in Southern Africa*. Cape Town: Oxford University Press. **1988**, 96.
[20] a) Jacot Guillarmod, A. *In Flora of Lesotho,* Lehre: Verlag von J. Cramer. **1971**, 451. b) Jessop, J.P. Studies in the Bulbous Liliaceae: 1. *Scilla, Schizocarphus* and *Ledebouria. Journal of South African botany* **1970**, *36*, 233.
[21] Watt, J.M.; Breyer-Brandwijk, M.G. In *Medicinal and poisonous plants of Southern and Eastern Africa* (2nd ed.) Edinburgh: E and S Livingstone Ltd. **1962**, 713.

Jäger *et al.*[13] reported the screening of *Scilla natalensis planch* for anti-inflammatory, antibacterial, antischistosomic, anticancer and anthelmintic activity, and both positive and negative results were observed (Summary in **Table 1**). Phytochemical screening for the presence of alkaloids, saponins and cardiac- glycosides were also performed. (Response in **Table 2**).

Table 1: Biological screenings for *Scilla natalensis planch*.

Plant / Screening	***Scilla natalensis planch***
Antibacterial	Poor result against both Gram-positive and negative bacteria
Anti-inflammatory	Good inhibition against both COX-1 and COX-2
Antischistosomic	Good activity against *Schistosoma haematobium*
Anticancer	No significant activity in BIA assay
Anthelmintic activity	Highest inhibitory effect against nematodes
Cytotoxicity	Extremely cytotoxic to VK cells

Table 2: Phytochemical screenings for *Scilla natalensis planch*.

Plant / Screening	***Scilla natalensis planch***
Alkaloids	No alkaloids detected in bulbs
Saponins	Having haemolytic activity with the haemolysis test
Cardiac glycosides	Contain bufadienolide proscillaridin A

From the above screenings it is evident that *Scilla natalensis* has pharmacological activity and contains important phytochemicals. However, this species must be used with caution as herbal medicine due to its toxicity.

2.2 Compounds isolated from *Scilla* species

From a phytochemical point of view it is evident that *Scilla* species are homoisoflavanone-rich plants **(Table 3)**[22]. Homoisoflavonoids (3-benzylchroman-4-ones) exhibit biological activities and are naturally occurring compounds structurally related to flavonoids[23], with their B- and C-rings connected by an additional CH_2 group **(Figure 6**).

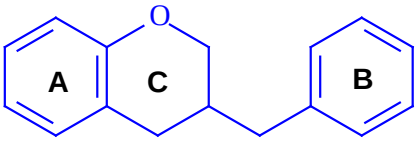


Figure 6: Basic skeleton for homoisoflavonoids.

Homoisoflavonoids have been shown to possess anti-inflammatory, anti-allergic, antihistaminic, angioprotective, antifungal[24], hypocholesterolemic[25], antimutagenic[26], antiviral activities[27] and are potent phosphodiesterase inhibitors[28].

Compound **(13)** was isolated from both *Scilla dracomontana* and *Scilla natalensis* respectively **(Table 3)**. A rare compound **(15)** was isolated from *Scilla dracomontana* as Eucomol with a unique hydroxyl group at C3 of heterocyclic ring. It is found that compound **(25)** has three unusual methoxy groups attached to the A-ring, while compound **(26)** has a hydroxyl group bonded to C8 and was only isolated from *Scilla nervosa*. Compound **(12)** and **(20)** are identical but isolated

[22] Pohl, T.; Koorbanally, C.; Crouch, N.R.; Mulholland, D.A. *Biochemical Systematics and Ecology* **2001**, *29*, 857.
[23] Lockhart, I.M. *In The Chemistry of Heterocyclic Compounds Chromenes, Chromanones and Chromones*; Ellis, G. P., Ed.; Wiley: New York, **1977**.
[24] Al Nakib, T.; Bezjak, V.; Meegan, M.J.; Chandy, R. *Eur. J. Med. Chem*. **1990**, *25*, 455.
[25] Kirkiacharian, B.S.; Gomis, M.; Koutsourakis, P. *Eur. J. Med. Chem*. **1989**, *24*, 309.
[26] Wall, M.E.; Wani, M.C.; Manikumar, G.; Taylor, H.; McGivney, R. *J. Nat, Prod.* **1989**, *52*, 774.
[27] Desideri, N.; Olivieri, S.; Stein, M.L.; Sgro, R.; Orsi, N.; Conti, C. *Antiviral Chem. Chemother*. **1997**, *8*, 545
[28] Amschler, G.; Frahm, A.W.; Hatzelmann, A.; Kilian, U.; Muller-Doblis, D.; Muller-Doblis, U. *Planta Medica*, **1996**, *62*, 534.

from two *Scilla* species (*Scilla kraussii* and *Scilla nervosa*). Compound **(17)** and **(27)** are also identical and found from *Scilla natalensis* and *Scilla plumbea*.

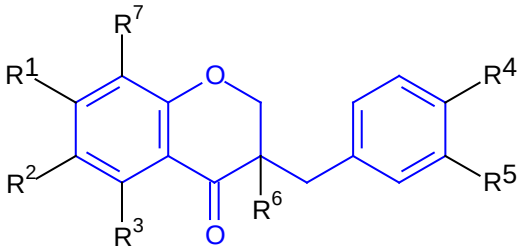


Table 3: Homoisoflavonoids isolated from *Scilla* species.

Plant	Substituent
Scilla kraussii[14]	**(12)** $R^1 = R^3 = R^5 = OH$, $R^2 = R^6 = R^7 = H$, $R^4 = OMe$
Scilla dracomontana[14]	**(13)** $R^1 = R^3 = R^4 = OH$, $R^2 = OMe$, $R^5 = R^6 = R^7 = H$ **(14)** $R^1 = R^3 = OH$, $R^2 = R^4 = OMe$, $R^5 = R^6 = R^7 = H$ **(15)** $R^1 = R^3 = OH$, $R^2 = R^5 = R^7 = H$, $R^4 = OMe$, $R^6 = OH$ **(Eucomol)**
Scilla natalensis[14]	**(13)** $R^1 = R^3 = R^4 = OH$, $R^2 = OMe$, $R^5 = R^6 = R^7 = H$ **(17)** $R^1 = R^3 = R^5 = OH$, $R^2 = R^4 = OMe$, $R^6 = R^7 = H$
Scilla nervosa[29]	**(18)** $R^1 = R^3 = R^4 = OMe$, $R^2 = R^5 = R^6 = R^7 = H$ **(19)** $R^1 = R^5 = OMe$, $R^2 = R^6 = R^7 = H$, $R^3 = R^4 = OH$ **(20)** $R^1 = R^3 = R^5 = OH$, $R^2 = R^6 = R^7 = H$, $R^4 = OMe$ **(21)** $R^1 = R^2 = R^5 = OMe$, $R^3 = R^4 = OH$, $R^6 = R^7 = H$ **(22)** $R^1 = R^3 = R^4 = OH$, $R^2 = OMe$, $R^5 = R^6 = R^7 = H$ **(23)** $R^1 = R^3 = OH$, $R^2 = R^6 = R^7 = H$, $R^4 = R^5 = OMe$ **(24)** $R^1 = R^3 = R^4 = OMe$, $R^2 = OH$, $R^5 = R^6 = R^7 = H$ **(25)** $R^1 = R^2 = R^3 = OMe$, $R^4 = OH$, $R^5 = R^6 = R^7 = H$ **(26)** $R^1 = R^3 = R^4 = OMe$, $R^2 = R^5 = R^6 = H$, $R^7 = OH$
Scilla plumbea[22]	**(27)** $R^1 = R^3 = R^5 = OH$, $R^2 = R^4 = OMe$, $R^6 = R^7 = H$
Scilla zebrina[30]	**(28)** $R^1 = R^5 = OMe$, $R^2 = R^3 = R^4 = OH$, $R^6 = R^7 = H$ **(Zebrinin A)** **(29)** $R^1 = R^3 = R^5 = OMe$, $R^2 = R^4 = OH$, $R^6 = R^7 = H$ **(Zebrinin B)** **(30)** $R^1 = R^3 = R^4 = OH$, $R^2 = R^6 = R^7 = H$, $R^5 = OMe$ **(31)** $R^1 = R^3 = OMe$, $R^2 = R^4 = OH$, $R^5 = R^6 = R^7 = H$ **(Zebrinin C)**

[29] Silayo, A.; Ngadjui, B.T.; Abegaz, B.M. *Phytochemistry* **1999**, *52*, 947.
[30] Mulholland, D.A.; Crouch, N.R.; Koorbanally, C.; Moodley, N.; Pohl, T. *Biochemical Systematics and Ecology* **2006**, *34*, 251.

There are four unsaturated bridge (3-benzylidene-chroman-4-one) compounds **(32)** – **(35)** isolated only from *Scilla nervosa* **(Table 4)**, one of the *Scilla* species represented in Southern Africa.

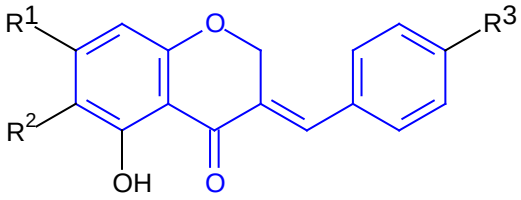
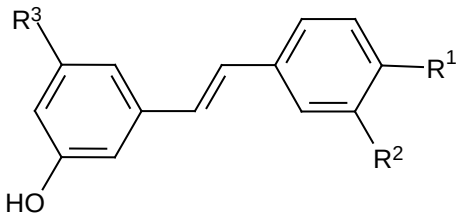


Table 4: Compound isolated from *Scilla nervosa*.

Plant	Substituent
Scilla nervosa[29]	**(32)** $R^1 = R^3 = OH$, $R^2 = OMe$ **(33)** $R^1 = OH$, $R^2 = R^3 = OMe$ **(34)** $R^1 = R^3 = OH$, $R^2 = H$ **(35)** $R^1 = OMe$, $R^2 = H$, $R^3 = OH$

The stilbenes **(36)**, **(37)**, and **(38)** were also isolated from the bulbs of *Scilla nervosa*, which is the only member known to occur in Botswana[29].



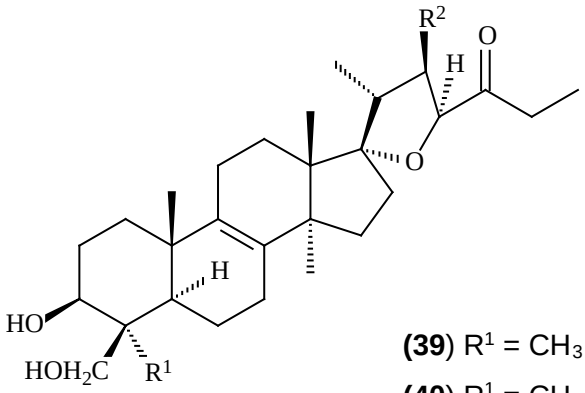
(36) $R^1 = OH$, $R^2 = R^3 = OMe$

(37) $R^1 = OMe$, $R^2 = R^3 = OH$ **(Rhapontigenin)**

(38) $R^1 = R^3 = OH$, $R^2 = OMe$ **(Isorhapontigenin)**

Figure 7: Stilbenes isolated from *Scilla nervosa*.

Mulholland *et al.*[30] has isolated two known nortriterpenoids **(39)** and **(40)** from *Scilla zebrina* distributed in South Africa mostly through the Mpumalanga and KwaZulu-Natal provinces.

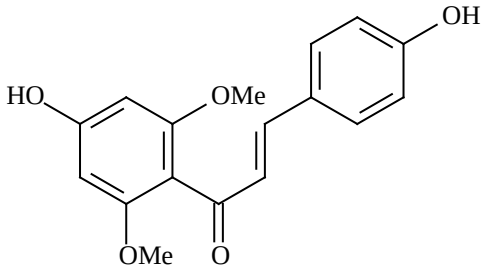


(39) $R^1 = CH_3$, $R^2 = OH$

(40) $R^1 = CH_2OH$, $R^2 = H$

Figure 8: Nortriterpenoids isolated from *Scilla zebrina*.

4,4'-dihydroxy-2',6'-dimethoxychalcone **(41)** previously isolated from *L. ovatifolia* was also isolated from *Scilla zebrina* by Mulholland *et al.*[22,30]



(41)

Figure 9: 4,4'-dihydroxy-2',6'-dimethoxychalcone isolated from *Scilla zebrina*.

CHAPTER 3

3. Proanthocyanidins

3.1 Introduction

Proanthocyanidins are metabolites formed by coupling of two or more flavanyl units and are also known as condensed tannins. Flavonoid polymers have a long history of use as tanning agents for animal skins, are determinants of flavour and astringency in teas, wines and fruit juices, and are increasingly recognized as having beneficial effects on human health. They are widely distributed in nature and often are the active compounds of the medicinal plants which exhibited anti-inflammatory, antiviral, antibacterial, enzyme-inhibiting, antioxidant, and radical-scavenging properties[31].

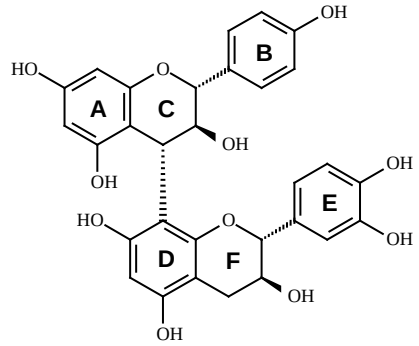
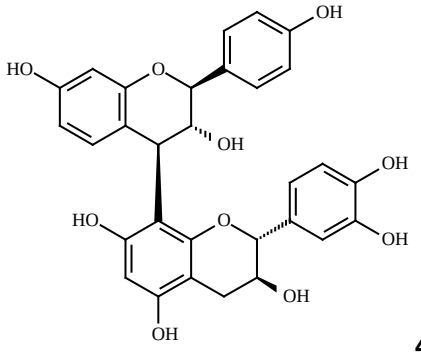
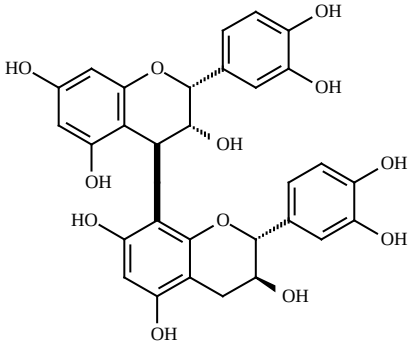
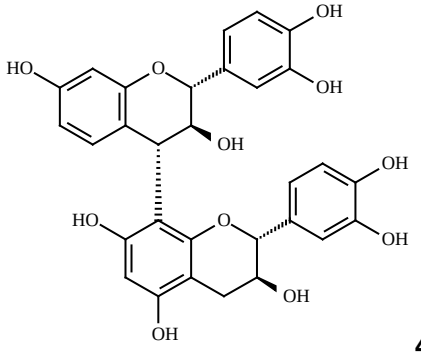
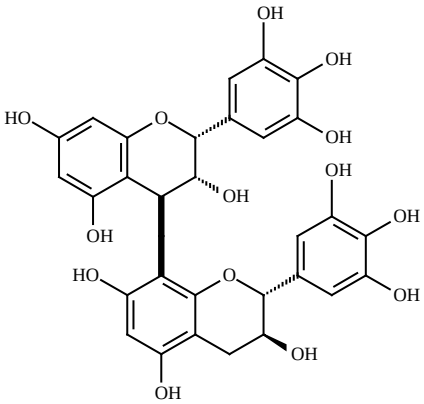
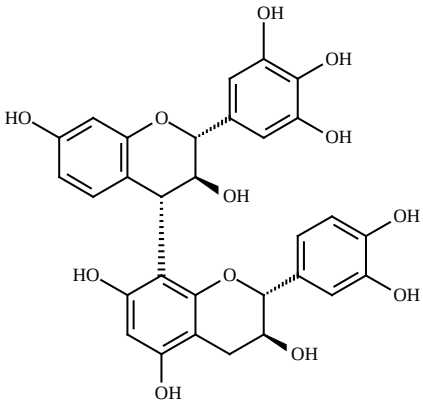
3.2 Nomenclature

The definition of the term proanthocyanidin was initially established by Freudenberg and Weinges[32] who used it to refer to 'all the colourless substances isolated from plants which, when treated with acid, form anthocyanidins'. Since (monomeric) flavan-3,4-diols comply with the Freundenberg-Weinges definition, they may be considered to be the simplest form of proanthocyanidins and are referred to as leucoanthocyanidins (precursors to anthocyanidins). Oligomeric proanthocyanidins are further classified on the basis of the hydroxylation pattern of the anthocyanidin produced on reaction with acid. The propelargonidins **(42)**, procyanidins **(43)**, and prodelphinidins **(44)** having a 5,7-dihydroxylated A-ring, are therefore grouped together since they all produce anthocyanidin on treatment with acid, while their 5-deoxy analogues constitute

[31] Ferreira, D.; Slade, D. *Nat. Prod. Rep*. **2002**, *19*, 517.
[32] Freudenberg, K.; Weinges, K. *Tetrahedron* **1960**, *8*, 336.

the proguibourtinidins **(45)**, profisetinidins **(46)**, and prorobinetinidins **(47)** respectively **(Table 5)**[33].

Table 5: Oligomeric proanthocyanidins of different hydroxylation pattern.

5,7-dihydroxylated A-ring	7-hydroxylated A-ring
 **42**	 **45**
 **43**	 **46**
 **44**	 **47**

Since mixed proanthocyanidins, containing different 'lower' and 'extender' units are now known, the Freundenberg-Weinges system of nomenclature has to a large extent become obsolete and has been replaced by a more systematic approach *vide infra*.

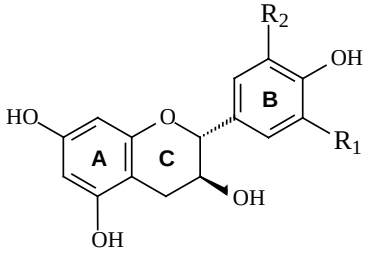
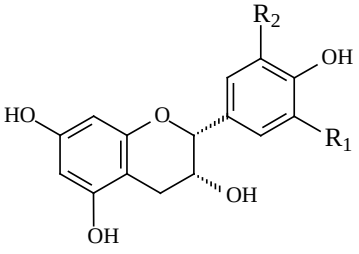
[33] Hemingway, R.W. *In Natural Products of Woody Plants I*,John W. Rowe (Ed.), Springer-Verlag, Berlin Heidelberg, New York, **1989**, 571.

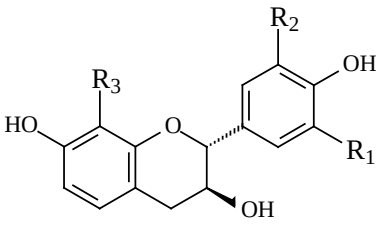
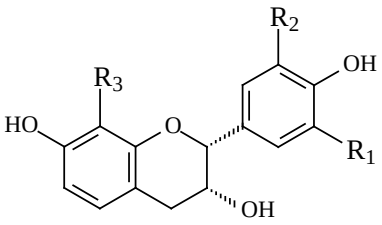
3.3 Monomers

3.3.1 Monomeric flavan-3-ols

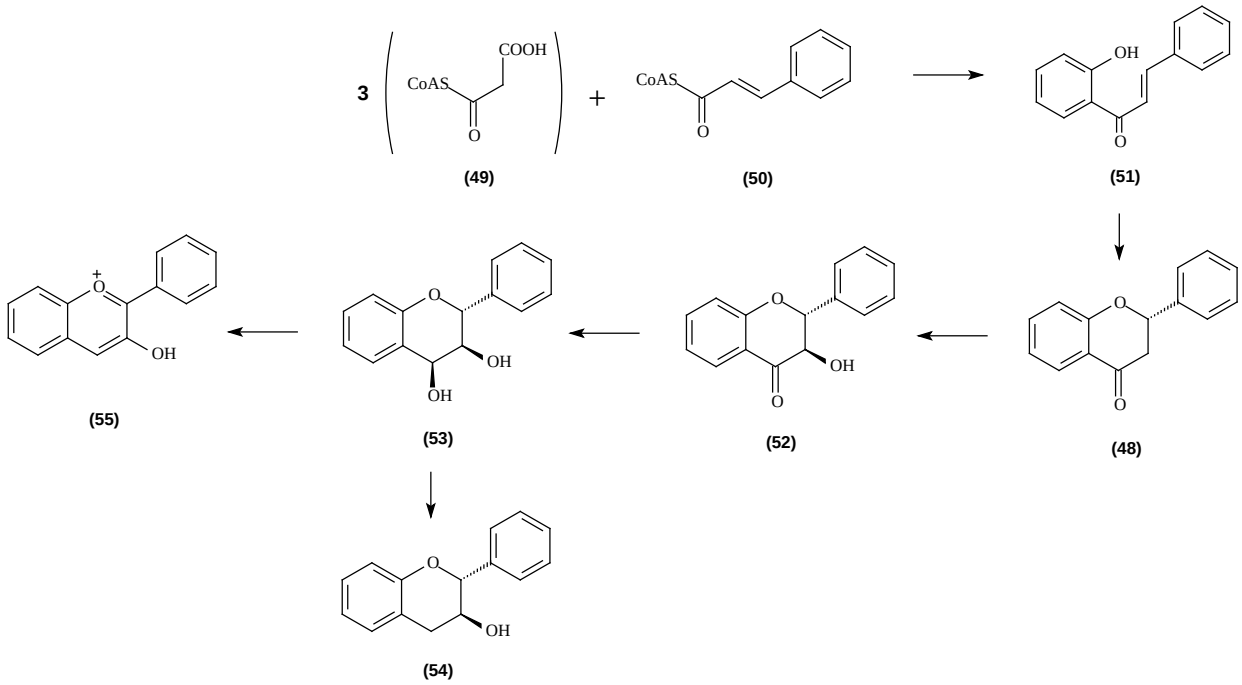
Like proanthocyanidins, variation in monomeric flavan-3-ols structures are depending on hydroxylation pattern of the A- and B-rings as well as the stereochemistry of the chiral centres displayed by the C-ring, while modifications such as esterification of the 3-hydroxyl group and/or methylation of the phenolic OH-groups may be present[32]. Since the trivial names of monomeric flavan-3-ol units are well established and much shorter than the systematic equivalents, these are widely used in condensed tannin literature. According to the trivial system, all compounds displaying a 2,3-*trans* relative stereochemistry, 2*R* absolute configuration and a 3',4',5,7-tetrahydroxy substitution pattern are indicated by the name catechin, while the analogues with only a *para*-hydroxy on the B-ring are called afzelechins and those with a 3,4,5-trihydroxy B-ring gallocatechins. In order to include relative stereochemistry into the common name, 2,3-*cis* isomers are indicated by the *epi*- prefix, while a 2S absolute configuration is designated by employing the *ent*- prefix. The same rules are applicable to the 5-deoxy analogues **(Table 6)**.

Table 6: Structures of the flavan-3-ol building blocks of proanthocyanidins.

5,7-dihydroxylated A-ring	
	
$R_1 = R_2 = H$, (+)-afzelechin	$R_1 = R_2 = H$, (-)-epiafzelechin
$R_1 = OH$, $R_2 = H$, (+)-catechin	$R_1 = OH$, $R_2 = H$, (-)-epicatechin
$R_1 = R_2 = OH$, (+)-gallocatechin	$R_1 = R_2 = OH$, (-)-epigallocatechin

7-hydroxylated and 7,8-dihydroxylated A-ring	
	
$R_1 = R_2 = R_3 = H$, (+)-guibourtinidol	$R_1 = R_2 = R_3 = H$, (-)-epiguibourtinidol
$R_1 = OH$, $R_2 = R_3 = H$, (+)-fisetinidol	$R_1 = OH$, $R_2 = R_3 = H$, (-)-epifisetinidol
$R_1 = R_2 = OH$, $R_3 = H$, (+)-robinetinidol	$R_1 = R_2 = OH$, $R_3 = H$, (-)-epirobinetinidol
$R_1 = R_3 = OH$, $R_2 = H$, (+)-mesquitol	$R_1 = R_3 = OH$, $R_2 = H$, (-)-mesquitol

All monomeric flavonoids derive their 15 carbon skeleton **(48)** from two basic metabolites, malonyl-CoA **(49)** and *p*-coumaroyl CoA **(50)**[34], which are enzymatically arranged into the common phenylbenzopyran structure ($C_6C_3C_6$) **(48)**. The biosynthetic route to flavan-3-ols **(54)** involves the reduction of dihydroflavonols **(52)** to flavan-3,4-diols **(53)** followed by a second reduction to the flavan-3-ols **(Scheme 1)**.



Scheme 1: Biosynthesis of the flavan-3-ols.

[34] Geisman, T.A.; Hinreiner, E. *Bot. Rev*. **1952**, *18*, 77.

The most commonly reported flavan-3-ols, catechin and epicatechin, are widely distributed in the leaves, woody parts and fruits of plants. Analogues carrying a pyrogallol B-ring, gallocatechin and epigallocatechin, are dominant in primitive plants. In comparison, afzelechins with a 4'-hydroxy B-ring are rare. Although a number of flavan-3-ols of the 2*S* configuration are known, their distribution is quite restricted.

3.4 Oligomers

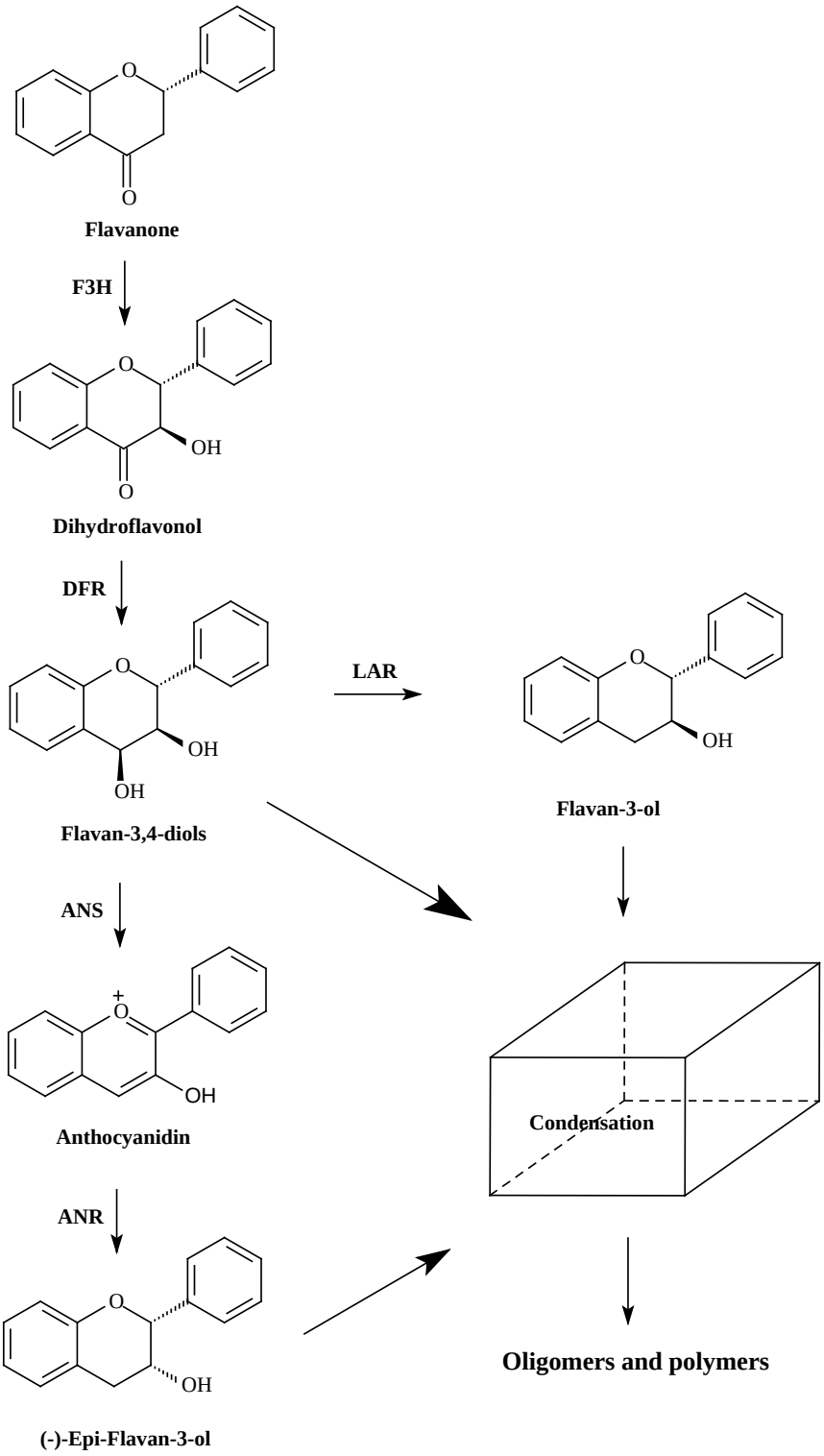
The oligomeric proanthocyanidins consist of between 2 and 4 monomeric units, which may or may not be the same. These compounds can be divided into three main groups, i.e. the A- and B-type proanthocyanidins and all other oligomeric compounds which display different types of bonds and bonds in different positions than those present in the condensed tannins. The isolation and especially structure elucidation of oligomeric flavonoids are hampered by vastly complicated NMR spectra mainly due to restricted rotation about the interflavanoid bond and conformational heterogeneity of the heterocyclic ring[35]. Even through the utilization of modern high field NMR techniques, this aspect of oligomeric flavonoid research still remains a challenge.

The biosynthetic pathway for formation of proanthocyanidins is represented in **(Scheme 2)**. In the first step flavanone 3-hydroxylase (F3H) is responsible for introducing the hydroxyl group into the 3-position; dihydroflavonol reductase (DFR) is responsible for converting the 3-hydroxyflavanone into the leucoanthocyanidin 'extender' unit, while leucoanthocyanidin reductase, (LAR), as well as anthocyanidin synthase and anthocyanidin reductase (ANS and ANR respectively) are responsible for formation of the flavan3-ol 'lower' unit. Finally nucleophylic displacement of the 4-OH in the 'extender' unit by the appropriate nucleophilic lower unit completes the biosynthesis of proanthocyanidins[36].

---

[35] Hemingway, R.W. *In Natural Products of Woody Plants I*, Springer-Verlag, Berlin Heidelberg, New York, **1989**, 612.

[36] Dixon, R.A.; Xie, D.Y.; Sharma, S.B. *New Phytologist* **2005**, *165*, 9.

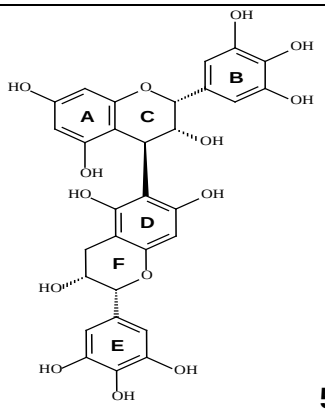
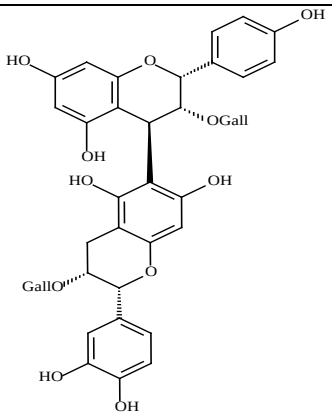


Scheme 2: Biosynthesis of procyanidins through a flavan-3,4-diol intermediate and oligomers are formed through condensation.

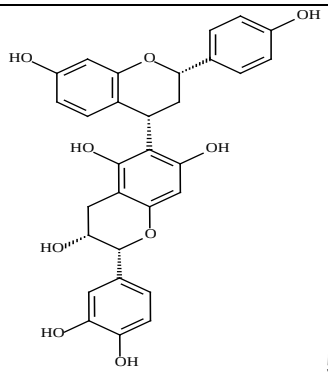
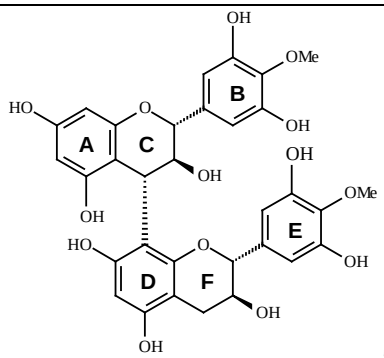
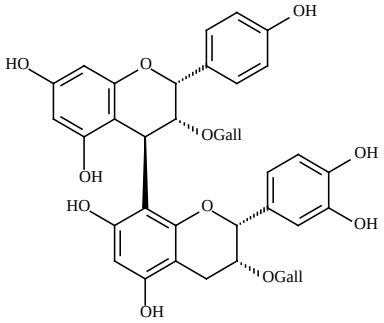
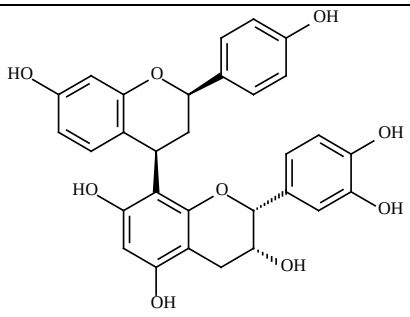
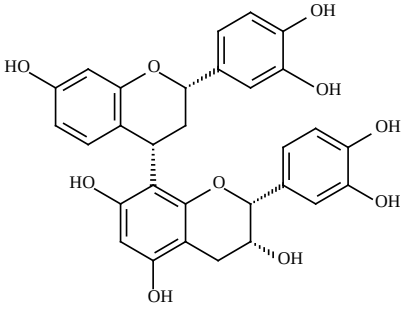
3.4.1 B-Type proanthocyanidins

Dimeric B-type proanthocyanidins are defined as those compounds exhibiting only one link between C-4 of the 'upper' unit and either C-6 or C-8 of the 'lower' unit **(Table 7)**. The interflavanyl bond configuration at C-4 may either be α or β and is, in accordance with the indication of relative orientation at the anomeric position in carbohydrate chemistry denoted as such. Thus, the familiar procyanidin B1 is called epicatechin-(4β→8)-catechin and the analogous prodelphindin is named epigallocatechin-(4 $\beta \rightarrow$ 8)-gallocatechin with the corresponding 2*S* enantiomer being *ent*-epicatechin-(4α→8)- *ent*-catechin[33].

Table 7: Examples of (4→6) and (4→8) proanthocyanidins.

Compounds	(4→6) dimers	Plant Source
Prodelphinidins	 **56**	*Stryphnodendron adstringens*[37] – Stem bark
Propelargonidins	 **57**	Green tea[38]

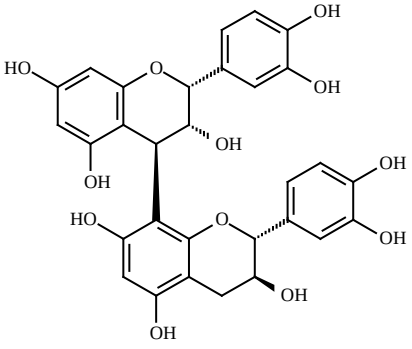
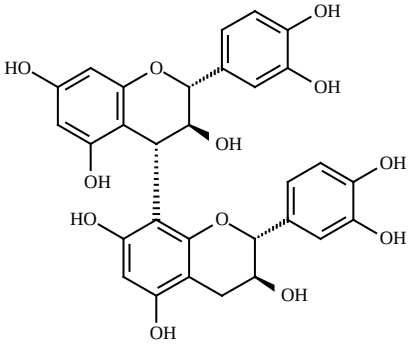
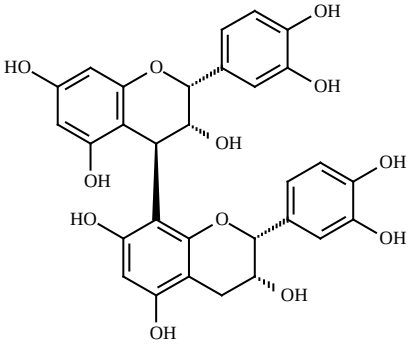
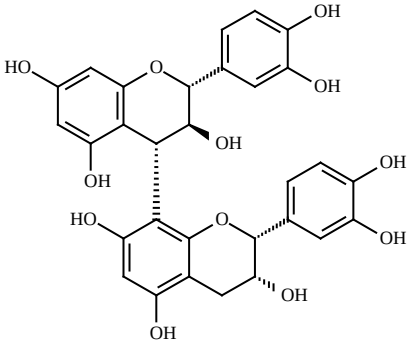
[37] De Mello, J.C.P.; Petereit, F.; Nahrstedt, A. *Phytochemistry* **1999**, *51*, 1105.
[38] Lakenbrink, C.; Engelhardt, U.H.; Wray, V. *J. Agric. Food Chem.* **1999**, *47*, 4621.

Procassinidins	 **58**	*Cassia petersiana*[39]
Compounds	**(4→8) dimers**	**Plant source**
Prodelphinidins	 **59**	*Stryphnodendron adstringens* – Stem bark
Propelargonidins	 **60**	Green tea
Procassinidins	 **61**	*Cassia petersiana*
Probutinidins	 **62**	*A. petersiana*[40]

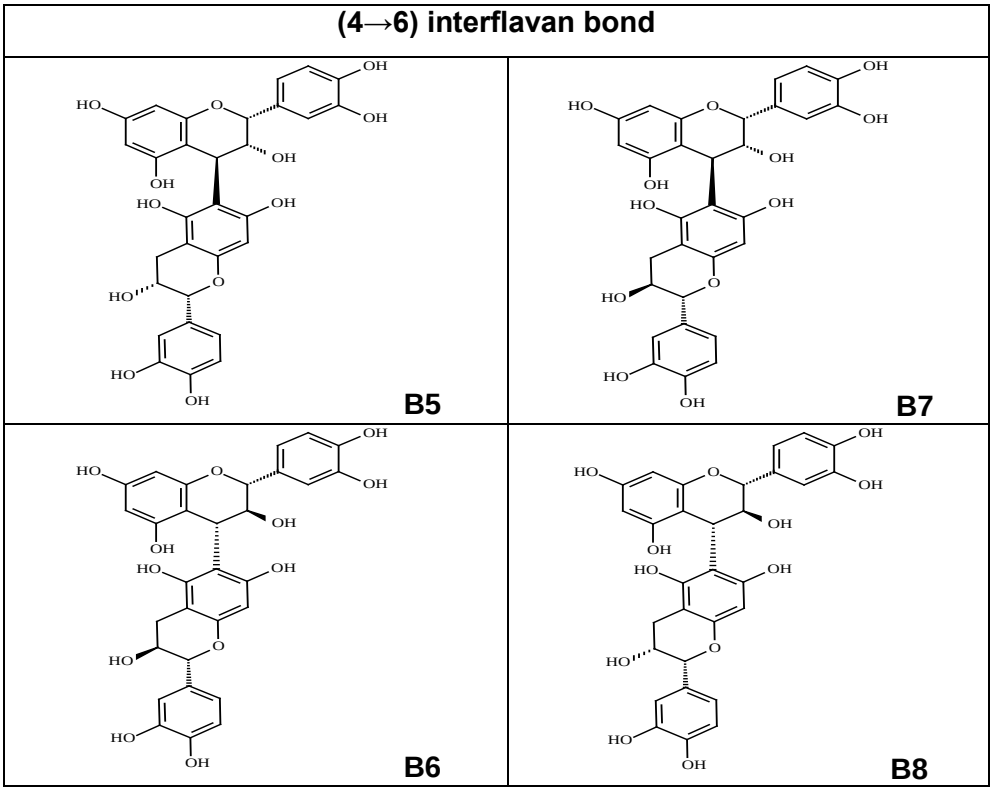
[39] Coetzee, J.; Mciteka, L.; Malan, E.; Ferreira, D. *Phytochemistry* **2000**, *53*, 795.
[40] Coetzee, J.; Mciteka, L.; Malan, E.; Ferreira, D. *Phytochemistry* **1999**, *52*, 737.

The procyanidins represent a dominant and widespread class of naturally occurring proanthocyanidins. Procyanidins (3,5,7,3',4'-pentahydroxylation) B1-B4 **(Table 8)** differ only in the arrangement of (+)-catechin and (-)-epicatechin starter and extender units and are examples of compounds with the (4→8) interflavan bond. Procyanidins B1, B2, B3 and B4 occur the most frequently in plant tissues and B1 is found in grape, sorghum and cranberry; B2 in apple, cocoa bean and cherry; B3 in strawberry, hops and willow catkins and B4 in raspberry and blackberry[41]. Procyanidins B5, B6, B7 and B8 **(Table 8)** are examples of compounds with the (4→6) interflavan bond also widespread in plants[42] although not as general as B1-4.

Table 8: Examples of B1-B8 procyanidins.

(4→8) interflavan bond	
 **B1**	 **B3**
 **B2**	 **B4**

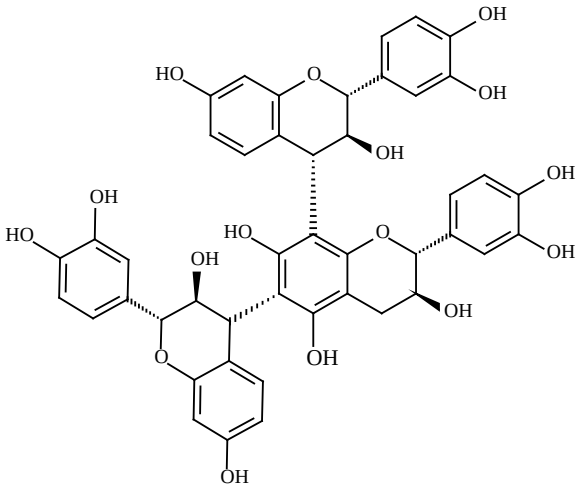
[41] Haslam, E. *Phytochemistry* **1977**, *16*, 1625.
[42] Ferreira, D.; Nel, R.J.J.; Bekker, R. Comprehensive Natural Products Chemistry, Elsevier, Kidlington, Oxford, **1999**, 791.



Procyanidins have the ability to bind strongly to proteins, reducing significantly the nutritional value of fodder when used in animal diets at high concentration[43]. Procyanidins are considered to be primarily responsible for the astringent properties of cider beverages[44].

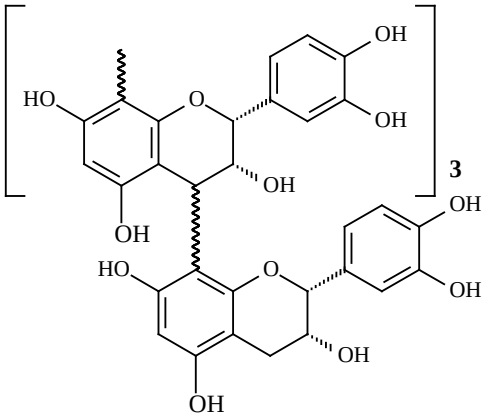
Furthermore, trimeric and tetrameric procyanidins are also classified as B-type proanthocyanidins. Most of these compounds are naturally occurring and could be isolated and synthesized. Solving the NMR spectra of this group is even more challenging due to the restricted rotation about the interflavanoid bond under normal temperature conditions[45]. Like other proanthocyanidins, the trimeric and tetrameric procyanidins that have both (4→8) and (4→6) bonds within the same molecule are designated as 'branch' type oligolymers, while 'linear' types are either connected in a (4→8) or (4→6) bonding fashion throughout all units **(Figure 10)**.

[43] Waghorn, G.C.; McNabb, W.C. *Proc. Nutr. Soc.* 62 (2) **2003**, 383.
[44] Guyot, S.; Marnet, N.; Sanoner, P.; Drilleau, J.F. *Methods Enzymol* **2001**, *335*, 57.
[45] DuPreez, I.C.; Rowan, A.C.; Roux, D.G.; Feeny, J. *J. Chem. Soc. Chem. Commun.* **1971**, 315.



Profisitinidol *(Colophospermum mopane)*

(trimeric-'branch' type)



Arabidopsis proanthocyanidin

(tetrameric-'linear' type)

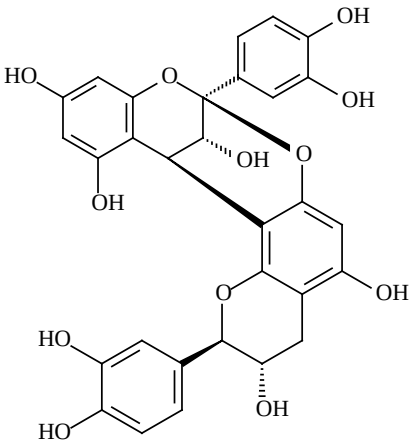
Figure 10: Examples of 'branch' type trimeric and 'linear' type tetrameric procyanidin.

3.4.2 A-Type proanthocyanidins

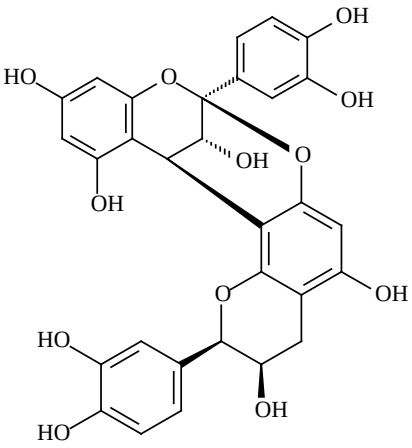
The A-type proanthocyanidins are compounds where two linkages between at least two of the repeating units are displayed. In these compounds the usual C4→C8 or C4→C6 bond between the 'upper' and 'lower' units are therefore accompanied by a second ether linkage usually between the oxygen at C7 of the 'lower' unit and C2 of the C-ring. Since they are not as frequently isolated from plants as the B-types, they have been considered as unusual structures.[46] The first identified A-type proanthocyanidin, procyanidin A2 **(64)**, was isolated from the shells of the fruit of *Aesculus hippocastanum*.[47] Since then, many more A-type proanthocyanidins have been found in plants, including dimers, trimers, tetramers, pentamers and ethers.[31] Procyanidin A1 **(63)** and A2 **(64)** are examples of simple A-type proanthocyanidins. The A-type procyanidin dimer **(65)** from peanut skins displays effective protection from hemorrhage[48], while the complex A-type proanthocyanidin **(66)** from cranberry (*Vaccinium macrocarpon*) has been shown to prevent urinary tract infections[49] **(Figure 11)**.

---

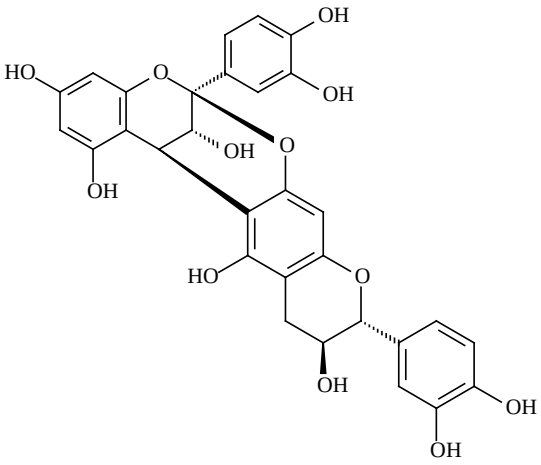
[46] Xie, D.Y.; Dixon, R.A. *Phytochemistry* **2005**, *66*, 2127.
[47] Mayer, W.; Goll, L.; Arndt, E.M.; Mannschreck, A. *Tetrahedron Letters* **1966**, *4*, 429.
[48] Lou, H.; Yamazaki, Y.; Sasaki, T.; Uchida, M.; Tanaka, H.; Oka, S. *Phytochemistry* **1999**, *51*, 297.
[49] Foo, L.Y.; Lu, Y.; Howell, A.B.; Vorsa, N. *Phytochemistry* **2000**, *54*, 173.



Procyanidin A1 (63)

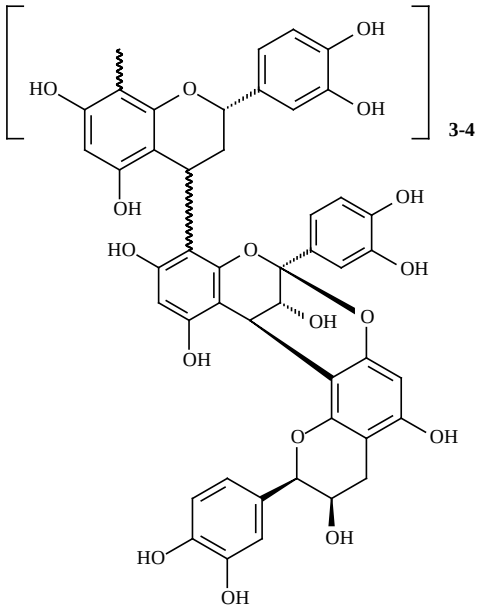


Procyanidin A2 (64)



Peanut skin

A-type procyanidin dimer **(65)**



Cranberry

A-type proanthocyanidin **(66)**

Figure 11: Examples of A-type proanthocyanidins.

3.5 Other oligomeric flavanoids

Although condensed tannins, i.e. A- and B-type proanthocyanidins represent the largest group of oligomeric flavonoids, structural diversity amongst the oligomeric flavonoids are by no means limited to these groups and many di- and oligomerinc compounds displaying other types of

linkages between the different units or containing other than flavanyl monomeric units have been isolated to date.

3,4,3',5'-tetrahydroxystilbene terminating unit is the group of proanthocyanidins obtained from *Guibourtia coleosperma*[50] **(Figure 12)**.

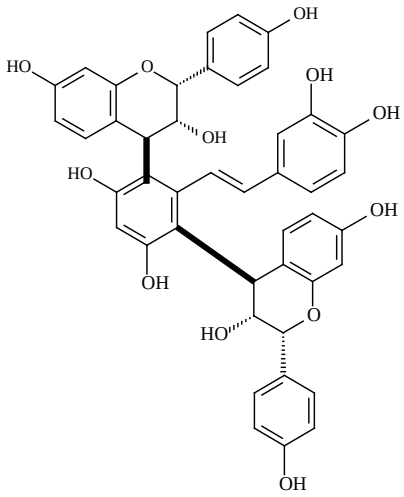


Figure 12: 3,4,3',5'-tetrahydroxystilbene terminating unit.

Another example of oligomeric flavanoids which composed of two dioxane-linked dimmers isolated from the heartwood of *Acacia mearnsii*[51,52] **(Figure 13)**.

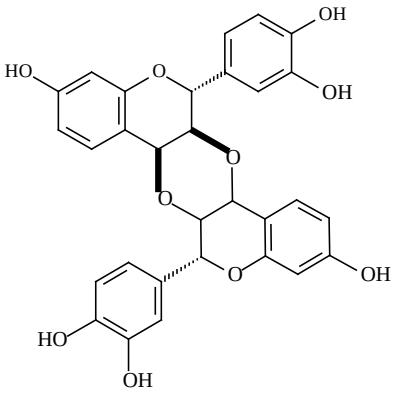
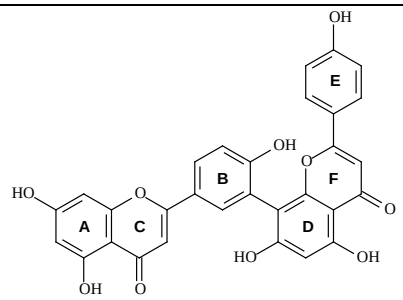
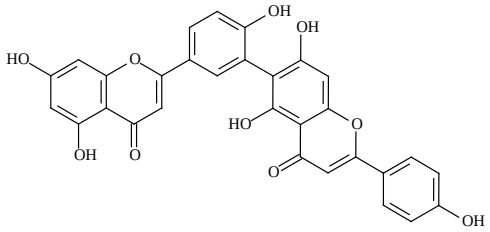
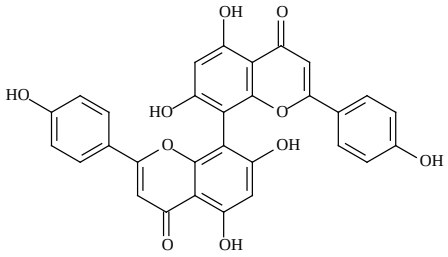
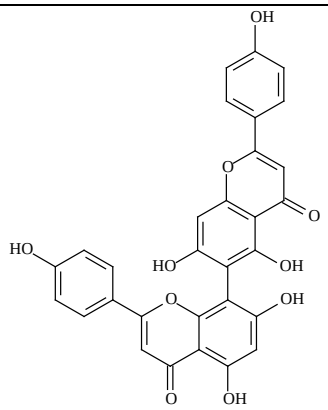


Figure 13: The dioxane-linked dimmer.

[50] Steynberg, J.P.; Ferreira, D.; Roux, D.G. *Tetrahedron Lett*. **1983**, *24*, 4147.
[51] Drewes, S.E.; Ilsley, A.H. *J. Chem. Soc. (C)* **1969**, 897.
[52] Young, D.A.; Ferreira, D.; Roux, D.G. *J. Chem. Soc. Perkin Trans I* **1983**, 2031.

In contrast to proanthocyanidins, the so-called biflavonoids always carry carbonyl functions at the C-4 positions[53] and exhibited great diversity in the location of the interflavonoid bond. Many of these compounds are formed through oxidative coupling reactions **(Table 9)**.

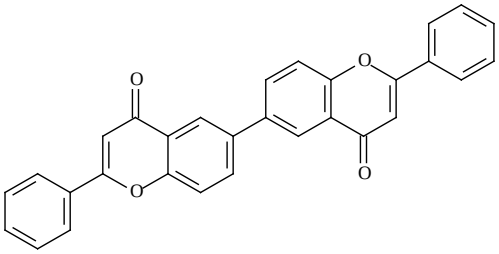
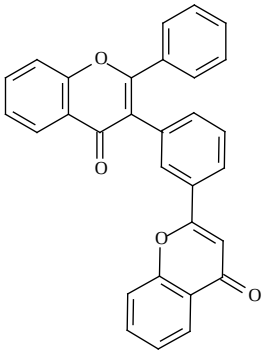
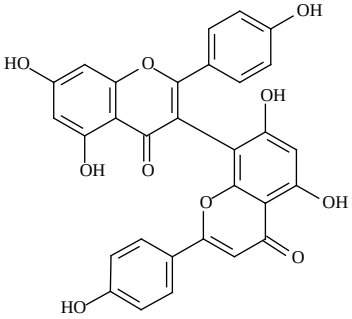
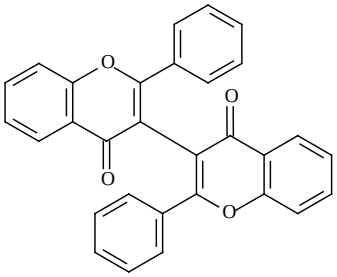
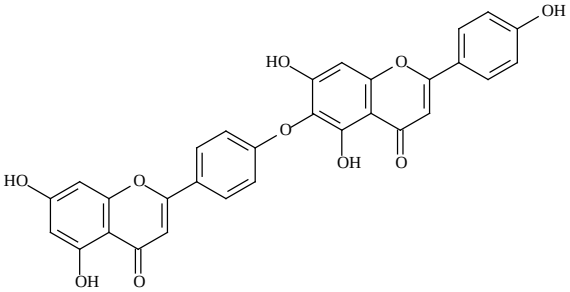
Table 9: Structural variations of biflavonoids.

Trivial name	Structure	Plant source
Amentoflavone	 **67**	*Semecarpus anacardium*[54]
Robustaflavone	 **68**	*Araucaria* and *Juniperus* of the gymnosperms[55]
Cupressuflavone	 **69**	*Mesua ferrea*[55]
Agathisflavone	 **70**	Araucariaceae[55]

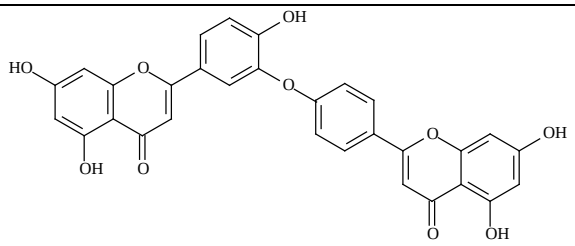
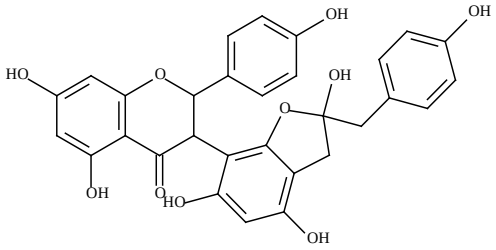
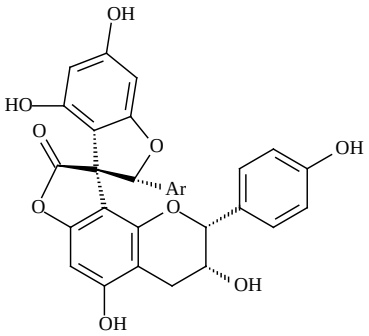
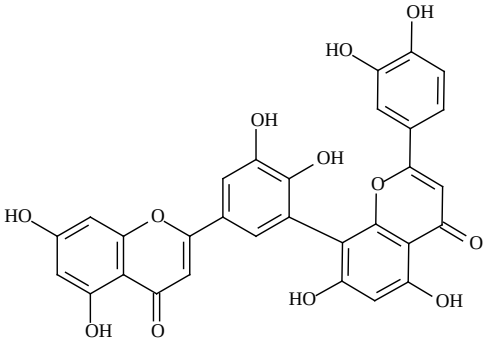
[53] Geiger, H.; Quinn, C. Biflavonoids. In: Harborne, J.B.; Mabry, T.J. *The flavonoids: Advances in research.* Chapman and Hall London, **1982**, 505.

[54] Murthy, S.S.N. *J. Chem.* **1983**, *22B*, 1167.

[55] Geriger, H.; Quinn, C. Biflavonoids. In: Harborne, J.B.; Mabry, T.J.; Mabry, H. *The flavonoids.* Academic Press New York, **1975**, 692.

Succedaneaflavone	 **71**	*Rhus succedanea*[56]
Taiwaniaflavone	 **72**	Taxodiaceae[57]
Volkensiflavone	 **73**	*Garcinia volkensii*[58]
Chamaejasmin	 **74**	*Stellera chamaejasme*[55]
Hinokiflavone	 **75**	Coniferales[55]

[56] Chen, F-C.; Lin, Y-M. *Phytochemistry* **1975**, *14*, 1644.
[57] Kamil, M.; Ilyas, M.; Rahman, W.; Hasaka, N.; Okigawa, M.; Kawano, N. *Taiwaniaflavone: a new series of naturally occurring biflavones from Taiwania cryptomerioides*, Chem Ind (London) **1977**, 160.
[58] Herbin, G.A.; Jackson, B.; Locksley, H.D.; Scheinmann, F.; Wolstenholme, W.A. *Phytochemistry* **1970**, *9*, 211.

Ochnaflavone	 **76**	*Ochna* species[59]
Zehyerin	 **77**	*Phyllogeiton* Species[60]
Larixinol	 **78**	*Larix gmelini*[61]
5',8''-Biluteolin[62]	 **79**	*Philonotis* species

[59] Okigawa, M.; Kawano, N.; Aqil, M.; Rahman, W. *Tetrahedron Lett*. **1973**, 2003.
[60] Volsteedt, F.; Roux, D.G. *Tetrahedron Lett*. **1971**, *20*, 1647.
[61] Shen, Z.; Falshaw, C.P.; Haslam, E.; Begley, M.J. *J. Chem. Soc. Chem. Commun.* **1985**, 1135.
[62] Nilsson, E. *Chem. Scripta* **1973**, *4*, 66.

CHAPTER 4

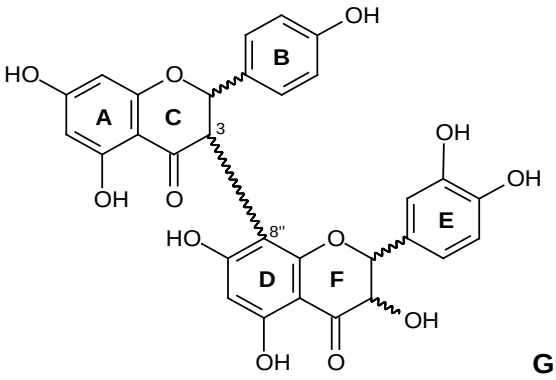
4. Determination of absolute stereochemistry of flavonoids

4.1 Introduction

Since many types of flavonoids contain one or more stereogenic centres {e.g. flavanones (one), flavan-3-ols (two), flavan-3,4-diols (three), flavanone-dihydroflavonols [like GB-2, **(140)**] (four), catechin-catechin [B3] (five) etc.}, structure elucidation of these compounds have always been plagued by determination of the absolute configuration at these stereocentres and have always included an element of optical measurement in order to define the stereochemistry. While optical rotation values have been used extensively for this purpose in early investigations, this method does not supply information as to the absolute configuration at individual chiral centres in molecules containing more that one stereogenic centre and is not that sensitive. Two techniques, i.e. optical rotation dispersion (ORD) and circular dichroism (CD), have been developed in recent times (since the middle 1960's) to assist in defining the absolute configuration(s) in chiral molecules. Circular dichroism can be defined as a form of spectroscopy that basically measures the differential absorption between left- and right-handed circularly polarised light as a function of wavelength. Depending on the stereochemistry of the sample molecule, which the light passes through, the difference in absorption between the left- and right-handed circularly polarised light can either be negative or positive and when plotted against wavelength can give rise to either a positive or negative curve, the so-called (+) or (-) Cotton-effect. Since CD basically represents an absorption technique, it is more sensitive than ORD, which is based on refractive index, and has therefore found widespread application in the determination of absolute configuration of molecules containing one or more chromophores[63]. Due to the fact that steroids were receiving a

[63] Djerassi, C., In : Snatzke, G. (Ed.), *Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry*. Heyden and Son Limited, London, **1967**, 16-17.

lot of attention from scientists during the middle of the previous century and these compounds contain a carbonyl group as well as at least one stereogenic centre, chiral carbonyl compounds were the first to be investigated with the 'new' technique of circular dichroism. In order to correlate the absolute configuration of a carbonyl containing compound with the observed Cotton-effect in the CD spectrum Moffitt *et al*.[64,65] formulated an empirical rule, the so-called Octant rule, which could be used to relate the sign of the observed CE with the absolute configuration of the molecule. Since the 1970's and 80's this technique was extended to include several other types of compounds and used together with several other empirical rules to define the absolute configuration of, amongst others, a number of non-planar flavonoids[66]. The utilization of CD together with empirical rules in the assignment of the stereochemistry of some flavonoids will be discussed in the rest of this chapter.



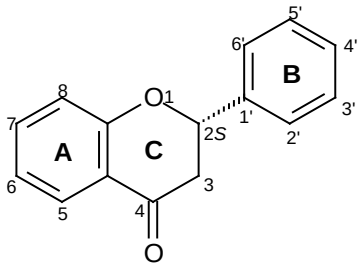
GB-2 (140)

4.2 Flavanones

Since flavanones **(e.g. 80)** represent one of the few classes of flavonoids containing only one stereogenic centre (at C-2) as well as a carbonyl chromophore, the flavanone naringenin became one of the first flavonoids to be subjected to the determination of absolute configuration by CD.

---

[64] Moffitt, W., Woodward, R.B., Moscowitz, A., Klyne, W., and Djerassi, C. *J. Am. Chem. Soc.* **1961**, 83, 4013.
[65] Snatzke, G. *Tetrahedron* **1965a**, *21*, 413-149.
[66] Slade, D.; Ferreira, D.; Marais, J.P.J. *Phytochem.* **2005**, *66*, 2177-2215.



(2*S*)-flavanone (80)

In the process of determining the absolute configuration at C2 of flavanones Gaffield[67] extended the modified octant rule for the relationship between the chirality of α,β-unsaturated ketones and the sign of the high wavelength CE [320 – 330 nm (associated with the n → π* transition)] to aryl ketones (acetophenones). Thus flavanones with 2*S*-configuration possessing a conformation with *P*-helicity of the heterocyclic ring and having a C2 equatorial aryl group **[(80) and Figure 14(a)]**[68], will exhibited a positive CE at the n → π* absorption band (320 – 330 nm) and negative CE at the π → π* transition band (270 – 290 nm). The advantage of using the n → π* absorption band for configurational assignment being that the sign of this transition is not effected by the substitution pattern of the aromatic ring system[69]. It must however be remembered that the n → π* transition at longer wavelengths tends to diminish with increasing amounts of the opposite enantiomers[70].

[67] Gaffield, W. *Tetrahedron* **1970**, *26*, 4093-4108.
[68] Gaffield, W. *Tetrahedron* **1970**, *26*, 4093-4108.
[69] Snatzke, G.; Znatzke, F.; Tökés, A.L.; Rákosi, M.; Bognár, R. *Tetrahedron* **1973a**, *29*, 909-912.
[70] Li, X.C.; Joshi, A.S.; Tan, B.; ElSohly, H.N.; Walker, L.A.; Zjawiony, J.K.; Ferreira, D. *Tetrahedron* **2002**, *58*, 8709-8717.

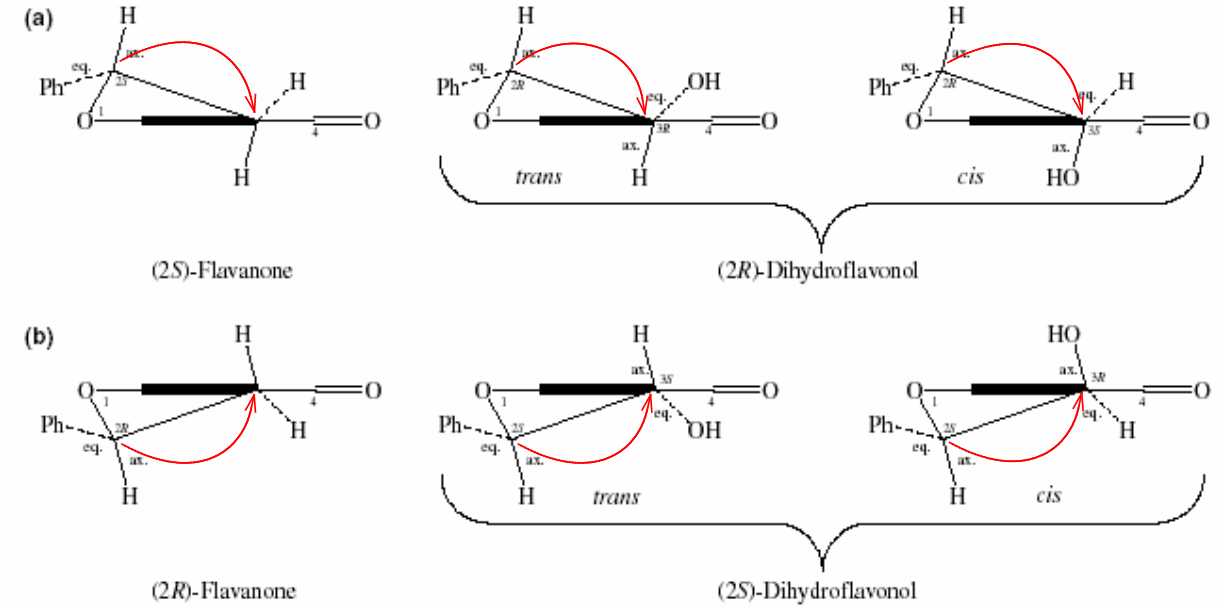
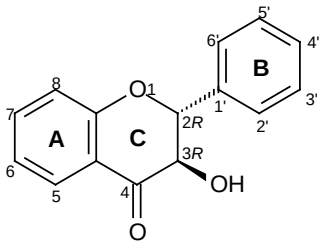


Figure 14: Hetero-ring conformations (helicities) of the two enantiometic flavanones and diastereomeric dihydroflavonols with equatorial C2-aryl groups.

4.3 Dihydroflavonols (3-hydroxyflavanones)



(81)

Dihydroflavonols **(e.g. 81)** which possess chiral centres at C2 and C3 can be viewed as flavanones with an additional OH substituent at C3. Having two stereocentres, dihydroflavonols exhibit four possible stereoisomers, i.e. (2*R*,3*R*), (2*R*,3*S*), (2*S*,3*R*), and (2*S*,3*S*). The assignment of absolute configuration to dihydroflavonols has to be done in two steps: In the first step NMR coupling constants ($J_{2,3}$) is utilised to identify the relative configuration of the C2 and C3 substituents as either *trans* or *cis*. For the *trans*-isomers the thermodynamically more stable conformation is the one that has both H2 and H3 axial, thus the absolute configuration (AC) has to be either (2*R*,3*R*) or (2*S*,3*S*), while the *cis*-configuration posses (2*R*,3*S*) or (2*S*,3*R*)

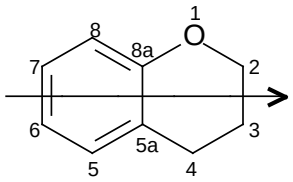
stereochemistry with H2 axial and H3 in the equatorial position **(Table 10)**. Subsequently, CD is used to determine the AC at C2 where a positive n $\rightarrow \pi^*$ CE at ca. 300 - 340 nm is indicative of a 2*R* configuration, whereas 2*S* configuration will show a negative n $\rightarrow \pi^*$ CE in that region **(Table 10)**. As for the flavanones, the sign of the n $\rightarrow \pi^*$ transition depends on the helicity of the heterocyclic ring, which in addition with the relative configuration and the equatorial orientation of the C2-aryl group establishes the AC. It should be emphasised that (2*R*,*3R*) dihydroflavonols and 2*S* flavanones are homochiral due to the change in Cahn-Ingold-Prelog priorities of the groups round the C2 chiral centre.

Table 10: Dihydroflavonol C2 and C3-geometry and configuration.

NMR: $J_{2,3}$	Result	CE at n $\rightarrow \pi^*$ (ca. 300 – 340 nm)	Result	Absolute configuration
trans	(2*R*, 3*R*) or	Positive	2*R*	(2*R*, 3*R*)
	(2*S*, 3*S*)	Negative	2*S*	(2*S*, 3*S*)
cis	(2*R*, 3*S*) or	Positive	2*R*	(2*R*, 3*S*)
	(2*S*, 3*R*)	Negative	2*S*	(2*S*, 3*R*)

4.4 Flavan-3-ols

Although flavan-3- and -4-ols do not contain a carbonyl group, the chroman chromophore **(82)** is found in these naturally occurring *O*-heterocycles and this entity can then be used for determining the AC of these compounds by CD. The achiral benzene A-ring chromophore in these compounds is chirally perturbed by the fused chiral heterocycle and the substituents attached to it. This gives rise to the observed CEs at ca. 260 - 280 nm (^1L_b band) and ca. 200 - 240 nm (^1L_a band). If the relationship between the helicity of the heterocyclic ring and the sign of the ^1L_b band is known, the chirality (conformation) of the C-ring can be deduced from the CD spectrum. This, in conjunction with the NMR coupling constants which give the relative stereochemistry of the groups attached to the C-ring, can then be used for determining the absolute configuration of the compound.

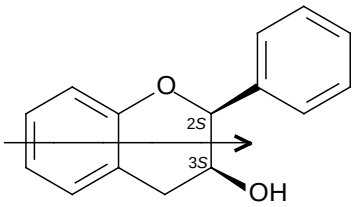


(82)

(The arrow indicates the direction of projection.)

Since the benzene rings in most of these natural products are substituted, the influence of the achiral substituents on the chiroptical properties had to be determined for each chromophore in order to be able to apply CD correctly. It was, however, found that methoxy and hydroxyl groups at C2, C3, C5 and C7 do not change the chroman helicity rule[71].

Flavan-3-ols, like catechins **(83)**, have two stereocentres and four possible diastereomers, namely, (2*R*,3*S*)-2,3-*trans*, (2*S*,3*R*)-2,3-*trans*, (2*R*,3*R*)-2,3-*cis*, and (2*S*,3*S*)-2,3-*cis* exist **(Figure 15)**.



(83)

[71] Antus, S.; Kurtán, T.; Juhász, L.; Kiss, L.; Hollósi, M.; Májer, Z. S. *Chirality* **2001**, *13*, 493-506.

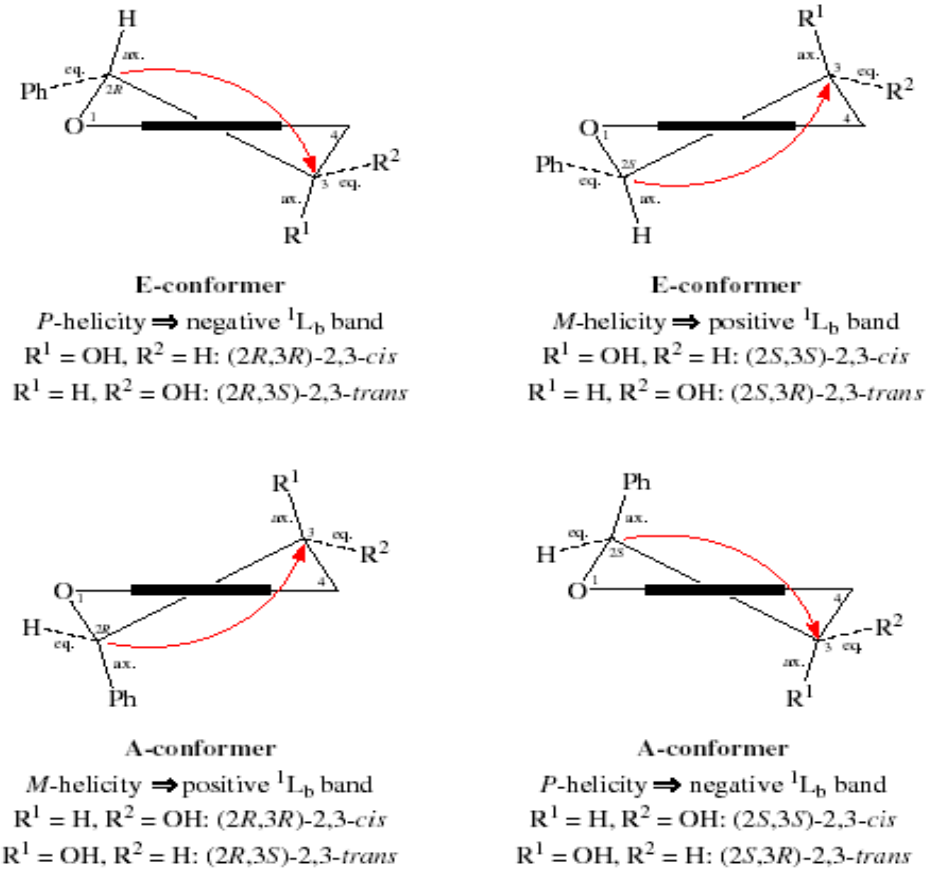


Figure 15: *P*- and *M*-helicity of the chroman C-ring of flavan-3-ols (83).

The chirality of the heterocyclic C-ring of flavan-3-ols not only determines the sign of the CE within each absorption band, but also plays a significant part in the magnitude. The chirality of the C-ring on the other hand is determined by the preference of the B-ring to be in the equatorial orientation, with the absolute configuration at the C3-OH only having a minor influence **(Figure 15)**[72]. Thus according to the helicity rule for the chroman ring system *M*-helicity and a subsequent (+)-CE in the ^1L_b band region is displayed by flavan-3-ols having a 2*S* AC. Flavan-3-ols with a 2*R* AC on the other hand display *P*-helicity and a negative CE in this region **(Figure 16)**. It should be noted that the helicity of the C-ring is inverted if the B-ring is forced into the axial orientation (A-conformer). The fact that the 3-OH group will, in this instance be, in the equatorial position for 2,3-*cis* flavan-3-ols allows the A conformer to be higher populated, which leads to a reduced magnitude for the ^1L_b band CE.

[72] Clark-Lewis, J.W. *Aust J Chem.* **1968**, *21*, 2059-2075.

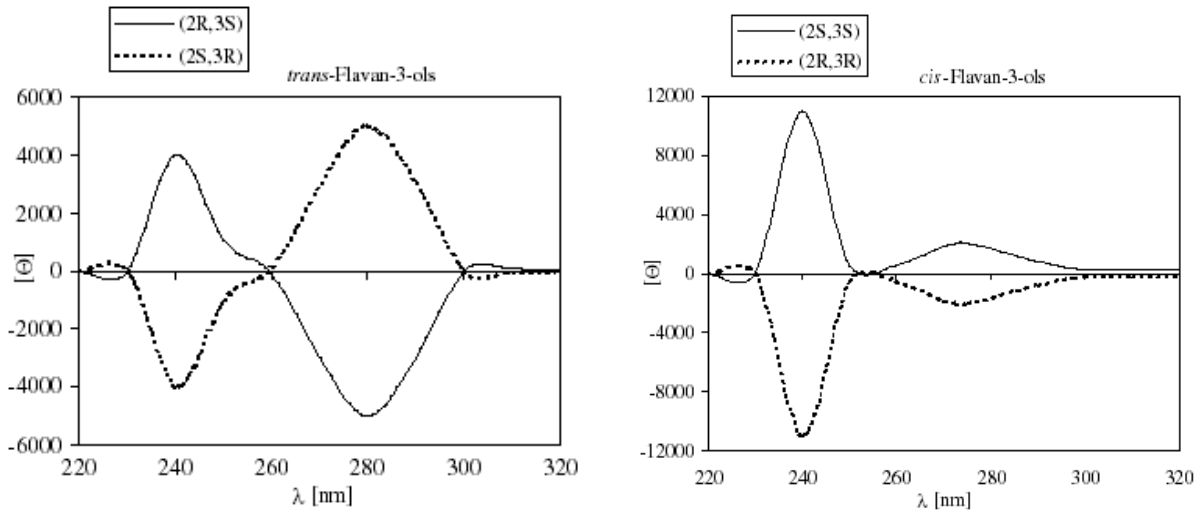
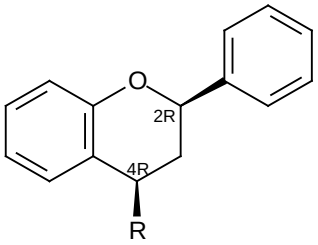


Figure 16: CD spectra of *trans*- and *cis*-flavan-3-ols.

In contrast to the ^1L_b transition (ca. 280 nm) where both sets of *cis*-and *trans*-enantiomers display opposite CEs, only the *trans* enantiomers [(2*R*,3*S*) and (2*S*,3*R*)] have CEs of opposite sign for the ^1L_a transition (ca. 240 nm). The *cis*-enantiomers [(2*R*,3*R*)- and (2*S*,3*S*)] in this instance display CEs of the same sign, thus leading to CD spectra as indicated in **figure 16**.

4.5 Flavan-4-ols

In contrast to flavan-3-ols where the half-chair is the preferred conformation of the heterocyclic C-ring, this ring of flavan-4-ols can either adopt a half-chair or sofa conformation **[(84), Figure 17 and 18]**.



(2*R*, 4*R*)-*cis*-flavan (84)

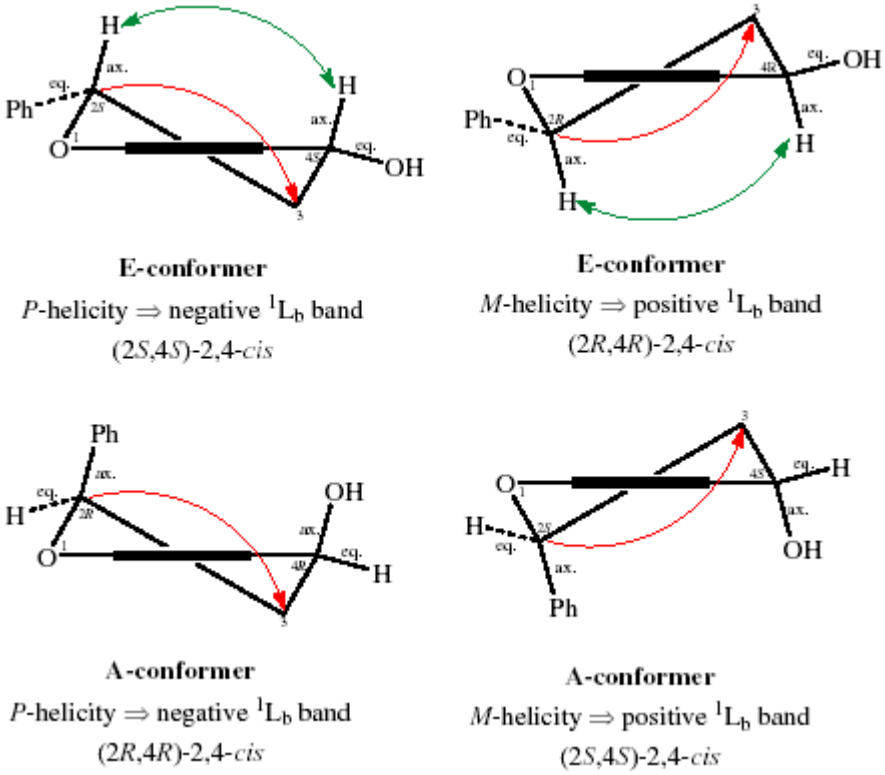


Figure 17: Half-chair conformations for 2,4-*cis*-flavan-4-ols.

With a half-chair conformation of the C-ring, however, the C4-OH of the 2,4-*cis* isomers would be forced into the unfavourable pseudo-equatorial position, while in the other possible half-chair conformation, the C2-phenyl group would adopt an equally unfavourable axial orientation **(Figure 17)**. In the sofa conformation, the C4-OH is oriented in such a way that *peri*-interaction is avoided, while the C2-phenyl group remains in the equatorial position. The sofa conformation therefore seems to be the preferred conformation for both 2,4-*cis* enantiomers **(Figure 18).**

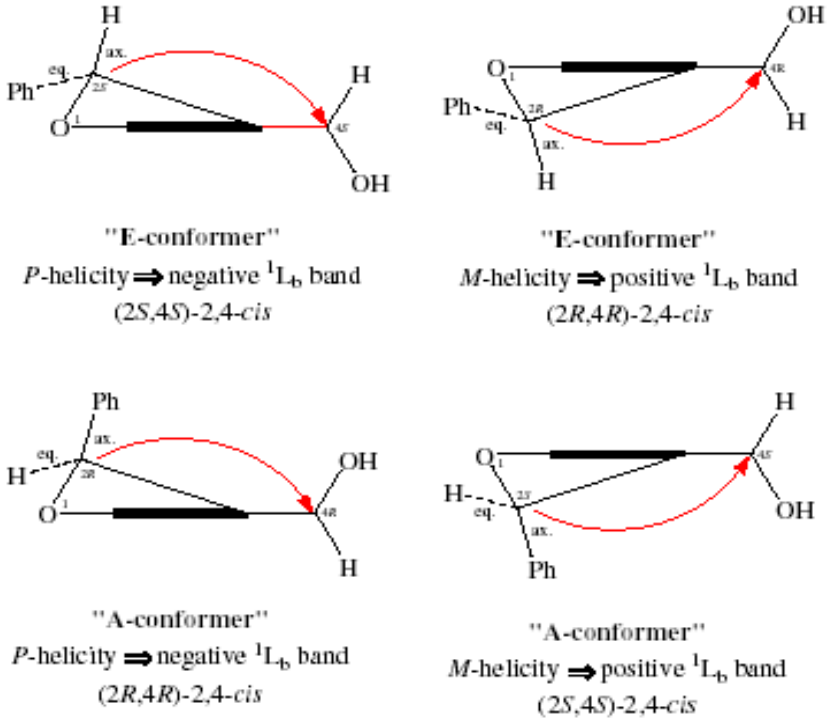


Figure 18: Sofa conformations for 2,4-*cis*-flavan-4-ols.

For the 2,4–*trans* isomers the situation is such that conformation analysis (by NMR) cannot differentiate between the sofa and half-chair conformations. While the C2-phenyl ring remains in the favourable equatorial orientation, the 4-OH now assumes a quasi-axial position in both the half-chair and sofa conformations.

In both conformations (half-chair and sofa) of both *cis*- and *trans*-flavan-4-ols, the helicity is governed by the equatorial orientation of the C2-phenyl group in both enantiomers. *P*- and *M*-helicity of the heterocyclic ring in the chroman chromophore of 2,4-*cis* isomers are reflected by negative – and positive CEs respectively within the ^1L_b band of the CD spectrum, while the opposite (*P* - positive and *M* - negative) is observed for the 2,4-*trans* isomers **(Figure 19 and 20)**.

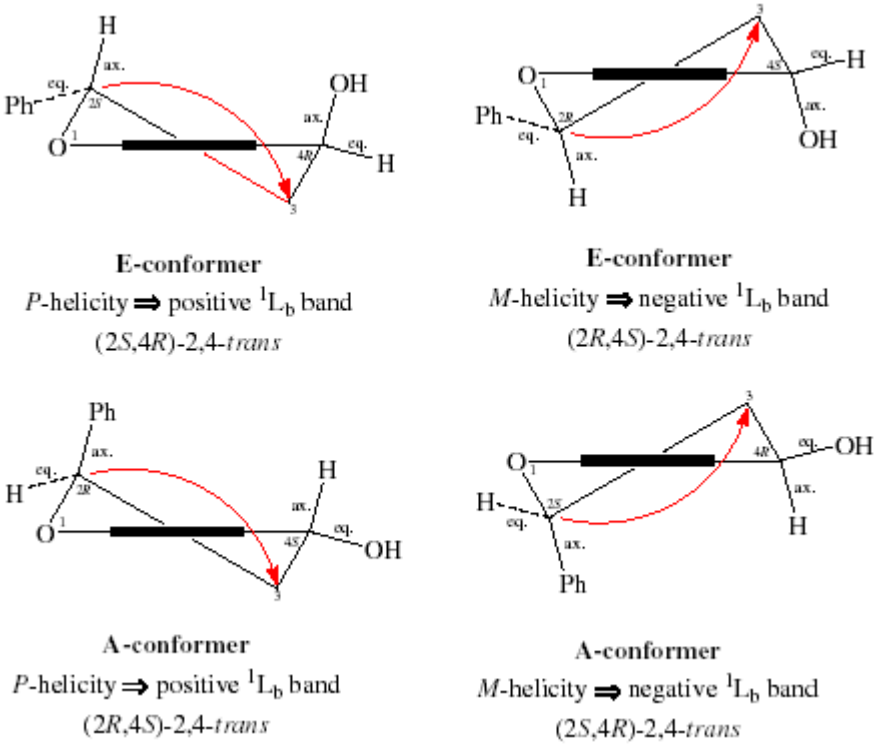


Figure 19: Half-chair conformations for 2,4-*trans*-flavan-4-ols.

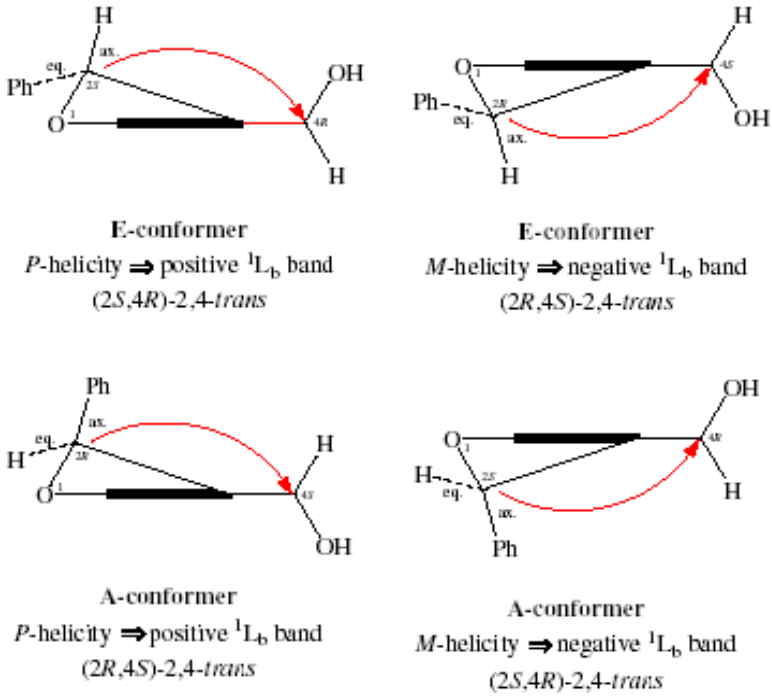


Figure 20: Sofa conformations for 2,4-*trans*-flavan-4-ols.

The final sign and amplitude of the CE of both ^1L_a and ^1L_b bands in the flavan-4-ols is also dependant on the contribution of the C4-OH. This substituent is expected to have a positive contribution to the CEs in both the ^1L_a and ^1L_b bands for the 4*R* stereoisomers and a negative contribution for the 4*S* isomers[73] **(Table 11 and Figure 21)**.

Table 11: CD spectra data of flavan-4-ols. Solvent: acetonitrile

Flavan-4-ol	λ_{max} ($\Delta\varepsilon$)
(2*R*, 4*R*)-*cis*	283 (+1.22), 276 (+1.28), 227 (+2.23)
(2*R*, 4*S*)-*trans*	282 (-1.17), 276 (-1.17), 226 (-7.62)
(2*S*, 4*R*)-*trans*	282 (+1.44), 276 (+1.41), 226 (+0.80)

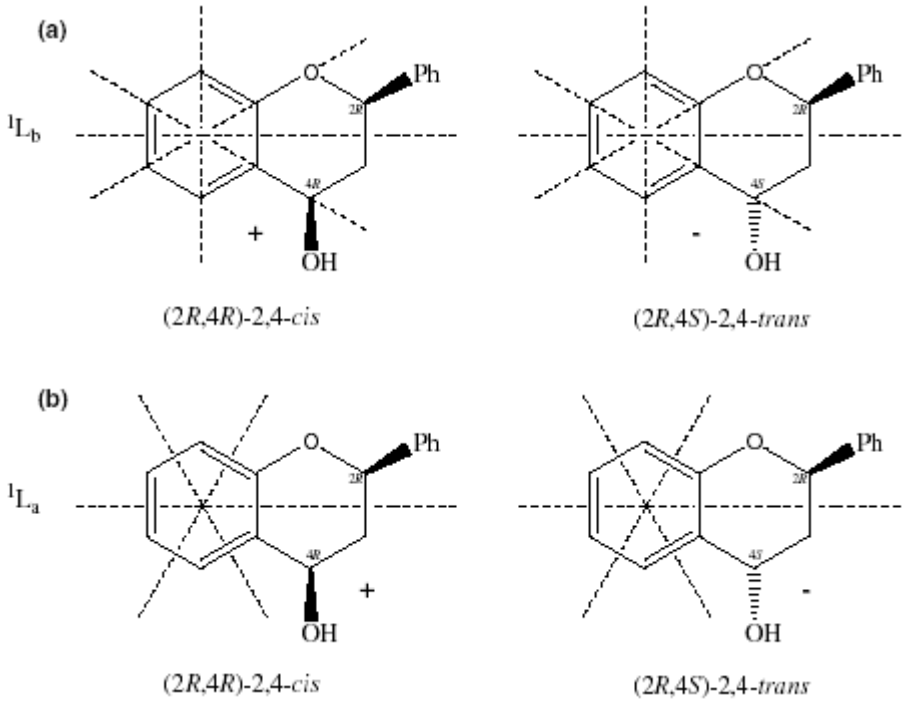
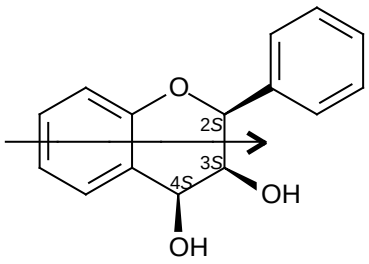


Figure 21: Sector rule for third-sphere contributions to the (a) ^1L_b and (b) ^1L_a bands.

[73] Snatzke, G.; Znatzke, F.; Tökés, A.L.; Rákosi, M.; Bognár, R. *Tetrahedron* **1973a**, *29*, 909-912.

4.6 Flavan-3,4-diols

As for the other classes of flavonoids, determination of the absolute configuration at the three stereogenic centres of flavan-3,4-diols **(85)** starts with the definition of the relative stereochemistry, which can easily be done by NMR. For most compounds the $^{3}J_{H,H}$ coupling constants of the C-ring protons give a clear indication as to the relative stereochemistry of the substituents attached to the C-ring. In cases where these values showed small differences, i.e. the 2,3-*cis*-3,4-*trans* and 2,3-*cis*-3,4-*cis* isomers, the relative stereochemistry can be confirmed by appropriate NOE experiments.



(85)

4.6.1 The $^{1}L_{b}$ transition

Similar to the flavan-3- and -4-ols, the sign and magnitude of the CE within each absorption band ($^{1}L_{a}$ and $^{1}L_{b}$) of the CD spectrum of flavan-3,4-diols are determined by the preferred conformation of the C-ring. As for the previous cases (flavan-3- and 4-ols) the preferred conformation of the C-ring would in this instance also be the half-chair/C2-sofa with the B-ring in the equatorial position. In general, this implies that *P*-helicity associated with a 2*R* configuration of the C-ring would lead to a negative $^{1}L_{b}$ CE, while *M*-helicity (2*S* configuration) would be associated with a positive CE for the same transition **(Figure 22)**. In agreement with what was concluded for the flavan-3-ols, the absolute configuration of the 3-OH group has a minor influence on the sign of the CE of the $^{1}L_{b}$ transition.

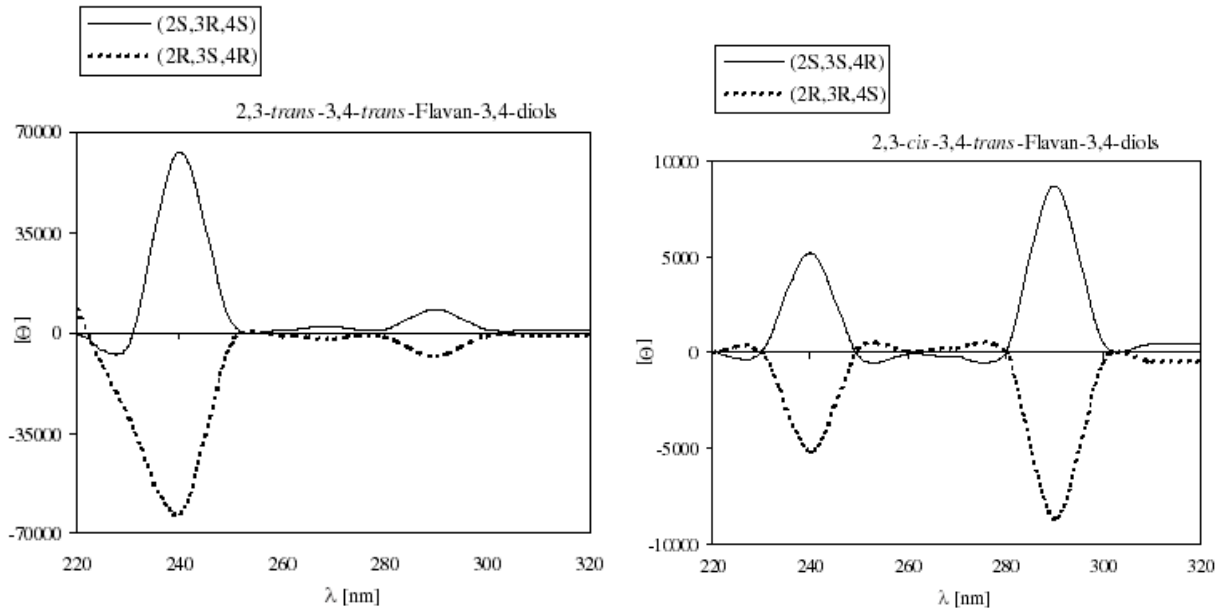
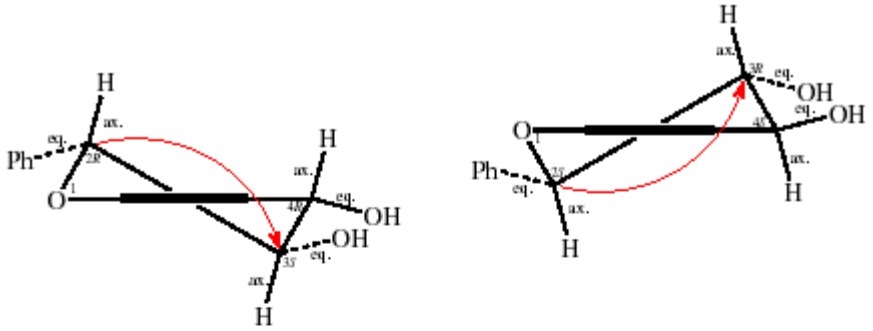


Figure 22: CD spectra of 2,3-*trans*(*cis)*-3,4-*trans*-flavan-3,4-diols.

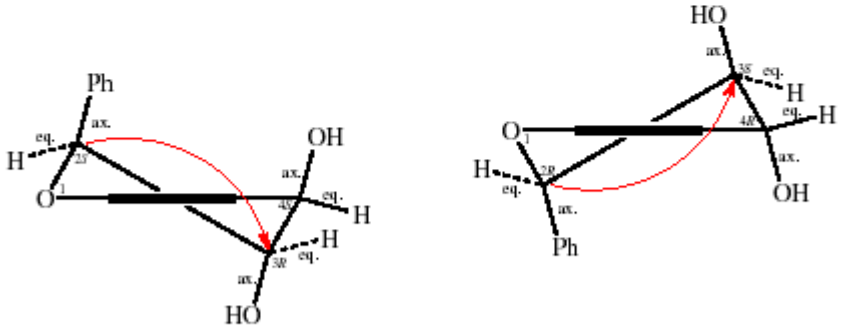
The E-conformer of the all-*trans* analogues, (2*R*,3*S*,4*R*)- and (2*S*,3*R*,4*S*)-2,3-*trans*-3,4-*trans*-flavan-3,4diol, however, experiences allylic strain between the C4-OH and H5. This results in a conformational change to alleviate the strain and leads to a relatively stable inversed half-chair/C2 sofa A-conformation [74] with *M*- as opposed to *P*-helicity for the 2*R*-all-*trans*-flavan-3,4-diols and vice versa for the 2*S* analogues **(Figure 23)**. The net effect of this conformational change is a reduced amplitude of the ^1L_b CE for the all-*trans* analogues compared to the 2,3-*trans*-3,4-*cis* compounds. The A-conformer of these flavan-3,4-diols, as well as the (2*R*,3*R*,4*R*)- and (2*S*,3*S*,4*S*)-2,3-*cis*-3,4-*cis* isomers (all the 2,4-*cis*-analogues) may be stabilised through hydrogen bonding between the C4-OH and the aromatic B-ring **(Figure 24).**

[74] Porter, L.J.; Wong, R.Y.; Benson, M.; Chan, B.G.; Vishwanadhan, V.N.; Gandour, R.D.; Mattice, W.L. *J Chem Res.* **1986**, 86-87.



E-conformer
P-helicity ⇒ negative ^1L_b band
(2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*

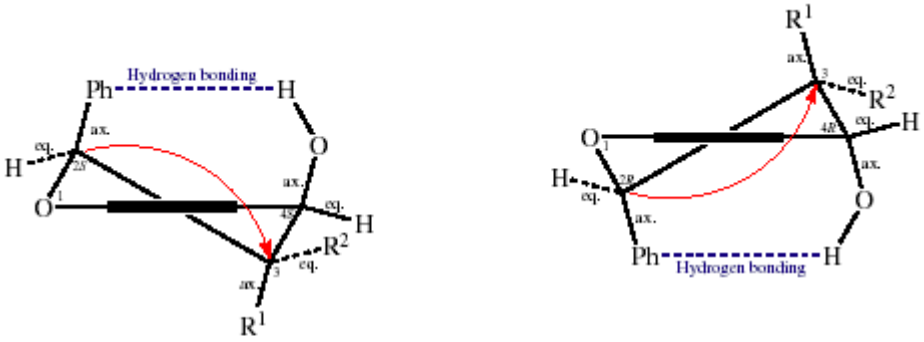
E-conformer
M-helicity ⇒ positive ^1L_b band
(2*S*,3*R*,4*S*)-2,3-*trans*-3,4-*trans*



A-conformer
P-helicity ⇒ negative ^1L_b band
(2*S*,3*R*,4*S*)-2,3-*trans*-3,4-*trans*

A-conformer
M-helicity ⇒ positive ^1L_b band
(2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*

Figure 23: A-conformer half-chair conformations for all-*trans*-flavan-3,4-diols.



A-conformer
P-helicity ⇒ negative ^1L_b band
R^1 = H, R^2 = OH: (2*S*,3*S*,4*S*)-2,3-*cis*-3,4-*cis*
R^1 = OH, R^2 = H: (2*S*,3*R*,4*S*)-2,3-*trans*-3,4-*trans*

A-conformer
M-helicity ⇒ positive ^1L_b band
R^1 = H, R^2 = OH: (2*R*,3*R*,4*R*)-2,3-*cis*-3,4-*cis*
R^1 = OH, R^2 = H: (2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*

Figure 24: A-conformation 2,4-*cis*-diaxial hydrogen bonding.

The E-conformer of the all-*cis* analogues [(2*R*,3*R*,4*R*)- and (2*S*,3*S*,4*S*) compounds] is also stabilized by hydrogen bonding between the axial C3-OH and the *O*-heteroatom of the C-ring **(Figure 25)**.

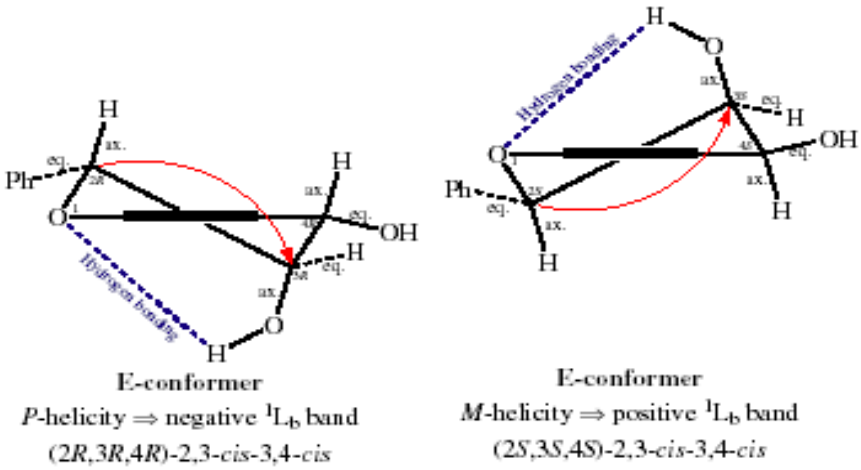


Figure 25: E-conformation all-*cis* hydrogen bonding.

4.6.2 The ^1L_a transition

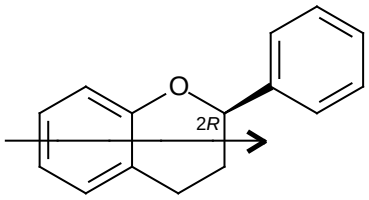
Ferreira *et al.*[75] reported the sign of the ^1L_a CE (ca 240 nm) to be the same as that of the ^1L_b CE (ca 280 nm) for the all-*trans*-, all-*cis*- and 2,3-*cis*-3,4-*trans*-flavan-3,4-diols, while the sign of the ^1L_a CE seems to be opposite to the ^1L_b CE for the 2,3-*trans*-3,4-*cis*-flavan-3,4-diols, except for 2,3-*trans*-3,4-*cis*-leucocyanidin. It was also concluded that the CEs of all the compounds under investigation did not obey the aromatic quadrant rule for correlating the sign of the ^1L_a CE with the absolute configuration at C4[76].

4.7 Flavans

Flavans are formed by a double reduction of a flavanone and their ORD and CD analysis revealed that the 2*S* absolute configuration can be assigned to all natural flavans. Like other flavonoids,

[75] Ferreira, D.; Marais, J.P.J.; Slade, D.; Walker, L.A. *Journal of Natural Products* **2004**, *67*, 174-178.
[76] DeAngelis, G.G.; Wildman, W.C. *Tetrahedron* **1969**, *25*, 5099-5112.

flavans adopt a half-chair conformation with the C2-phenyl group in an equatorial orientation and they follow the familiar rule of *P*- and *M*- helicity of the *O*-heterocyclic ring in the chroman chromophore resulting in negative - and positive CEs within the ^1L_b band respectively **(Figure 26)**. This implies that a negative ^1L_b CE is observed for 2*S* absolute configuration and a positive ^1L_b CE comes form a 2*R* configuration.



(86)

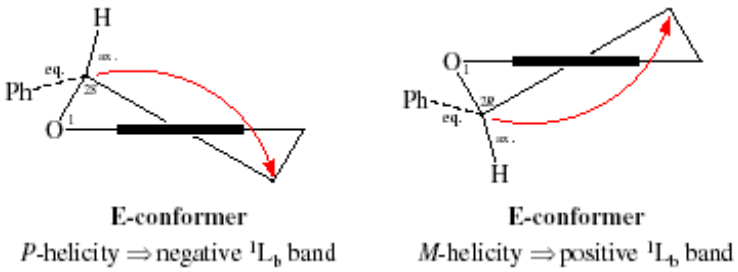
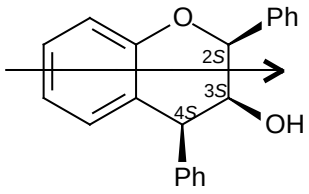


Figure 26: Flavan (86) *P*- and *M*-helicity.

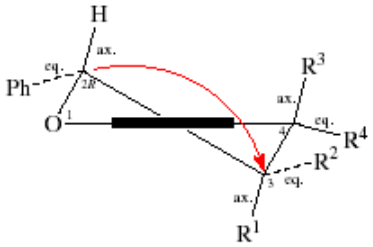
4.8 4-Arylflavan-3-ols (87)



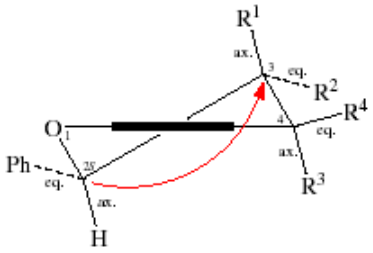
(87)

While the absolute configuration of the classes of flavonoids discussed thus far can easily be determined by application of the helicity rule and the preference of the C2-phenyl ring towards an equatorial orientation, the situation with 4-arylflavan-3-ols is much more complicated. In this

instance the preferred conformation of the C-ring is not that clear since three substituents, of which two are phenyl rings, are attached to adjacent carbon atoms and steric interaction (A- or 1,3-allylic strain) between the D-ring and a possible 5-substituentuent on the A-ring, which plays a significant role in the conformation of the heterocyclic C-ring, may be present. These facts in conjunction with the presence of another aromatic ring close to the A-ring chromophore have a profound influence on the observed Cotton effect(s). Although the helicity of the C-ring **(Figure 27 and 28)** can easily be concluded, if the preferred conformation of the C-ring (half-chair or C2 sofa) is known, very few reports where the CE has been related to the helicity is found in literature. In contrast to this, several workers utilised the aromatic quadrant rule[77] **(Figure 29)** in their efforts to relate the 220 to 240 nm CE with the absolute configuration of 4-arylflavan-3-ols.



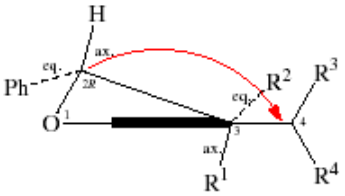
C2/C3	C3/C4	C2	C3	C4	R^1	R^2	R^3	R^4	Helicity
cis	*cis*	*R*	*R*	*R*	OH	H	H	Ph	*P*
trans	*trans*	*R*	*S*	*R*	H	OH	H	Ph	*P*
cis	*trans*	*R*	*R*	*S*	OH	H	Ph	H	*P*
trans	*cis*	*R*	*S*	*S*	H	OH	Ph	H	*P*



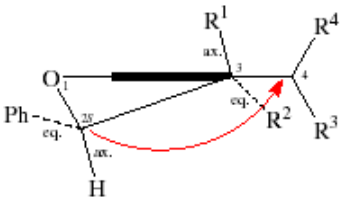
C2/C3	C3/C4	C2	C3	C4	R^1	R^2	R^3	R^4	Helicity
cis	*cis*	*S*	*S*	*S*	OH	H	H	Ph	*M*
trans	*trans*	*S*	*R*	*S*	H	OH	H	Ph	*M*
cis	*trans*	*S*	*S*	*R*	OH	H	Ph	H	*M*
trans	*cis*	*S*	*R*	*R*	H	OH	Ph	H	*M*

Figure 27: Half-chair conformation for 4-arylflavan-3-ols.

[77] DeAngelis, G.G.; Wildman, W.C. *Tetrahedron* **1969**, *25*, 5099-5112.



C2/C3	C3/C4	C2	C3	C4	R^1	R^2	R^3	R^4	Helicity
cis	*cis*	*R*	*R*	*R*	OH	H	H	Ph	*P*
trans	*trans*	*R*	*S*	*R*	H	OH	H	Ph	*P*
cis	*trans*	*R*	*R*	*S*	OH	H	Ph	H	*P*
trans	*cis*	*R*	*S*	*S*	H	OH	Ph	H	*P*



C2/C3	C3/C4	C2	C3	C4	R^1	R^2	R^3	R^4	Helicity
cis	*cis*	*S*	*S*	*S*	OH	H	H	Ph	*M*
trans	*trans*	*S*	*R*	*S*	H	OH	H	Ph	*M*
cis	*trans*	*S*	*S*	*R*	OH	H	Ph	H	*M*
trans	*cis*	*S*	*R*	*R*	H	OH	Ph	H	*M*

Figure 28: Sofa conformation for 4-arylflavan-3-ols.

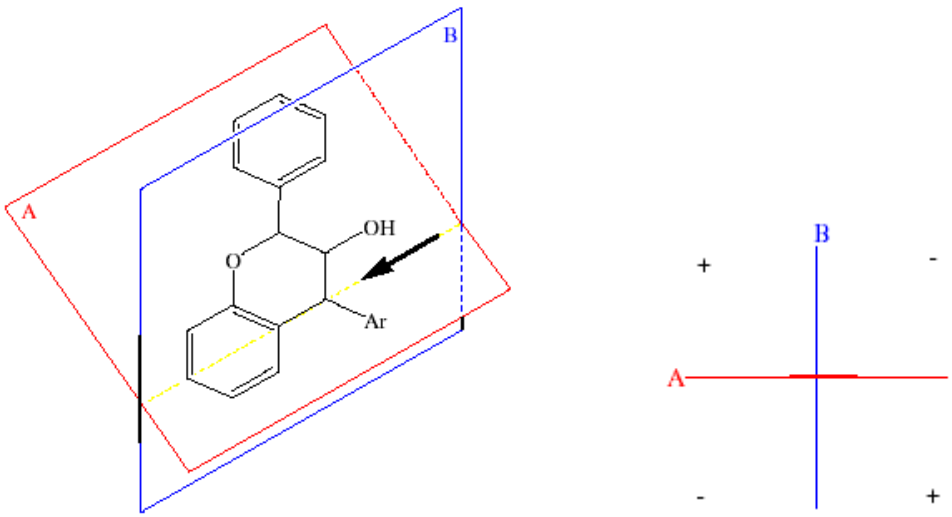
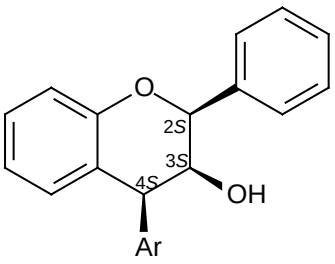
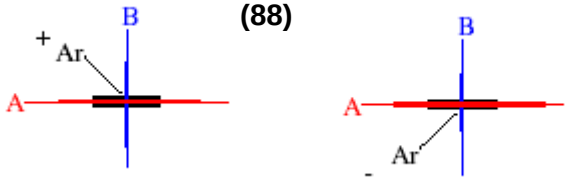


Figure 29: Aromatic quadrant rule.

Studies on a series of 2,3-*trans*- and 2,3-*cis*-4-arylflavan-3-ols where phloroglucinol and resorcinol were coupled to flavan-3,4-diols of known absolute configuration in indicated the CD spectra of these compounds to be dominated by multiple CEs contributed by the C4-aryl chromophore when compared to the CEs of flavan-3-ols[78]. The CD curves of the methyl ether 3-acetates of these compounds further indicated that CEs due to chirality at C2 and C3 are completely dominated by the high amplitude CEs of the C4-aryl chromophore (220 - 240 nm). Through application of the aromatic quadrant rule these workers determined that a positive CE represents a quasi-axial (extending above the plane of the A-ring) C4-aryl group and a negative CE indicates a quasi-equatorial (below the plane of the A-ring) C4-aryl group **(Figure 30)**.



(88)



Positive CE	**Negative CE**
(2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*cis*	(2*S*,3*R*,4*R*)-2,3-*trans*-3,4-*cis*
(2*R*,3*R*,4*S*)-2,3-*cis*-3,4-*trans*	(2*S*,3*S*,4*R*)-2,3-*cis*-3,4-*trans*
(2*S*,3*R*,4*S*)-2,3-*trans*-3,4-*trans*	(2*R*,3*S*,4*R*)-2,3-*trans*-3,4-*trans*

Figure 30: The aromatic quadrant rule for 2,3-*trans*-3,4-*trans*-, 2,3-*trans*-3,4-*cis*- and 2,3-*cis*-3,4-*trans*-4-arylflavan-3-ols.*

CD data of the methyl ether acetates of 2,3-*cis*-3,4-*cis*-4-arylflavan-3-ols and the catechin-4α-phloroglucinol analogue with abnormal coupling constants ($J_{2,3}$ 6.5, $J_{3,4}$ 5.5 Hz), however,

[78] Botha, J.J.; Ferreira, D.; Roux, D.G. *J Chem Soc.,* Chem Commu. **1978**, *16*, 698-700.

* Care should be taken with assignment of *R* and *S* absolute configuration at C4, since substituents on the D-ring influences the CIP priorities.

display inverse CEs to the above rule[79]. This indicates that deviations form the normal dihedral angles between substituents on the heterocyclic ring and therefore form the expected half-chair conformation significantly influences the sign of the CE. While these abnormal CEs were originally explained by invoking boat-conformations for these compounds **(Figure 31)**, modelling studies[80] indicated profound contributions by A-conformers to the C-ring conformation of isomers with a 2,4-*cis*-relative configuration when compared to 2,4-*trans*- isomers **(Figure 32)**. The CD data of 5-deoxy analogues do not show these irregularities indicating the predominance of the E-conformers and the absence of A-strain for both the 2,4-*trans*- and 2,4-*cis*-isomers. Although 1,3-diaxial arrangements are generally avoided on energetic grounds, the stability of A- relative to E-conformers for the 2,4-cis-sisomers appears to be an exception by virtue of π-stacking between the C2 and C4 aromatic rings **(Figure 33)**.

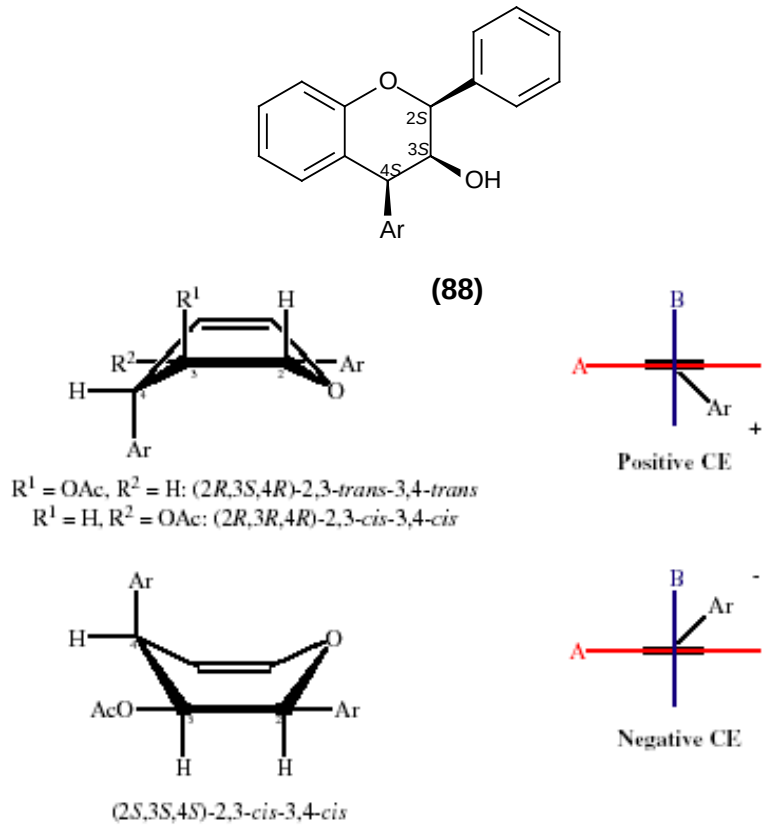


Figure 31: Boat-conformations for 2,3-*cis*-3,4-*cis*-4-arylflavan-3-ols and the catechin-4α-phloroglucinol analogue.

[79] Van der Westhuizen, J.H.; Ferreira, D.; Roux, D.G. *J Chem. Soc.*, Perkin Transactions 1 **1981**, 1220-1226.
[80] Steynberg, J.P.; Brandt, E.V.; Ferreira, D. *J Chem. Soc.*, Perkin Transactions 2, **1991**, 1569-1573.

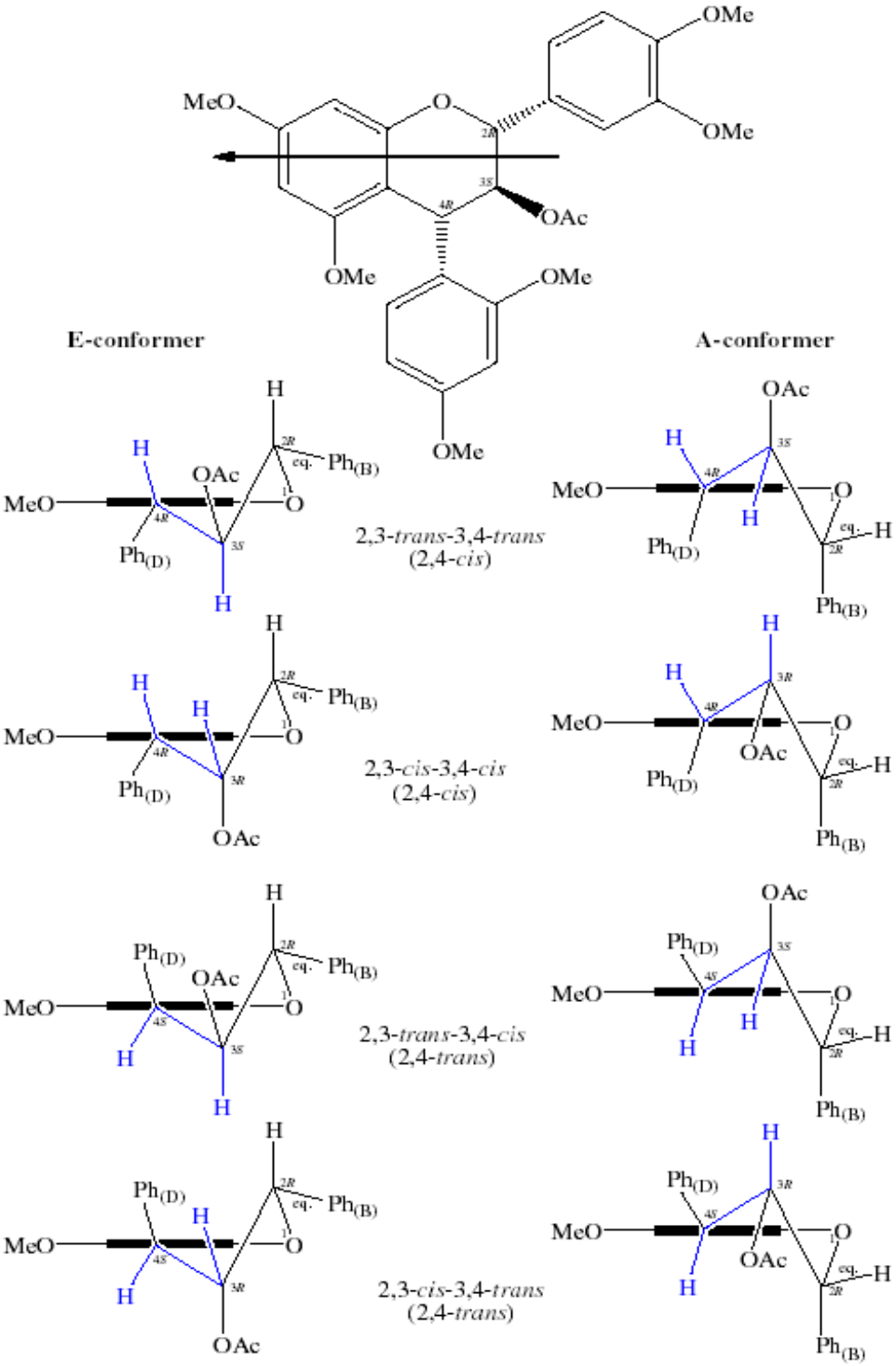


Figure 32: E- and A-conformations.

(H3-C3-C4-H4 angles indicated in blue.)

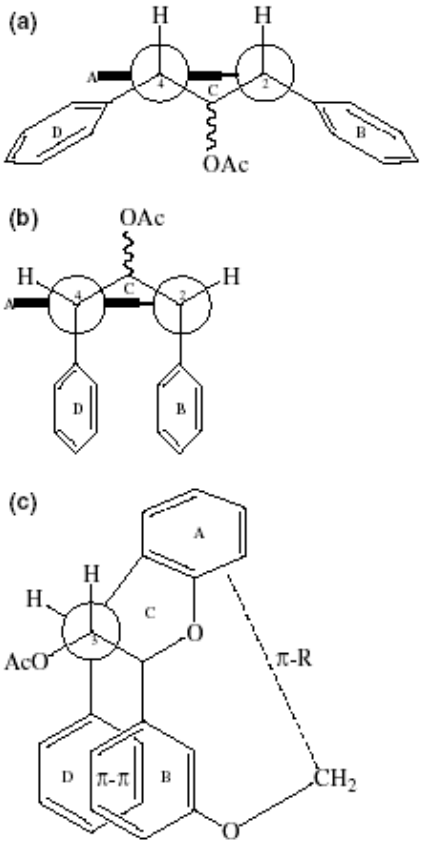


Figure 33: 2,4-*cis*-4-Resorcyl-5-oxyflavan-4-ols. (a) E-conformer viewed along the C2-O1 and C4-C5a bonds, (b) A-conformer viewed along the C2-O1 and C4-C5a bonds, and (c) A-conformer viewed along the C3-C4 bond.

Finally, it has to be pointed out that Van Zyl *et al.*[81] found that the CE at 220 – 240 nm of a (2*R*,3*S*)-2,3-*trans*-3,4-*trans*flavn-3-ol changes form negative for the free phenolic form to positive for the methyl ether acetate of the same compound.

4.9 Conclusion

Although CD has found wide application in the determination of the absolute configuration of different classes of flavonoids, this technique is to a large extent dependant on the conformation of the heterocyclic C-ring of the specific flavonoid. As was clearly shown in particular for the 4-arylflavan-3-ols, that the conformation of the C-ring can change dramatically with small changes

[81] Van Zyl, P.W.; Steynberg, J.P.; Brandt, E.V.; Ferreira, D. *Magnetic Resonance in Chemistry* **1993**, *31*, 1057-1063.

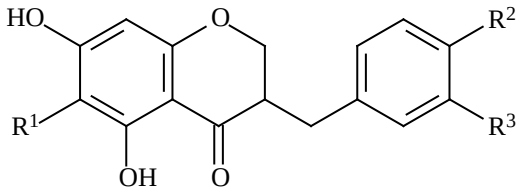
in the relative orientation of the substituents attached to this ring and/or substituents on the A-ring of the flavonoid. The fact that the NMR coupling constants, which are used to determine the relative stereochemistry, and the interpretation of the CE spectrum depend on the same phenomenon, i.e. conformation of the C-ring, casts some doubt as to the accuracy of results obtained in this way. If some uncertainty in this regard exists for the monomeric flavonoids, it has to be pointed out that the situation would be worse for the oligomeric analogues where two or more heterocyclic rings, each with its own conformation and absolute configuration at least two chiral centres, are present in the molecule. Methodology or a technique that would allow the assessment of the absolute configuration at each chiral centre independently would therefore make an important contribution to this area of natural product research.

CHAPTER 5

5. Isolation and characterization of compounds from *Scilla natalensis*

5.1 Introduction

Scilla natalensis planch is one of the plants that are widely used in traditional medicines and it grows naturally over large parts of Kwazulu-Natal, the eastern Free State and Gauteng in South Africa, as well as Lesotho and Swaziland. From phytochemical investigations, Jäger *et al.*[82] found this plant to contain a variety of biologically active compounds that show anti-inflammatory, antibacterial, antischistosomic, anthelmintic and cytotoxicity activity. Apart from flavonoids, like two homoisoflavonoids **(89 and 90) (Figure 34)**, compounds such as saponins[83] and cardiac-glycosides (bufadienolides)[84] were also found in the bulbs of the plant.



Cpd	R^1	R^2	R^3
(89)	OCH_3	OH	H
(90)	OCH_3	OCH_3	OH

Figure 34: Homoisoflavonoids isolated from *Scilla natalensis* by Crouch *et al*.[85]

[82] Sparg, S.G.; van Staden, J.; Jäger, A.K. *Journal of Ethnopharmacology* **2002**, *80*, 95.
[83] Hutchings, A.; Haxton Scott, A.H.; Lewis, S.G.; Cunningham, A. **1996**, Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg.
[84] van Wyk, B.E.; van Oudtshoorn, B.; Gericke, N. **1997**, Medicinal Plants of South Africa. Briza Publications, Pretoria, 232.
[85] Crouch, N.R.; Bangani, V.; Mulholland, D.A. *Phytochemistry* **1999**, *51*, 943.

Despite numerous phytochemical investigations into the chemical components of this plant, it was felt that due to the renewed interest in the medicinal properties of natural products, it would be worth a while to revisit the chemical constituents of *Scilla natalensis*. The methanol extract of two parts of this plant, i.e. above soil growth (1-AS) and root & bulb (2-RB), were obtained from Prof. Pretorius at the Department of Oil, Crop and Climate Science of this University. After acetylation of fraction E of the 1-AS methanol extract of this plant, seven compounds, (a) – (g), were isolated as the peracetates. While compounds (c) and (e) were obtained from a natural source for the first time, (a), (b), (d), (f) and (g) were isolated from *Scilla natalensis* for the first time during the current study.

(a) 3',4'-Di-*O*-acetylchavicol **(91)**

(b) 4',4''-Di-*O*-acetyl-3''-methoxynyasol **(92)**

(c) 5,7-Diacetoxy-3-(4'-methoxybenzyl)-6-hydroxychroman-4-one **(93a)** or
5,6-diacetoxy-3-(4'-methoxybenzyl)-7-hydroxychroman-4-one **(93b)**

(d) 5,7-Diacetoxy-3-(3'-acetoxy-4'-methoxybenzyl)-chroman-4-one **(95)**

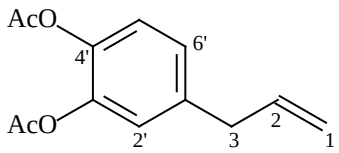
(e) 5,6,7-Triacetoxy-3-(3',4'-dimethoxybenzyl)-chroman-4-one **(96)**

(f) 4'-*O*-acetyl-5,7-di-*O*-methylnaringenin **(97)**

(g) 2'',3'',4'',5,6''-Penta-*O*-acetyl-4'-*O*-methylapigenin-7-*O*-*β*-D-glucopyranoside **(98)**

Although all four of the homoisoflavanones mentioned above contain a stereogenic centre at C-3, none was isolated in optically active form. This result is probably explicable in terms of the pyridine used during acetylation, which caused racemization to occur at the carbon α to the C-4 carbonyl function.

5.2 3’,4’-Di-*O*-acetylchavicol (91)



91

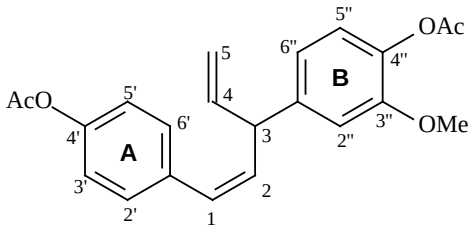
The title compound, 3’,4’-di-*O*-acetylchavicol **(91)**, was obtained as a light yellow solid[86] (3.6 mg, R*f* 0.56). The ^{1}H NMR spectrum **(Plate 1a, Table 12)** of compound **(91)** obtained as the peracetate, displayed the typical aromatic ABX spin-system of a 1,3,4-trisubstituted aromatic ring as well as five non-aromatic protons, three of which corresponded to the characteristic chemical shift values of a 3-substituted allyl system. These resonances were accompanied by the signals from two acetoxy groups thus indicating the isolated compound to be 3’,4’-di-*O*-acetylchavicol **(91)**. The structure of 3’,4’-di-*O*-acetylchavicol **(91)**, found in *Scilla natalensis* for the first time, was confirmed by ^{13}C NMR **(Plate 1b)** and coupling between proton 3 and carbons 1, 1’, 2, 2’, and 6’ in the HMBC spectrum **(Plate 1c)**. The free phenolic form of 3’,4’-di-*O*-acetylchavicol, eugenol, was previously isolated by Lee-Chen *et al.*[87] from betel and bay leaves.

[86] Shenoy, N.R.; Choughuley, A.S.U. *J. Agric. Food Chem.* **1989**, *37*, 721.
[87] Lee-Chen, S.F.; Chen, C.L.; Ho, L.Y.; Hsu, P.C.; Chang, J.T.; Sun, C.M.; Chi, C.W.; Liu, T.Y. *Mutagenesls* **1996**, *11*, 519.

Table 12: NMR data of 3',4'-di-*O*-acetylchavicol.

Proton, ^{1}H		Carbon, ^{13}C		HMBC
No.	**δ_H (ppm)**	**No.**	**δ_C (ppm)**	**^{2}J and ^{3}J (H→C)**
1	5.10 (d, *J* 1.0 Hz)	1	116.7	
1	5.15 (dd, *J* 7.0 and 1.0 Hz)			
2	6.0 - 5.9 (m)	2	136.4	
3	3.40 (d, *J* 7.0 Hz)	3	39.5	C-1, C-2', C-6', C-2, C-1'
1'	-	1'	138.9	
2'	7.03 (d, *J* 2.1 Hz)	2'	123.1	
3'	-	3'	141.9	
4'	-	4'	140.8	
5'	7.13 (d, *J* 8.4 Hz)	5'	123.4	
6'	7.09 (dd, *J* 8.4 and 2.1 Hz)	6'	126.7	
$COCH_3$ (C3')	2.30	$COCH_3$ (C3')	20.1	
		$COCH_3$ (C3')	168.4	
$COCH_3$ (C4')	2.30	$COCH_3$ (C4')	20.1	
		$COCH_3$ (C4')	168.3	

5.3 4',4''-Di-*O*-acetyl-3''-methoxynyasol (92)



92

In addition to 3',4'-di-*O*-acetylchavicol **(91)**, the methanol extract also contained compound **(92)**, a coniod or norlignan, as a light yellow solid[88] (5.8 mg, R*f* 0.42). The ^{1}H NMR spectrum **(Plate 2a, Table 13)** of **(92)** displayed the signals from seven aromatic, five olefinic, and one allylic protons in addition to two aromatic acetoxy groups and one methoxy entity. The resonances from the

[88] Zhang, H.J.; Sydara, K.; Tan, G.T.; Ma, C.; Southavong, B.; Soejarto, D.D.; Pezzuto, J.M.; Fong, H.H.S. *J. Nat. Prod*. **2004**, *67*, 194.

aromatic protons resembled those of a typical AA'BB' spin system for the A-ring and an ABX spin system for the B-ring. The carbon skeleton of the compound was confirmed by the ^{13}C NMR spectrum **(Plate 2b)**, which showed the presence of 22 carbons of which 16 were in the aromatic region, accounting for the A- and B-rings as well as four alkene carbons. Two carbonyl carbon signals, together with two alkyl carbon signals, account for the two acetoxy groups, whereas the signals at δ 47.4 ppm and δ 55.9 ppm could be assigned to C-3 and the OMe-group, respectively.

^{1}H-^{1}H coupling constants and the ^{1}H-^{1}H COSY spectrum **(Plate 2e)** confirmed the low field alkene proton H-1 (δ_H 6.62, d, *J* 11.0 Hz) to be coupled to H-2 (δ_H 5.80, t, *J* 11.0 Hz), whereas H-2 showed coupling to both H-1 and H-3 (δ_H 4.54, m). H-3 coupled to both H-2 and H-4 (δ_H 6.05, m), whereas H-4 coupled to both H-3 and two doublets assigned to the 5 protons (δ_H 5.22 and δ_H 5.24, *J* 5.6 and 1.4 Hz); thus defining the aliphatic part of the molecule as a vinyl substituted propene moiety. The HMBC spectrum **(Plate 2c)** confirmed the allocation of H-3 and placement of the B-ring by correlation of C-3 to H-1, H-5, H-2'' and H-6'' over three bonds and of C-3 to H-2 and H-4 over two bonds. HMBC ^{3}J correlations of H-2 to C-1', C-4 and C-1'' confirmed the allocation of H-2 as well as attachment of the A-ring to C-1, while the ^{2}J correlations of H-5 to C-4 supports the assignment of H-5 **(Figure 35)**.

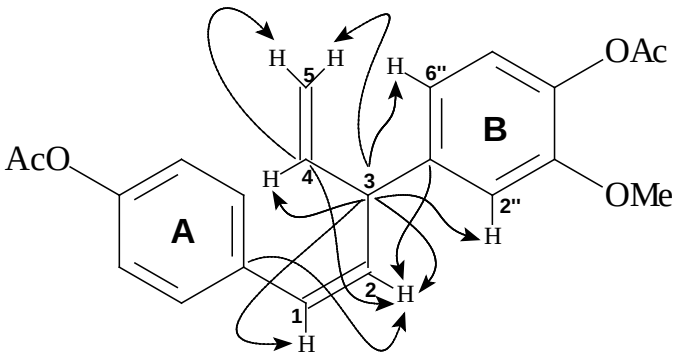


Figure 35: Relevant HMBC correlations for 4',4''-di-*O*-acetyl-3''-methoxynyasol.

In the NOESY spectrum **(Plate 2f)**, strong association between the aromatic proton, H-2'', and the methoxy group confirmed attachment of the latter to C-3" of the B-ring. The assignment of H-3 was confirmed by association from H-3 to H-6'' and H-6'. A weak association between protons H-5 and H-6' confirmed the allocation of C-4 and C-5 in previous experiments **(Figure 36)**.

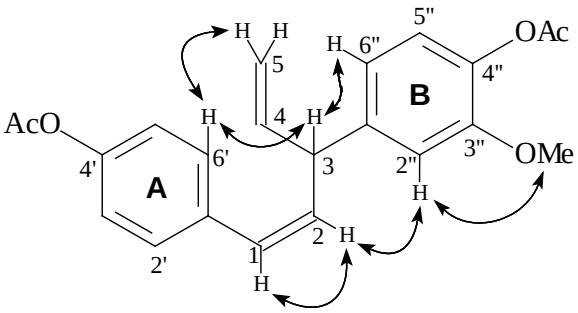


Figure 36: Relevant NOESY correlations for 4',4''-di-*O*-acetyl-3''-methoxynyasol.

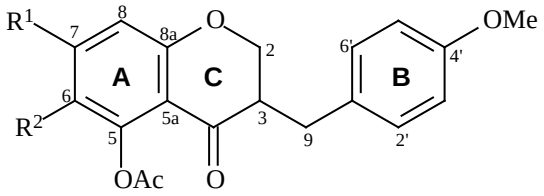
The above NMR data thus supports the structure of the isolated compound to be that of 4',4''-di-*O*-acetyl-3''-methoxynyasol **(92)** and is also corroborated by NMR data of a similar compound, (-)-4'-*O*-methylnyasol.[89] This C_{17}-phenolic compound was first isolated by Erdtman and Vorbrüggen in 1960 from the Tasmanian conifer *Athrotaxis selaginoides.*[90]

Table 13: NMR data of 4',4''-di-*O*-acetyl-3''-methoxynyasol.

[89] Su, B.N.; Zhu, Q.X.; Jia, Z.J. *Phytochemistry* **2000**, *53*, 1103.

Proton, ^{1}H		Carbon, ^{13}C		HMBC
No.	**δ_H (ppm)**	**No.**	**δ_C (ppm)**	**^{2}J and ^{3}J (H→C)**
1	6.62 (d, *J* 11 Hz)	1	128.9	C-3, C-2’ (C-6’), C-1’
2	5.80 (dd, *J* 10 and 11 Hz)	2	132.8	C-3, C-1’, C-4, C-1’’
3	4.54 (m)	3	47.4	C-4, C-1’’
4	6.05 (m)	4	140.0	
5	5.24 (dd, *J* 5.6 and 1.4 Hz)	5	115.9	C-3, C-4
5	5.22 (d, *J* 1.4 Hz)			
1’	-	1’	134.7	
2’	7.32 (d, *J* 8.6 Hz)	2’	129.9	C-3’ (C-5’), C-1, C-4’
3’	7.07 (d, *J* 8.6 Hz)	3’	121.6	C-3’ (C-5’), C-1’, C-4’
4’	-	4’	149.7	
5’	7.07 (d, *J* 8.6 Hz)	5’	121.6	C-3’ (C-5’), C-1’, C-4’
6’	7.32 (d, *J* 8.6 Hz)	6’	129.9	C-3’ (C-5’), C-1, C-4’
1’’	-	1’’	142.0	
2’’	6.84 (d, *J* 1.8 Hz)	2’’	119.9	
3’’	-	3’’	151.1	
4’’	-	4’’	138.4	
5’’	6.98 (d, *J* 8.6 Hz)	5’’	122.8	C-4’’, C-1’’, C-3’’
6’’	6.84 (dd, *J* 8.6 and 1.8 Hz)	6’’	112.1	C-3, C-6’’, C-2’’, C-5’’, C-4’’, C-1’’, C-3’’
$COCH_3$ (C4’)	2.30	$COCH_3$ (C4’)	20.7	
		$COCH_3$ (C4’)	169.2	
$COCH_3$ (C4’’)	2.30	$COCH_3$ (C4’’)	21.1	
		$COCH_3$ (C4’’)	169.6	
OCH_3	3.79	OCH_3	55.9	

5.4 5,7-Diacetoxy-3-(4’-methoxybenzyl)-6-hydroxychroman-4-one (93a) or 5,6-diacetoxy-3-(4’-methoxybenzyl)-7-hydroxychroman-4-one (93b)



Cpd	R^1	R^2
93a	OAc	OH
93b	OH	OAc

[90] Norin, T. *in Natural Products of Woody Plants I*, Springer-Verlag, Berlin Heidelberg, New York, **1989**, 517.

The title compound **(93a or b)** (R*f* 0.58) was isolated as a *light yellow solid* (2.6 mg). The ^{1}H NMR spectrum **(Plate 3a, Table 14)** displayed a singlet, allocated to H-8 (δ 6.37 ppm) of the A-ring, while the B-ring was characterised by an AA'BB' spin-system and thus *para*-substitution. The spectrum also showed two acetoxy- , one hydroxy- (δ 6.36 ppm) and one methoxy signals. In addition to the three heterocyclic protons of a flavanone or isoflavanone, the heterocyclic region of the spectrum also contained an additional methylene system indicating the compound to be a homoisoflavanone. The multiplet assigned to proton H-3 (δ 2.84, m) of the C-ring, showed coupling to proton H-2 (δ 4.44, dd, *J* 11 and 4 Hz), H-2 (δ 4.24, dd, *J* 11 and 8 Hz) as well as the exocyclic-CH_2 protons [(δ 3.29, dd, *J* 14 and 4 Hz, H-9) and (δ 2.66, dd, *J* 14 and 11 Hz, H-9)]; thus confirming the homoisoflavonoid nature of the product.

The homoisoflavonoid skeleton of **(93)** was confirmed by the ^{13}C NMR **(Plate 3b, Table 14)** and HMBC spectra **(Plate 3d)**, which revealed a carbonyl carbon at δ 191.1, two C=O carbons from acetoxy groups (δ 170.6 and δ 170.3) and seven non-hydrogen substituted carbons C-5 (δ 155.6), C-8a (δ 155.3), C-4' (δ 150.1), C-6 (δ 147.8), C-1' (δ 136.2), C-7 (δ 133.0) and C-5a (δ 108.5) in the aromatic region. Two sets of equivalent CH-carbons C-2' and C-6' (δ 130.5) and C-3' and C-5' (δ 122.2) and another CH carbon C-8 (δ 105.0) could also be distinguished. C-2 (δ 70.2), C-3 (δ 48.4), C-9 (δ 32.3), the methoxy carbon (δ 62.0) and the methyl carbons from two acetoxy groups (δ 21.7) resonated in the non-aromatic region of the spectrum. The assignment of C-5a (δ 108.5) and C-8a (δ 155.3) were based on results published by Silayo *et al*.[91] for homoisoflavanone **(95)** (*vide infra*).

In the NOE spectrum **(Plate 3c)**, the strong association observed between the heterocyclic protons H-2 and H-3, as well as the relation between H-2 and H-9, supported the assignment of H-2. Protons H-2' and H-6' on the B-ring showed association with protons H-2, H-3 as well as H-9, the latter supporting assignment of the methylene bridge protons **(Figure 37)**. An NOE effect

[91] Silayo, A.; Ngadjui, B.T.; Abegaz, B.M. *Phytochemistry* **1999**, *52*, 947.

between the methoxy group and H-3',5' confirmed the position of the methoxy group as being on the B-ring and thus the acetoxy - and hydroxy groups to be attached to ring A. While the exact positions of the two acetoxy groups on the A-ring could not be determined, it was assumed that either the 6- or 7 hydroxyl function was not acetylated during the isolation process, since no evidence of a low field hydrogen bonded OH could be picked up in the ^{1}H-NMR spectrum of **(93)**. Since the compound probably existed in the plant as the free phenol and re-acetylation confirmed the presence of third hydroxyl function [triacetate **(94)**, **Plate 3g, Table 15**], it was considered not crucial to determine the exact position of the remaining free OH group at this stage.

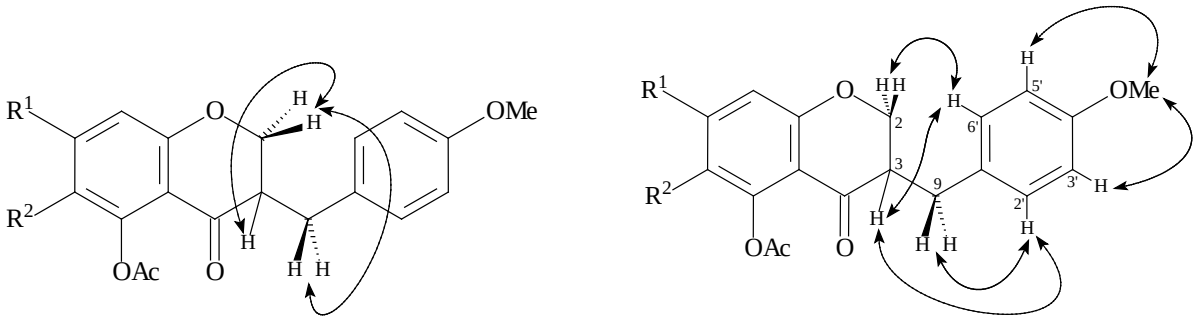


Figure 37: Relevant NOE associations for (93).

HMBC correlations **(Plate 3d)** ^{3}J of H-8 to C-5a and C-6 and ^{2}J of H-8 to C-8a and C-7 confirmed assignment of H-8, while, the correlations of H-9 to C-1', C-2', C-6', C-3, C-2 and C-4 supported the assignment of H-9. The ^{3}J correlations of H-3 to C-1' and ^{2}J of H-3 to C-9 corroborated the allocation of H-3 and the assignment of H-2 was confirmed by the correlations ^{3}J of H-2 to C-4, C-8a and C-9 and ^{2}J of H-2 to C-3 **(Figure 38)**.

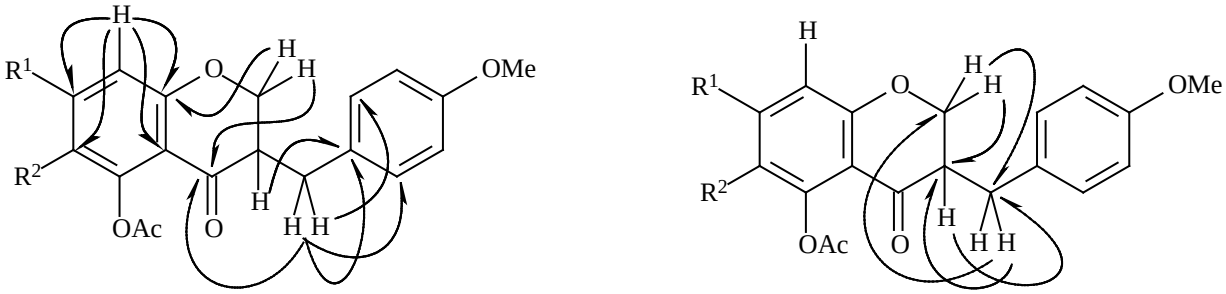


Figure 38: Relevant HMBC correlations for (93).

Through HMQC correlations **(Plate 3e)**, the carbon at δ_C 105.0 could be assigned to carbon 8 and the shielded carbons at δ_C 70.2, δ_C 48.4 and δ_C 32.3 to C-2, C-3 and C-9 respectively. The equivalent carbons (C-2' and C-6') resonated at δ_C 130.5 and C-3' and C-5' at δ_C 122.2 **(Figure 39)**.

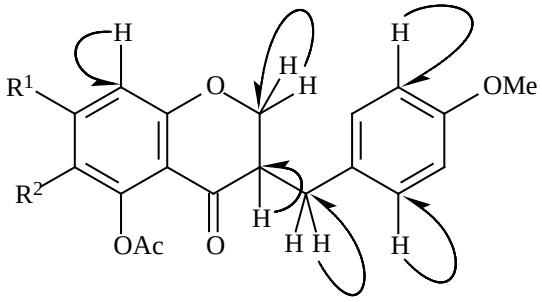
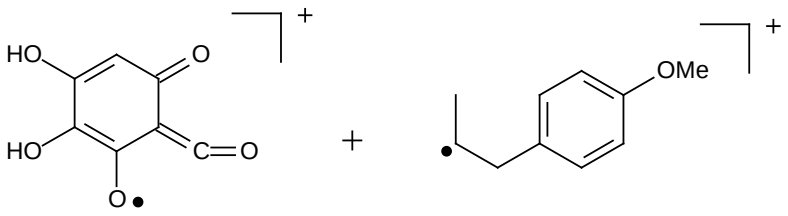


Figure 39: Relevant HMQC correlations for (93).

In the COSY spectrum **(Plate 3f)** strong correlations between aromatic protons H-2', H-6' and H-3', H-5' could be observed, confirming the AA'BB' spin-system on the B-ring, while large coupling ^3J of H-2 to H-3 and ^3J H-3 to H-9 confirmed the assignment of H-3.

In agreement with structure **(93)** assigned to the isolated homoisoflavanone, the IR spectrum displayed strong stretching frequencies at 1744 cm^{-1} and 1664 cm^{-1} indicated the presence of ester and ketone carbonyl respectively, while the EIMS data showed fragments *m/z* 167 and *m/z* 149 indicative of retro-Diels-Alder fragmentation of the C-ring after loss of the acetyl groups **(Scheme 3)**.

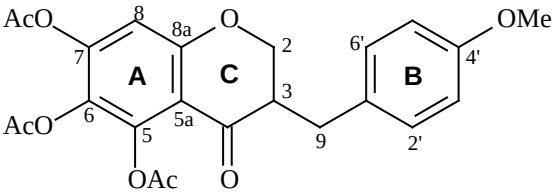


m/z 167 *m/z* 149

Scheme 3: Retro-Diels-Alder fragmentation (93).

Table 14: NMR data of (93a) or (93b).

Proton, ^{1}H		Carbon, ^{13}C		HMBC
No.	**δ_H (ppm)**	**No.**	**δ_C (ppm)**	**^{2}J and ^{3}J (H→C)**
1	-	1	-	
2	4.44 (dd, *J* 11 and 4 Hz)	2	70.2	C-9, C-3, C-8a, C-4
2	4.24 (dd, *J* 11 and 8 Hz)			
3	2.84 (m)	3	48.4	C-1', C-9
4	-	4	191.1	
5	-	5	155.6	
5a	-	5a	108.5	
6	-	6	147.8	
7	-	7	133.0	
8	6.37 (s)	8	105.0	C-5a, C-7, C-6, C-8a
8a	-	8a	155.3	
9	3.29 (dd, *J* 14 and 4 Hz)	9	32.3	C-2', C-6', C-1', C-4, C-3, C-2
9	2.66 (dd, *J* 14 and 11 Hz)			
1'	-	1'	136.2	
2'	7.23 (d, *J* 8.5 Hz)	2'	130.5	C-9, C-6', C-4', C-3', C-5'
3'	7.06 (d, *J* 8.5 Hz)	3'	122.2	C-5', C-1', C-4'
4'	-	4'	150.1	
5'	7.06 (d, *J* 8.5 Hz)	5'	122.2	C-3', C-1', C-4'
6'	7.23 (d, *J* 8.5 Hz)	6'	130.5	C-9, C-2', C-4', C-3', C-5'
O<u>H</u>	6.36 (s)			
CO<u>C</u>H$_3$ (C5)	2.34 (s)	CO<u>C</u>H$_3$ (C5)	21.7	
		<u>CO</u>CH$_3$ (C5)	170.6	
CO<u>C</u>H$_3$ (C6)	-	CO<u>C</u>H$_3$ (C6)	-	
		<u>CO</u>CH$_3$ (C6)	-	
CO<u>C</u>H$_3$ (C7)	2.39 (s)	CO<u>C</u>H$_3$ (C7)	21.7	
		<u>CO</u>CH$_3$ (C7)	170.3	
O<u>C</u>H$_3$	3.91	O<u>C</u>H$_3$	62.0	



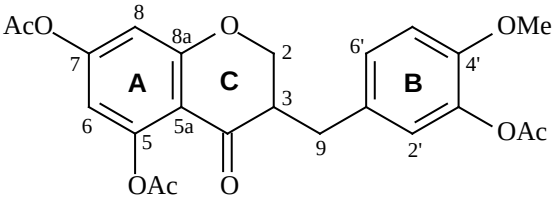
94

Table 15: NMR data of 5,6,7-Triacetoxy-3-(4'-methoxybenzyl)-chroman-4-one.

Ring	**Proton**	**$CDCl_3$, 298 K**
A	H-8	6.49 (s)
B	H-2', H-6'	7.23 (d, *J* 8 Hz)
	H-3', H-5'	7.06 (d, *J* 8 Hz)
C	H-2	4.47 (dd, *J* 12 and 4 Hz)
	H-2	4.27 (dd, *J* 12 and 9 Hz)
	H-3	2.88 (m)
	H-9	3.28 (dd, *J* 14 and 4 Hz)
	H-9	2.66 (dd, *J* 14 and 11 Hz)
	OAc	2.40 – 2.32 (x 3) (s)
	OMe	3.85 (s)

The allocation of H-8 is further corroborated by the chemical shift of this proton resonance in 5,6,7-triacetoxy-3-(4'-methoxybenzyl)-chroman-4-one **(94)** being identical to that in 5,6,7-triacetoxy-3-(3',4'-dimethoxybenzyl)-chroman-4-one **(96)** (*vide infra*).

5.5 5,7-Diacetoxy-3-(3'-acetoxy-4'-methoxybenzyl)-chroman-4-one (95)



95

Acetylation and purification of fraction E of the 1-AS methanol extract afforded the title compound **(95)** (R*f* 0.43), which was previously isolated in the free phenolic form from the bulbs of *Scilla nervosa,* as a white solid[92] (4.5 mg). In contrast to the spectrum of the previous compound, the ^{1}H NMR spectrum **(Plate 4a, Table 16)** this benzylchromanone displayed a *meta*-coupled AB spin-system [H-6 (δ 6.30, d, *J* 2.0 Hz) and H-8 (δ 6.22, d, *J* 2.0 Hz)] on the A-ring and an ABX system on the B-ring [H-2' (δ 6.90, d, *J* 2.0 Hz), H-5' (δ 6.93, d, *J* 8.0 Hz) and H-6' (δ 7.05, dd, *J* 8.0 and 2.0 Hz)] together with one methoxy and three acetoxy signals. The COSY experiment **(Plate 4c)** revealed coupling between protons H-2 and H-3 and protons H-3 and H-9, suggesting H-3 to be bonded to a carbon between H-2 and H-9. The NOE spectrum **(Plate 4b)** showed strong association between the methoxy group and H-5' (on the B-ring) as well as association of H-2' to H-3 and H-9; thus confirming the homoisoflavanone structure of the product **(Figure 40)**.

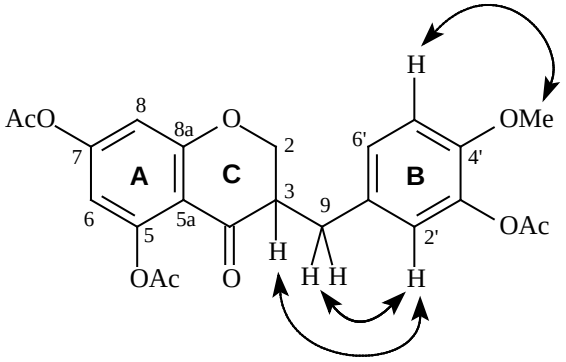


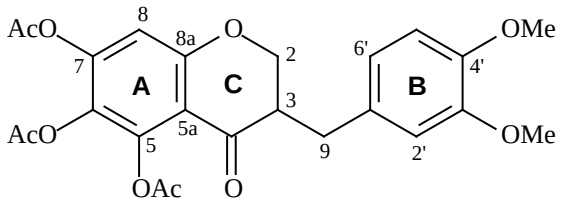
Figure 40: NOE of 5,7-diacetoxy-3-(3'-acetoxy-4'-methoxybenzyl)-chroman-4-one (95).

[92] Silayo, A.; Ngadjui, B.T.; Abegaz, B.M. *Phytochemistry* **1999**, *52*, 947.

Table 16: NMR data of 5,7-diacetoxy-3-(3'-acetoxy-4'-methoxybenzyl)-chroman-4-one.

Ring	Proton, ^{1}H	$CDCl_3$, 298K
A	H-8	6.22 (d, *J* 2.0 Hz)
	H-6	6.30 (d, *J* 2.0 Hz)
B	H-2'	6.90 (d, *J* 2.0 Hz)
	H-5'	6.93 (d, *J* 8.0 Hz)
	H-6'	7.05 (dd, *J* 8.0 and 2.0 Hz)
C	H-2	4.33 (dd, *J* 12.0 and 5.0 Hz)
	H-2	4.15 (dd, *J* 12.0 and 8.2 Hz)
	H-3	2.74 (m)
	H-9	3.18 (dd, *J* 14.0 and 5.0 Hz)
	H-9	2.60 (dd, *J* 14.0 and 9.3 Hz)
	OMe	3.83 (s)
	OAc	2.20 – 2.52 (x 3) (s)

5.6 5,6,7-Triacetoxy-3-(3',4'-dimethoxybenzyl)-chroman-4-one (96)



96

The title compound was afforded as a *light yellow solid* (3.7 mg) upon acetylation and TLC purification (R*f* 0.53). The ^{1}H NMR spectrum **(Plate 5a, Table 17)** of this homoisoflavanone **(96)** was identical to that of the triacetate of **(93)** with respect to the heterocyclic region, the A-ring proton (H-8) at δ 6.49 and three acetoxy groups. The spectrum, however, displayed an extra methoxy group (δ 3.85) as well as an ABX spin-system [H-2', δ 6.90 (d, *J* 2.0 Hz); H-5', δ 6.94 (d, *J* 9.0 Hz); H-6', δ 7.06 (dd, *J* 9.0 and 2.0 Hz)] for the B-ring.

The NOE spectrum **(Plate 5b)** confirmed the allocation of the two methoxy groups on the B-ring next to protons H-2' and H-5', while, both H-2' and H-6' showed strong correlations to H-3, H-9 and H-2. The strong correlation observed between proton H-8 and H-2 was crucial in the assignment of H-8 **(Figure 41)**.

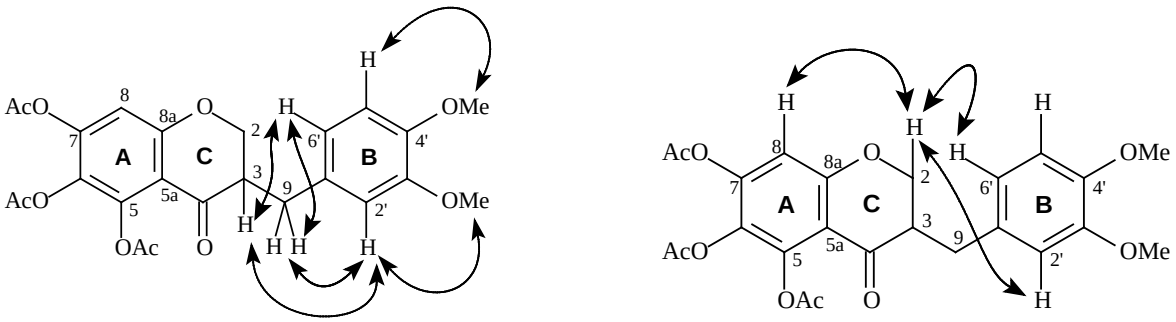


Figure 41: NOE of 5,6,7-triacetoxy-3-(3',4'-dimethoxybenzyl)-chroman-4-one.

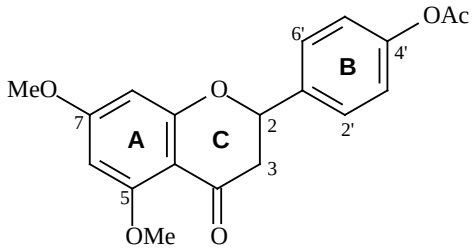
The ^{13}C NMR spectrum **(Plate 5d, Table 17)** confirmed the presence of a carbonyl carbon (C-4) at δ 190.92 along with three acetoxy C=O carbons at δ 169.48, δ 168.92 and δ 167.87. Seven non-oxygen substituted carbons C-8a (δ 156.43), C-8 (δ 110.92), C-5a (δ 112.58), C-1' (δ 130.34), C-2' (δ 127.26), C-5' (δ 112.71) and C-6' (δ 123.41) as well as five non-hydrogen bonded carbons C-5 (δ 145.65), C-6 (δ 148.55), C-7 (δ 150.00), C-3' (δ 138.84) and C-4' (δ 139.83) resonated in the aromatic region. C-2 (δ 69.57), C-3 (δ 47.56), C-9 (δ 29.69), two methoxy carbons (δ 55.98) and the methyl carbons from three acetoxy groups (δ 20.65) resonated in the non-aromatic region of the spectrum.

Additional evidence for structure **(96)** came from the IR spectrum where strong stretching frequencies at 1610 cm^{-1} and 1767 cm^{-1} indicated the presence of ketone and ester carbonyl respectively, while the MS data displayed molecular ions of $[M+Na]^+$ at *m/z* 495 and $[M+H]^+$ at *m/z* 473.

Table 17: NMR data of 5,6,7-Triacetoxy-3-(3',4'-dimethoxybenzyl)-chroman-4-one.

Proton, ^{1}H		**Carbon, ^{13}C**	
No.	**δ_H (ppm)**	**No.**	**δ_C (ppm)**
1	-	1	-
2	4.48 (dd, *J* 12 and 5 Hz)	2	69.57
2	4.27 (dd, *J* 12 and 8 Hz)		
3	2.85 (m)	3	47.56
4	-	4	190.92
5	-	5	145.65
5a	-	5a	112.58
6	-	6	148.55
7	-	7	150.00
8	6.49 (s)	8	110.92
8a	-	8a	156.43
9	3.22 (dd, *J* 15.0 and 4.0 Hz)	9	29.69
9	2.61 (dd, *J* 15.0 and 10.0 Hz)		
1'	-	1'	130.34
2'	6.90 (d, *J* 2.0 Hz)	2'	127.26
3'	-	3'	138.84
4'	-	4'	139.83
5'	6.94 (d, *J* 9.0 Hz)	5'	112.71
6'	7.06 (dd, *J* 9.0 and 2.0 Hz)	6'	123.41
$COCH_3$	2.32 – 2.43 (x 3) (s)	$COCH_3$ (x 3)	20.65
		$COCH_3$ (x 3)	169.48-167.87
OCH_3	3.85 (x 2) (s)	OCH_3 (x 2)	55.98

5.7 4'-*O*-acetyl- 5,7-di-*O*-methylnaringenin (97)



97

The title compound **(97)** was isolated as a white solid[93] (R*f* 0.65, 1.5 mg). The ^{1}H NMR spectrum **(Plate 6a, Table 18)** of **(97)** displayed six aromatic protons, two of which being typical as from a *meta*-coupled AB spin-system on the A-ring of a flavonoid nucleus [H-6, δ 6.34 (d, *J* 2.1 Hz) and

[93] Shoja, M., *Z. Kristallogr. New cryst. Struct.* **1997**, *212*, 127.

H-8, δ 6.47 (d, *J* 2.1 Hz)] and the other four (representative of a B-ring) an AA'BB'-spin system [H-2',6', δ 7.48 (d, *J* 8.4 Hz) and H-3',5', δ 7.16 (d, *J* 8.4 Hz)]. These resonances were accompanied by two methoxy signals and a single acetoxy signal. NOE association **(Plate 6b)** between the two methoxy groups and H-6 and 8 respectively, placed the methoxy groups on the A-ring with the acetoxy attached to C-4' of the B-ring **(Figure 42)**. The flavanone/isoflavanone character became apparent from the typical heterocyclic three-proton spin system [H-2, δ 5.46 (dd, *J* 8.3 and 2.8 Hz); H-3, δ 3.05 (dd, *J* 16.0 and 8.3 Hz) and H-3, δ 2.86 (dd, *J* 16.0 and 2.8 Hz)] associated with this type of compound; the chemical shift of the methine proton (δ 5.46 vs δ 4.78 of an isoflavanone) defining it as a flavanone.

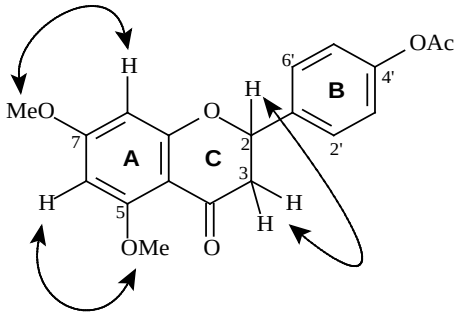
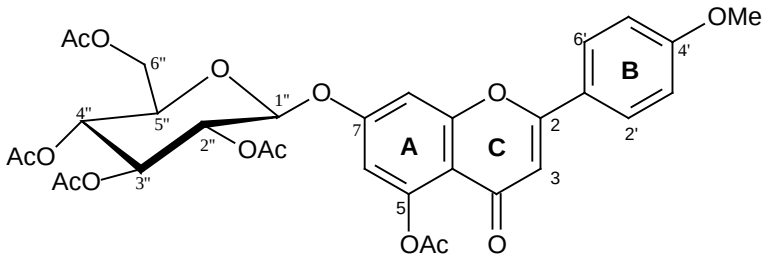


Figure 42: NOE of 4'-*O*-acetyl- 5,7-di-O-methylnaringenin.

Table 18: NMR data of 4'-*O*-acetyl- 5,7-di-O-methylnaringenin.

Ring	**Proton**	**$CDCl_3$, 298 K**
A	H-6	6.34 (d, *J* 2.1 Hz)
	H-8	6.47 (d, *J* 2.1 Hz)
B	H-2', H-6'	7.48 (d, *J* 8.4 Hz)
	H-3', H-5'	7.16 (d, *J* 8.4 Hz)
C	H-2	5.46 (dd, *J* 8.3 and 2.8 Hz)
	H-3	3.05 (dd, *J* 16.0 and 8.3 Hz)
	H-3	2.86 (dd, *J* 16.0 and 2.8 Hz)
	OAc (arom.)	2.31 (s)
	OMe	3.85-3.95 (x 2) (s)

5.8 2”,3”,4”,5,6”-Penta-*O*-acetyl-4’-*O*-methylapigenin-7-*O*-*β*-D-glucopyranoside (98)



98

2”,3”,4”,5,6”-Penta-*O*-acetyl-4’-*O*-methylapigenin-7-*O*-*β*-D-glucopyranoside **(98)** (R*f* 0.54) was isolated as a white amorphous solid[94] (4.8 mg) from the methanol extract of *Scilla natalensis.* The ^{1}H NMR spectrum **(Plate 7a, Table 19)** of compound **(98)** displayed a sharp singlet characteristic of the vinylic proton of a flavone at δ 6.56 ppm and AB [H-6, δ 6.70 (d, *J* 2.3 Hz) and H-8, δ 7.02 (d, *J* 2.3 Hz)] as well as AA’BB’ spin-systems [H-2’,6’, δ 7.82 (d, *J* 8.3 Hz) and H-3’,5’, δ 7.04 (d, *J* 8.3 Hz)] in the aromatic region. These resonances were accompanied by five acetoxy signals (four aliphatic and one aromatic) and one aromatic methoxy singlet as well as seven protons (δ 4.00 – δ 5.35 ppm) in the carbohydrate area of the spectrum; thus defining the compound as having a flavone glycoside skeleton. The NOE spectrum **(Plate 7b)** displayed associations between H-3 and H-2’,6’ of the B-ring as well as between the methoxy group and the other ortho coupled B-ring doublet (δ 7.04) placing the methoxy group at C-4’ and thus the aromatic acetoxy function at either C-5 or C-7. Assignment of the linkage between the sugar unit and the aglycone was possible due to a strong NOE association between the anomeric proton (H-1”, δ 5.22, $CDCl_3$) of the glycoside and both A-ring aromatic protons (H-6, δ 6.70) and (H-8, δ 7.02) **(Figure 43)**. Individual sugar protons could be allocated according to coupling constants and in analogy to similar systems in analogous compounds **(Plate 7d, C_6D_6)**. The large coupling constants between H-1” and H-2” (J 8.0 Hz), H-2” and H-3” (J 10.0 Hz), H-3” and H-4” (J 10.0 Hz) and H-4” and H-5” (J 10.0 Hz) indicate that each pair is orientated *trans*-diaxially to each other and,

[94] Kaloshina, N.A. *Khimiya Prirodnykh Soedinenii* **1988**, *3*, 453.

together with the resonances of two geminally coupled H-6" protons, prove that the sugar is derivatised glucose in the pyranose form. The absolute D-configuration follows from the natural occurrence of the sugar, whereas the anomeric β-configuration and the C-O coupling of the sugar follows from the vicinal coupling constant between H-1" and H-2" (J 8.0 Hz)[95].

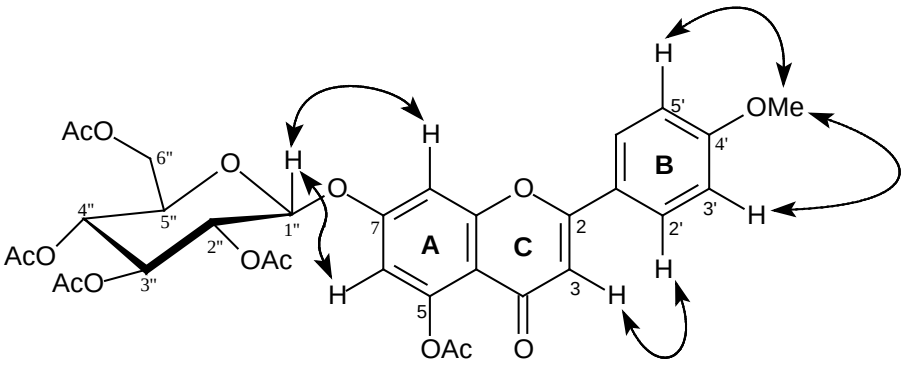


Figure 43: NOE of 2",3",4",5,6"-Penta-*O*-acetyl-4'-*O*-methylapigenin-7-*O*-*β*-D-glucopyranoside (98).

[95] Marais, C., Struktuur en sintese van metaboliete uit rooibostee (*Aspalathus linearis*). Fisiologiese aktiwiteit en biomimetiese model vir die fermentasieproses, Ph.D dissertation, University of the Orange Free State, **1995**, 35, 136.

Table 19: ^{1}H NMR of 2”,3”,4”,5,6”-Penta-*O*-acetyl-4’-*O*-methylapigenin-7-*O*-*β*-D-glucopyranoside (98) in $CDCl_3$ and C_6D_6.

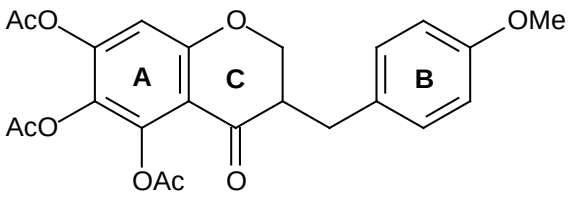
$CDCl_3$, 298 K			C_6D_6, 298 K		
Ring	**Proton**	**δ_H (ppm)**	**Ring**	**Proton**	**δ_H (ppm)**
A	H-8	7.02 (d, *J* 2.3 Hz)	**A**	H-8	6.74 (d, *J* 2 Hz)
	H-6	6.70 (d, *J* 2.3 Hz)		H-6	6.69 (d, *J* 2 Hz)
B	H-2’, H-6’	7.82 (d, *J* 8.3 Hz)	**B**	H-2’, H-6’	7.37 (d, *J* 9 Hz)
	H-3’, H-5’	7.04 (d, *J* 8.3 Hz)		H-3’, H-5’	6.64 (d, *J* 9 Hz)
C	H-3	6.56 (s)	**C**	H-3	6.47 (s)
Glucose	H-1”, H-4”	5.22 (m)	**Glucose**	H-1”	4.77 (d, *J* 8 Hz)
	H-2”, H-3”	5.35 (m)		H-2”	5.51 (dd, *J* 10 and 8 Hz)
	H-6” (x 2)	4.30 (m)		H-3”	5.41 (t, *J* 10 Hz)
	H-5”	4.00 (m)		H-4”	5.19 (t, *J* 10 Hz)
				H-5”	3.18 (m)
				H-6”	4.08 (dd, *J* 12 and 6 Hz)
				H-6”	4.01 (dd, *J* 12 and 2 Hz)
	OAc (arom.)	2.46 (s)		OAc (arom.)	2.35 (s)
	OAc (aliph.)	2.06-2.12 (x 4) (s)		OAc (aliph.)	1.75-1.65 (x 4) (s)
	OMe	3.89 (s)		OMe	3.20 (s)

CHAPTER 6

6. Synthesis of 5,6,7-triacetoxy-3-(4'-methoxybenzyl)chroman-4-one (94), a derivatised homoisoflavonoid from *Scilla natalensis* (Hyacinthaceae)

6.1 Introduction

Several homoisoflavonoids (3-benzylchroman-4-ones) have been isolated from *Scilla natalensis* during the current study. Since homoisoflavonoids have been found to possess antifungal, hypocholesterolemic, antimutagenic and antiviral activities[96] and due to the isolation of a new member of this family of natural products, it was decided to confirm the structure of the isolated compound **(94)** by synthesis.



94

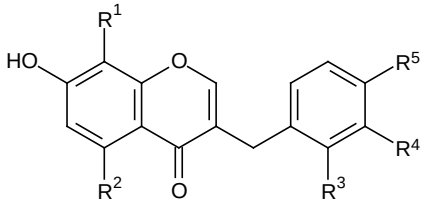
6.2 Literature approaches to the synthesis of homoisoflavanones

6.2.1 Retro-synthetic approach I

Since, the obvious and easiest way of synthesising homoisoflavonoids would be to utilise the corresponding dihydrochalcone (DHC) **(101)** as key precursor **(Scheme 4)**, this was the approach towards the synthesis of compounds (a) to (h) followed by most of the earlier workers in the field

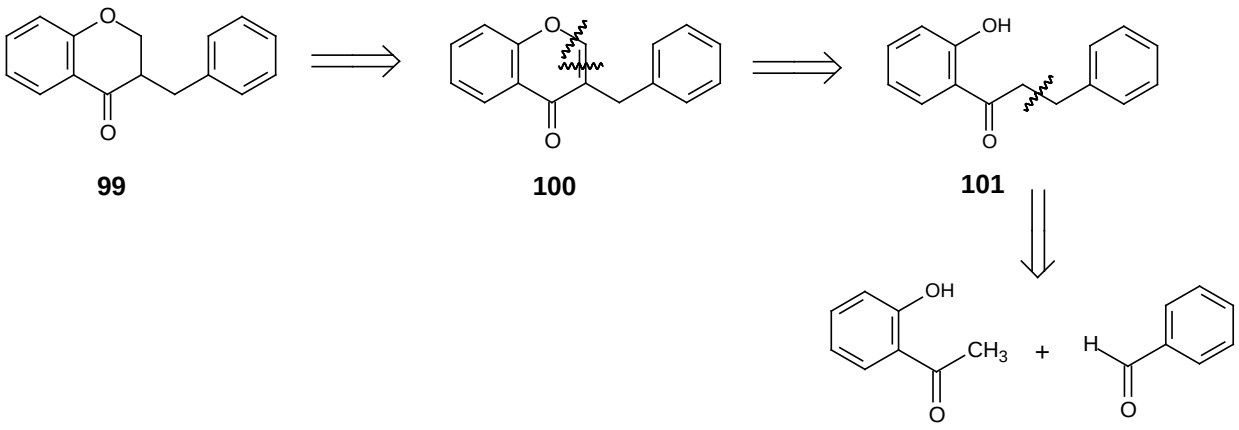
[96] Siddaiah, V.; Rao, C.V.; Venkateswarlu, S.; Krishnaraju, A.V.; Subbaraju, G.V. *Bioorganic & Medicinal Chemistry* **2006**, *14*, 2545.

(Figure 44). If the flavanone analogue **(99)** is the desired product, this compound can then be reached easily by selective reduction of the intermediate homoisoflavone **(100)**.



Cpd	R^1	R^2	R^3	R^4	R^5
a	H	H	H	H	OH
b	H	H	H	H	OCH_3
c	H	H	H	OH	OH
d	H	H	H	OCH_3	OCH_3
e	H	H	OCH_3	H	OCH_3
f	OH	H	H	H	OH
g	OH	H	H	H	OCH_3
h	H	OH	H	H	OH

Figure 44: Homoisoflavones synthesised through DHC.



Scheme 4: Retro-synthesis *via* DHC.

The pivotal step in this approach would be attachment of the C-1 fragment to the α-carbon of the DHC. Several reagent systems like BF_3-etherate/DMF/PCl_5 and HCO_2Et/Na have been reported in literature for this purpose[97,98]. Subsequent cyclization between the 2'-OH function of the DHC and a leaving group on the newly introduced carbon followed by hydrogenation of the double bond, if necessary, would then complete the synthesis and give the desired product **(99)**.

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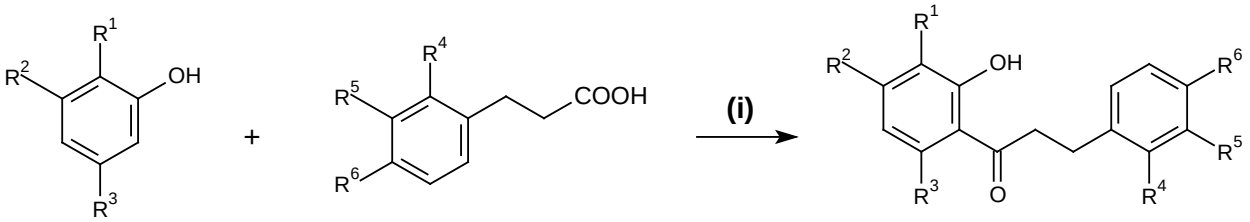
[97] Siddaiah, V.; Rao, C.V.; Venkateswarlu, S.; Subbaraju, G.V. *Tetrahedron* **2006**, *62*, 841.
[98] Davis, .F.A.; Chen, B-C. *J. Org. Chem*. **1993**, 58, 1751.

Although the required dihydrochalcone was obtained in most instances by aldol condensation between an appropriate acetophenone and benzaldehyde *via* the chalcone intermediate, some workers utilised other methods for reaching this key intermediate:

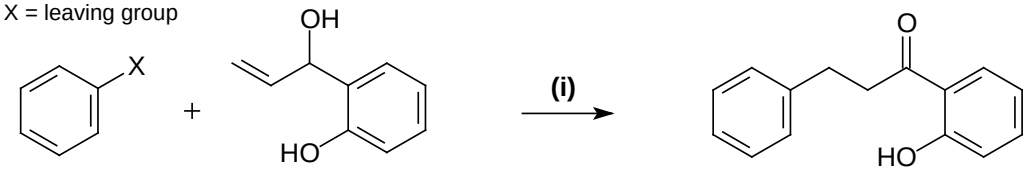
In an improvement on the multi-step chalcone approach, Siddaiah *et al.*[99] utilised a Friedel-Crafts acylation reaction between phenol **(104)** and a dihydrocinnamic acid derivative **(105)** to prepare the polyhydroxydihydrochalcone **(106)** in a single step (30-71 % yield) **(Scheme 5)**.



104 **105** **106**

Scheme 5: (i) $BF_3 \cdot Et_2O$, 80-90˚C.

Briot *et al*.[100] utilised the Heck-reaction in their quest to synthesise dihydrochalcones in a single step. Thus an aryl halide **(107)** and the allyl alcohol **(108)** were reacted to give the required dihydrochalcone **(109)** in 12-80 % yield **(Scheme 6)**. Although this methodology represents a single reaction step for the formation of the dihydrochalcone, 1-aryl-2-propen-1-ols with the required hydroxylation pattern like **(108)** are not that freely available and may themselves require quite a few steps to be prepared.



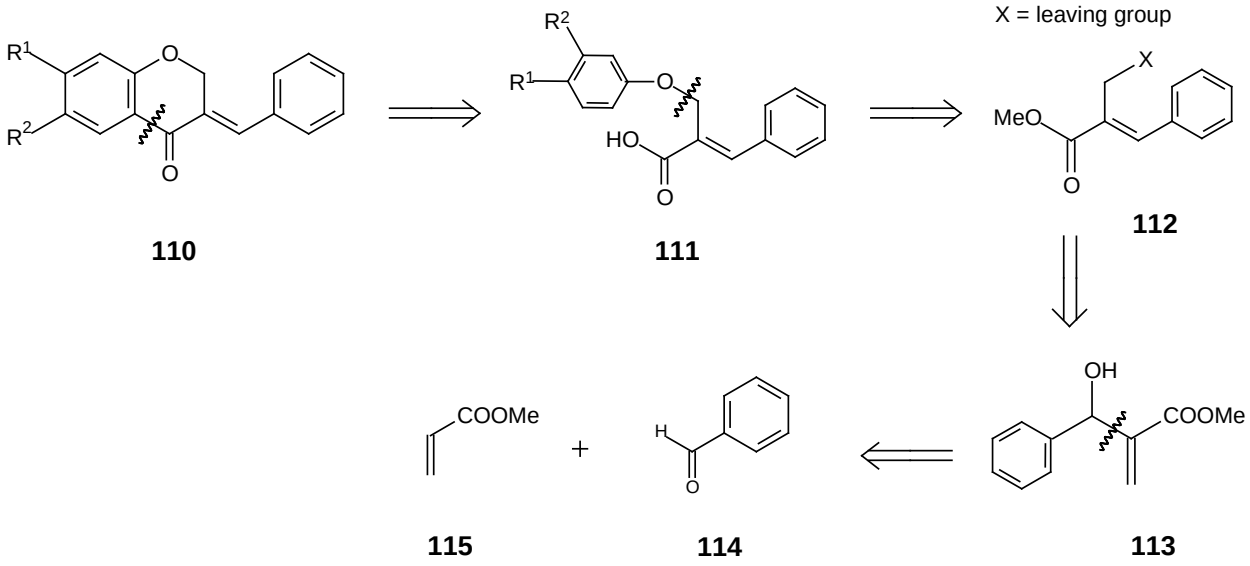
107 **108** **109**

Scheme 6: (i) $Pd(OAc)_2$, NEt_3, CH_3CN.

[99] Siddaiah, V.; Rao, C.V.; Venkateswarlu, S.; Subbaraju, G.V. *Tetrahedron* **2006**, *62*, 841.
[100] Briot, A.; Baehr, C.; Brouillard, R.; Wagner, A.; Mioskowski, C. *J. Org. Chem.* **2004**, *69*, 1374.

6.2.2 Retro-synthetic approach II

Although the dihydrochalcone methodology is frequently used to prepare homoisoflavanones, two other routes have recently been developed in this regard. Since polyhydroxylated acetophenones are not always available, Basavaiah *et al.*[101] followed a protocol where a phenol is attached to the β-carbon of a propionic acid derivative **(112)** containing a benzylidene substituent on the α-carbon **(Scheme 7)**. This compound would then become available through the utilization of the Baylis-Hillman reaction between an acrylic acid derivative **(115)** and a substituted benzaldehyde **(114)**.



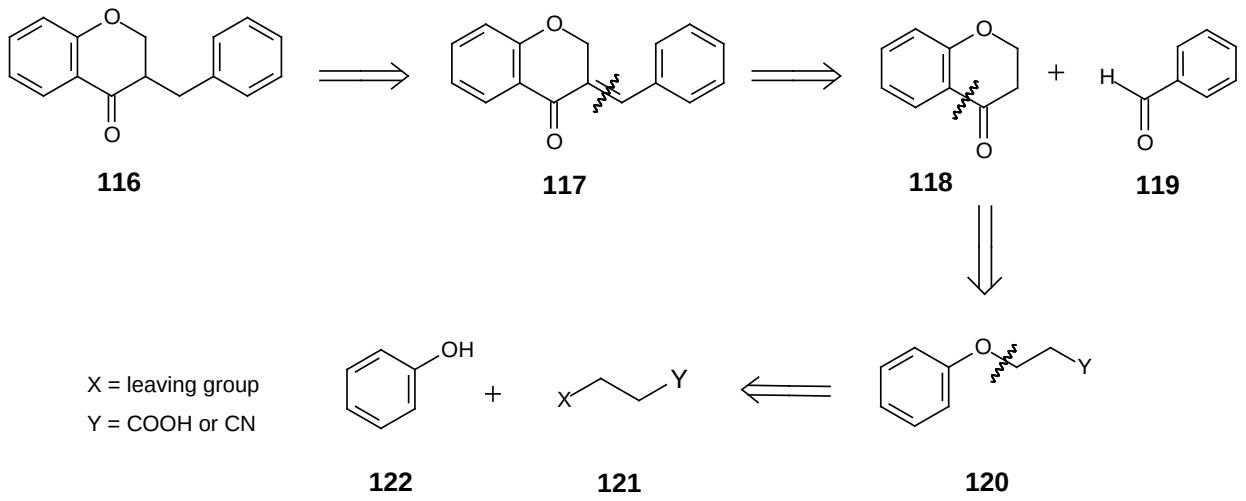
Scheme 7: Retro-synthesis *via* Baylis-Hillman reaction.

6.2.3 Retro-synthetic approach III

In a variation of the Basavaiah methodology, Siddaiah *et al.*[102] described a strategy for homoisofavanone synthesis where the chromanone **(118)** was identified as the key intermediate **(Scheme 8)**. Since polyhydroxy chromanones are not freely available, methodology for the

[101] Basavaiah, D.; Bakthadoss, M.; Pandiaraju, S. *Chemistry Commun*. **1998**, 1639.
[102] Siddaiah, V.; Rao, C.V.; Venkateswarlu, S.; Krishnaraju, A.V.; Subbaraju, G.V. *Bioorganic & Medicinal Chemistry* **2006**, *14*, 2545.

synthesis of these intermediates had to be developed as well. In their first paper, the chromanone **(118)** was prepared by cyclization of a *β*-phenoxy propionic acid derivative (**120**, Y = COOH), which was obtained from the reaction of the corresponding halo-propionic acid **(121)** with a substituted phenol **(122)**. In an attempt to use more benign conditions and to improve on the yield in the formation of the key chromanone **(118)**, Siddaiah *et al.*[103] in a subsequent report, changed their strategy towards the utilization of a *β*-halo acrylonitrile in stead of the *β*-halo propionic acid. Yields were, however, only marginally improved from 46 % to 54 % in this process which utilised H_2SO_4 and not polyphosphoric acid.



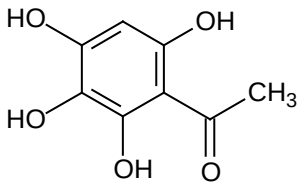
Scheme 8: Retro-synthesis *via* chromanone.

6.3 Model reactions for the synthesis of 5,6,7-triacetoxy-3-(4'-methoxybenzyl)-chroman-4-one (94)

In order to confirm the structure of the isolated compound **(94)** and in particular the position of the third OH on the A-ring, the synthesis of this homoisoflavanone was embarked upon. After careful consideration of the different methodologies available (*vide supra*), it was decided to follow the dihydrochalcone route due to the following reasons: (a) The Baylis-Hillman reaction, which forms

[103] Siddaiah, V.; Maheswara, M.; Rao, C.V.; Venkateswarlu, S.; Subbaraju, G.V. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, 1288.

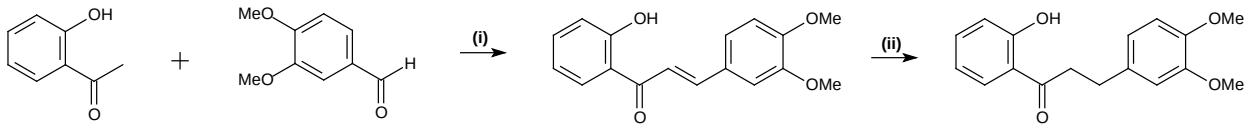
part of route II **(scheme 7)**, is notorious for being a very slow reaction. (b) While considerable expertise does exist in the Bloemfontein group on the preparation and reactions of chalcones, the synthesis of chromanones, which forms the key intermediate in route III **(scheme 8)**, will be new. One major concern with the DHC route, however, was the availability of acetophenone **A** owing to the fact that phenols with a 1,2,3,5 hydroxylation pattern are not commercially available. It was therefore decided to test the synthesis on a model compound, 2-hydroxyacetophenone, first and to determine the appropriate C-1 fragment to be used before attempting the difficult synthesis of the required acetophenone **A**.



A

Standard Claisen-Schmidt condensation [104] between 2-hydroxyacetophenone **(123)**, and 3,4-dimethoxybenzaldehyde **(124)** afforded the required chalcone **(125)** in 68 % yield **(Scheme 9)**. The ^{1}H NMR spectrum **(Plate 8)** confirmed the chalcone nature of **(125)** by displaying the typical resonances of an α,β-unsaturated carbonyl system appearing as doublets at δ 7.52 (d, 1 H, *J* 16 Hz, α-H) and δ 7.89 (d, 1 H, *J* 16 Hz, β-H). Subsequent hydrogenation of the chalcone over 5 % Pd/C yielded the dihydro equivalent **(126)** quantitatively **(Scheme 9)**. In the ^{1}H NMR spectrum **(Plate 9)** of **(126)** the signals from the α, β- unsaturated system was replaced with the resonances from two methylene (CH_2) groups at δ 3.32 (t, 2 H, *J* 8 Hz, α-CH_2) and δ 3.04 (t, 2 H, *J* 8 Hz, β-CH_2) thus confirming the product to be a DHC.

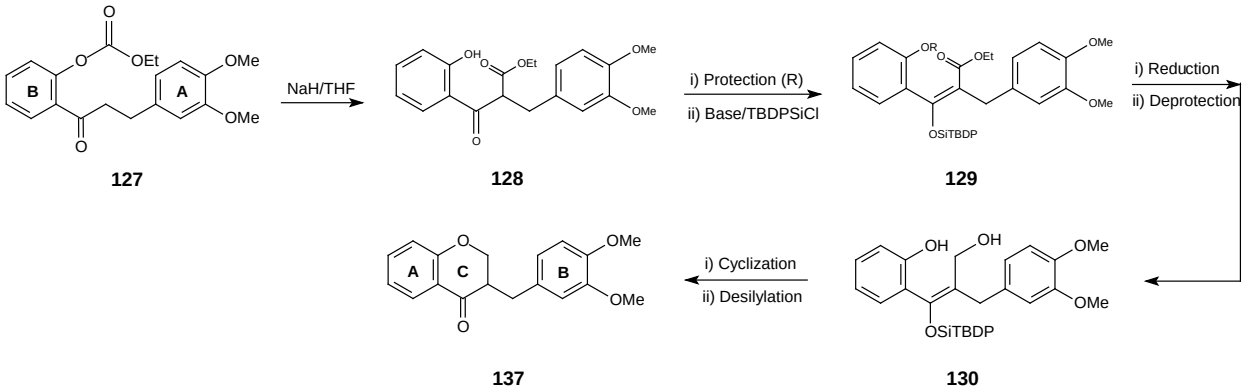
[104] Davis, .F.A.; Chen, B-C. *J. Org. Chem*. **1993**, 58, 1751.



123 **124** **125** **126**

Scheme 9: (i) 60% KOH, EtOH, rt (ii) H_2, 5% Pd/C, acetone.

In order to introduce C-2 into the dihydrochalcone moiety, it was decided to utilise a modified Baker-Venkataraman rearrangement[105] strategy followed by reduction of the ester functionality and subsequent Mitsunobu cyclization[106,107] **(Scheme 10)**. Since the ketone moiety would be more susceptible to reduction than the ester, it was envisage that the ketone could be protected as a silyl enol ether before the reduction step. After cyclization liberation of the enol would then re-instigate the required carbonyl functionality without the involvement of another reagent.



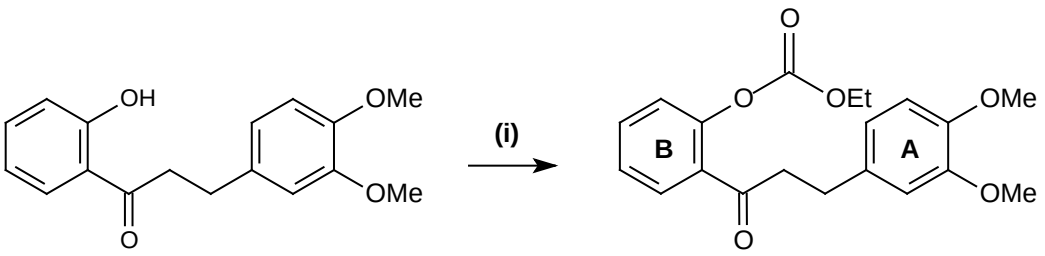
127 **128** **129** **137** **130**

Scheme 10: Baker-Venkataraman rearrangement and Mitsunobu cyclization.

Thus 2'-hydroxy-3,4-dimethoxydihydrochalcone **(126)** was reacted with ethylchloroformate and pyridine in DCM to give the required ester **(127)** (66 % yield), which was subjected to treatment with NaH in THF **(Scheme 11 and 10)**. The ^{1}H NMR spectrum **(Plate 10a)** of 2'-ethoxycarbonyloxy-3,4-dimethoxydihydrochalcone **(127)** displayed a quartet (δ 4.31) and triplet (δ 1.37, *J* 7 Hz) as well as deshielded B-ring aromatic protons [δ 7.74 (dd, 1 H, *J* 8 and 1

[105] Kalinin, A.V.; Da Silva, A.J.M.; Lopes, C.C.; Lopes, R.S.C.; Sniekus, V. *Tet. Lett*. **1998**, *39*, 4995.
[106] Shih, T.L.; Wyvratt, M.J.; Mrozik, H. *J. Org. Chem*. **1987**, *52*, 2029.
[107] Versteeg, M.; Bezuidenhoudt, B.C.B.; Ferreira, D. *Tetrahedron* **1999**, *55*, 3365.

Hz, H-6'); 7.52 (m, 1 H, H-5'); 7.31 (m, 1 H, H-4'); 7.21 (dd, 1 H, *J* 8 and 1 Hz, H-3') vs δ 7.76 (dd, 1 H, *J* 8 and 2 Hz, H-6'); 7.48 (m, 1 H, H-5'); 6.89 (m, 1 H, H-4'), 7.00 (dd, 1 H, *J* 8 and 1 Hz, H-3') for the hydroxy compound]; thus confirming the presence of the ester group at C-2'. The spectrum **(plate 11a)** of the product **(133)** from the hydride reaction could, however, not be reconciled with the expected keto-ester **(128) (Scheme 10)**.



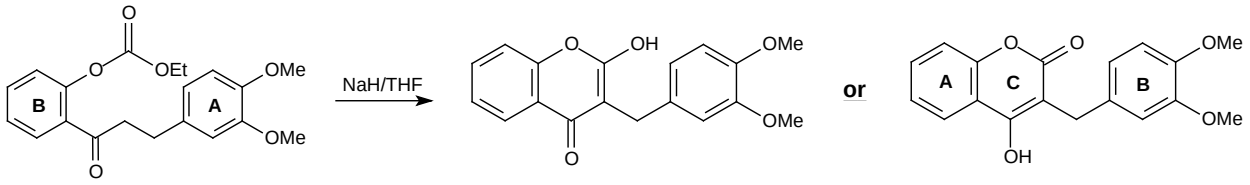
126 **127**

Scheme 11: (i) ClCOOEt, pyridine, DCM.

Careful examination of the ^{1}H-NMR spectrum **(Plate 11a, Table 20)** of this product **(133)** indicated the presence of the expected benzylic methylene group [δ 4.00 (s)], but no ethoxy resonances and no deshielded methine proton, which should be attached to C-α. The MS spectrum also showed no molecular ion at *m/z* 358 as would be expected for compound **(128)**, but an M^+ peak at *m/z* 335 $[M+Na]^+$ instead. It was therefore clear that the ethoxy group was displaced from the starting material. The ^{13}C NMR **(Plate 11b, Table 20)** confirmed the presence of a carbonyl group with a resonance at δ 160.7 (C-2), indicative of an ester carbonyl[108], as well as another low field carbon resonance at δ 163.8 (C-4) and a methylene carbon at δ 30.1 (C-9) apart from the expected aromatic carbon peaks. The IR spectrum confirmed the presence of an ester carbonyl with a stretching frequency band at 1669 cm^{-1}, while it also indicated the presence of an OH group with a stretching frequency of 3455 cm^{-1}. It was therefore concluded that the rearrangement was accompanied by a transesterification reaction involving the 2'-hydroxy - and ethoxy groups as well

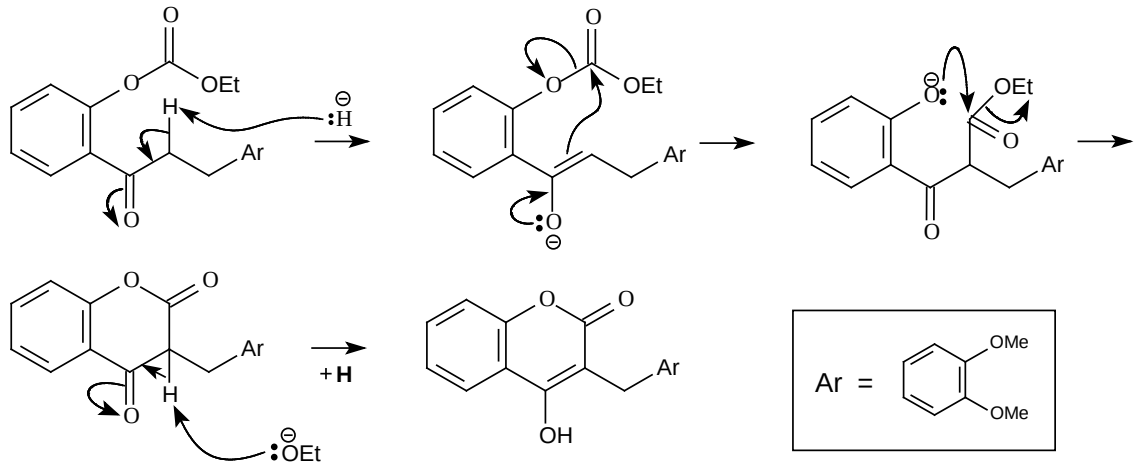
[108] Kamara, B.I, Structure and synthesis of polyphenols from honeybush tea (*Cyclopia intermedia*) and the potential of flavonoids as active oxygen scavengers, Ph.D dissertation, University of the Free State, **1999**, 42.

as enol formation. The product from the hydride reaction, which was obtained in 33% yield, was therefore identified as either the unlikely compound **(132)** or 3-(3',4'-dimethoxybenzyl)-4-hydroxycoumarin **(133) (Scheme 12)**. The formation of **(133)** can be explained in terms of the mechanism indicated in **Scheme 13** and was confirmed by silylation of the 4-OH functionality **(Scheme 14)** and the ^{1}H-NMR spectra of the silylated product **(134)** (*vide infra*).

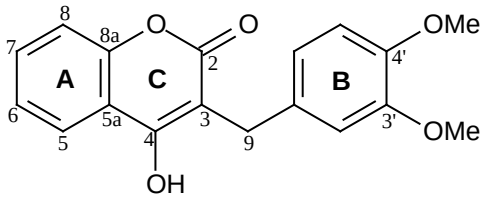


127 **132** **133**

Scheme 12: Possible products from the rearrangement of keto-ester (127).



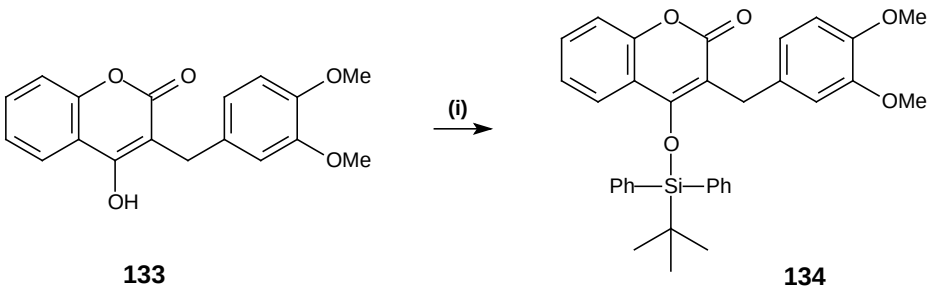
Scheme 13: Mechanism for the formation of (133).



133

Table 20: NMR data for compound (133).

Proton, ^{1}H		Carbon, ^{13}C	
No.	**δ_H (ppm)**	**No.**	**δ_C (ppm)**
1	–	1	–
2	–	2	160.72
3	–	3	104.24
4	–	4	163.80
5	7.76 (dd, 1 H, *J* 8 and 2 Hz)	5	123.94
5a	–	5a	115.76
6	7.55 (m, 1 H)	6	122.78
7	7.28 (m, 1 H)	7	131.95
8	7.35 (dd, 1 H, *J* 8 and 2 Hz)	8	116.54
8a	–	8a	152.55
9	4.00 (s, 2 H, CH_2)	9	30.06
1’	–	1’	129.80
2’	6.88 (br d, 1 H, *J* 2 Hz)	2’	111.65
3’	–	3’	148.56
4’	–	4’	149.95
5’	6.84 (d, 1 H, *J* 8 Hz)	5’	111.66
6’	6.93 (br dd, 1 H, *J* 8 and 2 Hz)	6’	120.20
OCH_3	3.88-3.86 (s, x 2).	OCH_3	55.94-55.96 (x 2)



133 **134**

Scheme 14: (i) NaH/THF, TBDPSiCl, rt, 20 %.

While the ^{1}H-NMR spectrum **(Plate 12a)** of **(134)** displayed two additional multiplets at δ 7.65 (4 H) and δ 7.25 (6 H) for the two phenyl groups of the silyl protecting unit as well as a singlet resonating at δ 0.90 (9 H, ^{t}Bu), the ^{13}C NMR spectrum **(Plate 12b)** displayed a quaternary carbon at δ 13.4 (C-10) together with 12 additional aromatic carbons [δ 127.4 (x 6) and δ 134.7 (x 6)] and the methyl carbons of the *tert*-butyl group at δ 26.1 (x 3). Assignment of the silyloxy group to C-4 was confirmed by NOE association between one phenyl ring of the silyl protecting group and protons H-6 and H-7 in the NOE spectrum **(Plate 12c and Figure 45)**. Confirmation of the proposed structure **(134)** also came from the IR spectrum, where an enol ester stretching frequency band at 1620 cm^{-1} was clearly visible, while the MS spectrum contained a $[M+Na]^{+}$ peak at *m/z* 573.

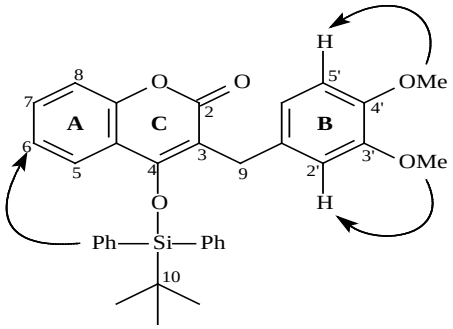
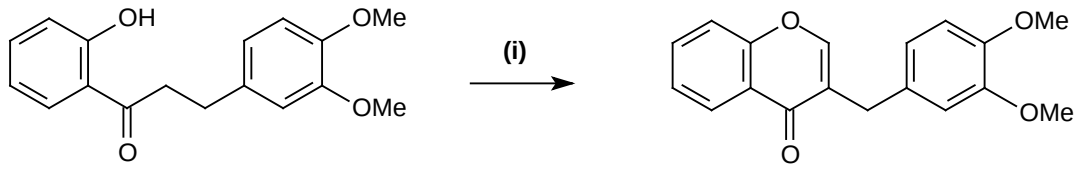


Figure 45: NOE for 3-(3',4'-dimethoxybenzyl)-4-*O*-(*tert*-butyldiphenylsilyl)-coumarin (134).

In order to complete the synthesis of the homoisoflavanone from this point, a selective 1,2-reduction of the ester functionality followed by re-cyclization would be needed. We, however, became aware of the latest work by Siddaiah *et al.*[109] and due to the fact that some uncertainty about the selectivity of the reduction step existed and the process would take 4 steps to be completed any way, it was decided to abandon the current synthetic route in favour of the Siddaiah approach. 2'-Hydroxy-3,4-dimethoxydihydrochalcone **(126)** was therefore treated with BF_3 etherate before it was reacted with the Vilsmeier reagent (prepared from N,N-dimethylformamide and PCl_5) **(Scheme 15 and 16)** to give 3-(3',4'-dimethoxybenzyl)-

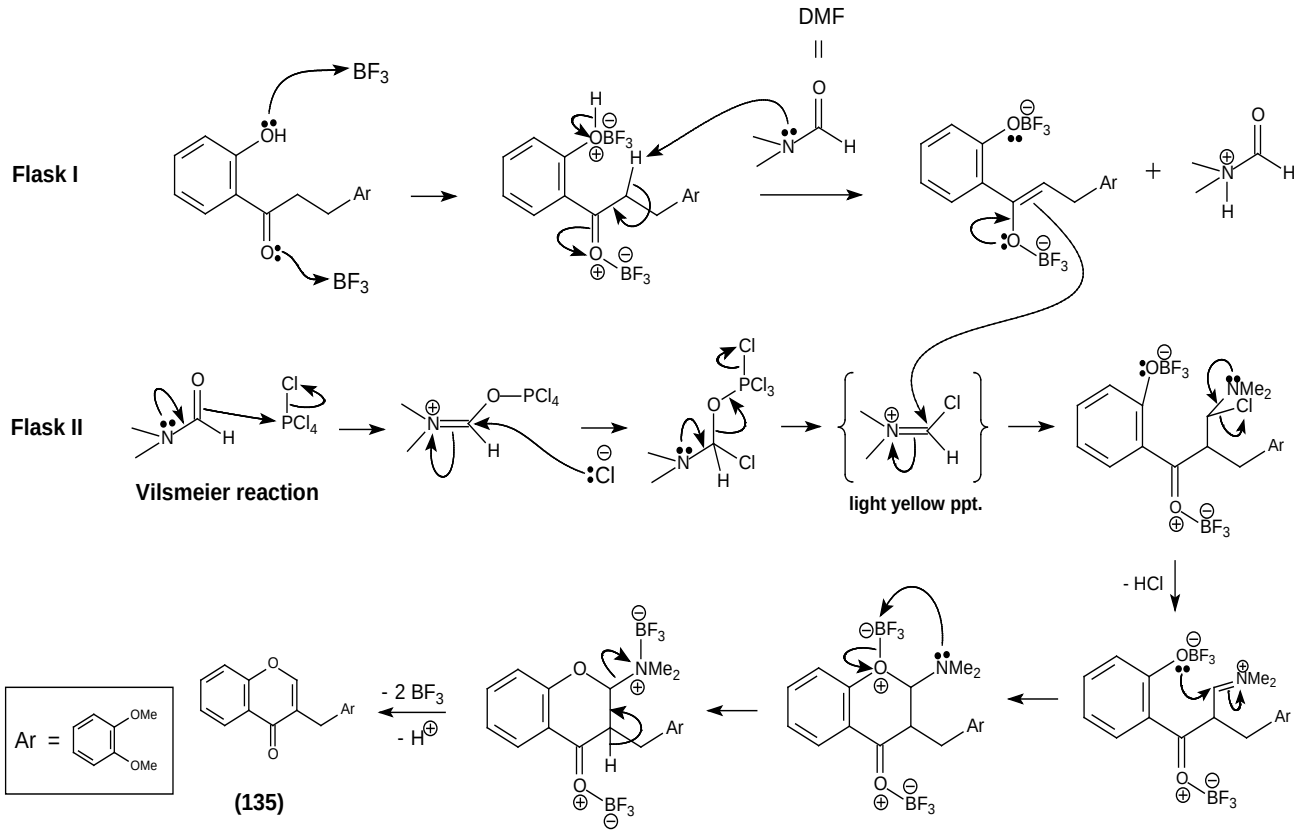
[109] Siddaiah, V.; Rao, C.V.; Venkateswarlu, S.; Subbaraju, G.V. *Tetrahedron* **2006**, *62*, 841.

chromen-4-one **(135)** in 40 % yield, after acidification and work-up.



126 **135**

Scheme 15: (i) DMF/$BF_3 \cdot Et_2O$, DMF/PCl_5, rt.

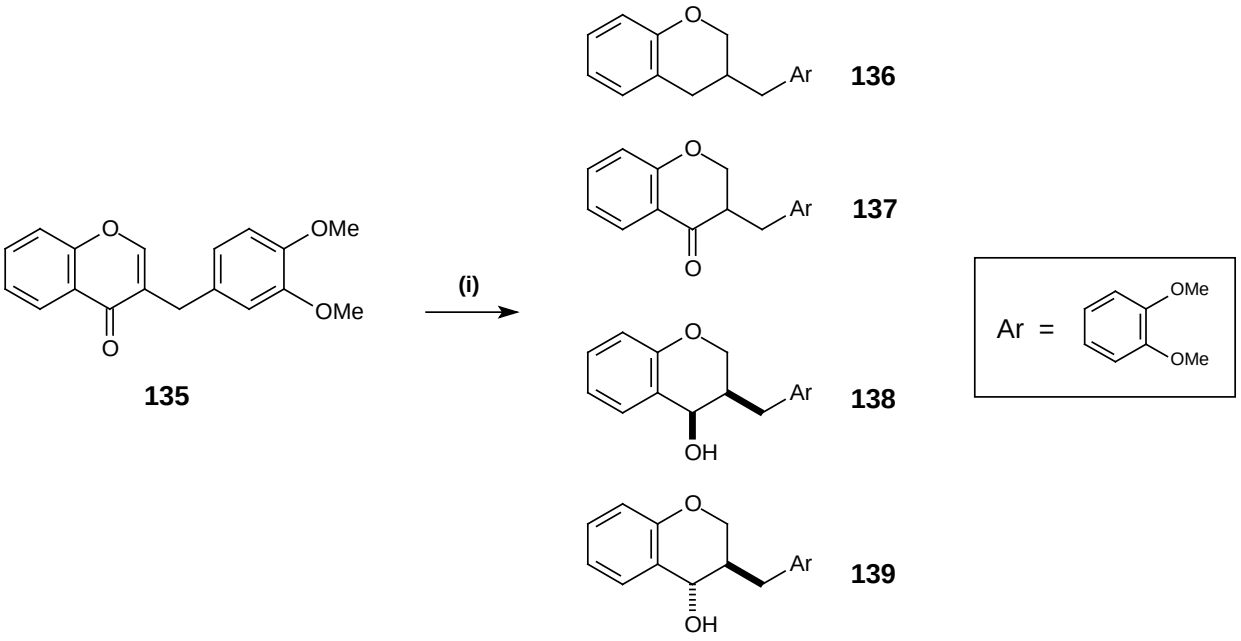


Scheme 16: Mechanism for the formation of product (135).

In addition to the expected aromatic signals, the ^{1}H NMR spectrum **(Plate 13a)** of **(135)** displayed a methylene peak at δ 3.78 (s, 2 H, H-9) and a singlet at δ 7.61 corresponding to the characteristic 2-H resonance of an isoflavone. The ^{13}C NMR spectrum **(Plate 13b)** displayed a carbonyl carbon at δ 177.50 (C-4) as well as an α,β-unsaturated system at δ 112.40 (C-3) and δ 153.10 (C-2) together with a methylene carbon [δ 31.30 (C-9)]; thus confirming **(135)** as the

desired product. An IR stretching frequency at 1638 cm^{-1} also indicated the presence of an α,β-unsaturated ketone, while the MS spectrum showed the molecular ion at *m/z* 297 {$[M+H]^+$}; thus giving additional credence to structure **(135)** as that of the product from the reaction.

In the final step for the preparation of the homoisoflavanone, product **(135)** was hydrogenated in acetone over 10 % Pd/C. Four products of Rf = 0.42 (43 %), Rf = 0.36 (3 %), Rf = 0.26 (3 %), and Rf = 0.22 (3 %) were obtained from the reaction **(Scheme 17)**.



Scheme 17: H_2, 10 % Pd/C, Acetone, rt.

The ^{1}H-NMR spectrum **(Plate 14c)** of the (Rf 0.42) product displayed the signals from three methylene groups at δ 4.19 (ddd, 1 H, *J* 11 and 3 and 2 Hz*)[110] and 3.83 (dd, 1 H, *J* 11 and 9 Hz) (2-CH_2), δ 2.81 (dd, 1 H, *J* 16 and 5 Hz) and 2.52 (dd, 1 H, *J* 16 and 8 Hz)(9-CH_2), and δ 2.66 (dd, 1 H, *J* 14 and 8 Hz) and 2.57 (dd, 1 H, *J* 14 and 8 Hz)(4-CH_2) together with a multiplet at δ 2.31 for H-3 (1 H). Structure **(136)** could therefore be assigned to this product. The ^{1}H-NMR spectrum **(Plate 14a)** of the second product (Rf 0.36) showed very close resemblance to that of the

[110] Arnaud, C. *J. Chem. Ed.* **1974**, *51*, 819.
* W-coupling between H-2 equatorial and H-4 equatorial.

homoisoflavanone natural products **(Plate 3a, 4a and 5a)** and structure **(137)** could therefore be assigned to the second product from the reaction. The ^{13}C NMR spectrum **(Plate 14b)** supported structure **(137)** as that of the second product, since it displayed two additional alkane carbons at δ 69.4 (C-2) and δ 47.9 (C-3) indicating successful reduction of the double bond. Since a product with the carbonyl group still intact **(137)** as well as one where the carbonyl was completely reduced to a C-4 methylene function **(136)** was already identified from the reaction mixture, it was concluded that the remaining two products might be the two isomeric 4-hydroxy compounds **(138 and 139) (Scheme 17)**. The ^{1}H-NMR spectra **(Plate 14d and 14e)** of the remaining two products were very similar and, apart from the benzylic CH_2 [δ 2.85 (dd, 1 H, *J* 14 and 8 Hz) and 2.65 (dd, 1 H, *J* 14 and 8 Hz)(9-CH_2) and δ 2.67 (dd, 1 H, *J* 14 and 7 Hz) and 2.51 (dd, 1 H, *J* 14 and 9 Hz)(9-CH_2) respectively], showed another methylene group at δ 4.12 (m, 2 H, 2-CH_2) and δ 4.25 (dd, 1 H, *J* 12 and 3 Hz) and 4.01 (dd, 1 H, *J* 12 and 4 Hz)(2-CH_2) respectively. The methylene groups were accompanied by a set of methine protons at δ 4.54 (d, 1 H, *J* 3 Hz, H-4) and 2.33 (m, 1 H, H-3) and δ 4.52 (d, 1 H, *J* 4 Hz, H-4) and 2.23 (m, 1 H, H-3) respectively, in each compound. It was therefore concluded that the last two products were in fact the *cis*– and *trans*-4-hydroxy-3',4'- dimethoxyhomoisoflavans **(138)** and **(139)**, arising from partial reduction of the C-4 carbonyl group.

Although the desired product **(137)** was obtained from the hydrogenation of the 3-benzylisoflavone in only 3% yield, this result is explicable in terms of over-reduction probably due to the small scale (30 mg) on which the reaction was executed resulting in a relatively large excess of Pd-catalyst having been used. Since it was only a model reaction, the reaction was, however, not repeated on larger scale as it already served the purpose of establishing that a homoisoflavanone can indeed be formed in this way. With methodology for the synthesis of a homoisoflavanone from a chalcone in hand, attention during the candidate's PhD study will be directed at the synthesis of the required acetophenone **A** to complete the final structural proof for the novel homosioflavanone **(94)** isolated form *Scilla natalensis* during the current investigation.

CHAPTER 7

7. Synthesis of selected Procyanidins through C-4 functionalization of Flavan-3-ols

7.1 Introduction

It has been known for many decades that flavonoids demonstrate potentially significant biological activities such as antiviral, antibacterial, molluscidal, enzyme-inhibiting, anti-oxidant and radical-scavenging properties.[111] Owing to the renewed interest in the biological and pharmaceutical properties of natural products the study of flavonoids and proanthocyanidins in particular, has lately received renewed attention from researchers around the world. Since the 3-dimensional structure and thus stereochemistry of physiologically active compounds is of paramount importance to the activity of these compounds, determining the absolute configuration of these compounds is therefore equally important.

Although Circular Dichroism (CD), which is measured in the near to far UV ranges (250 – 350 and 190 – 250 nm), still finds widespread application as the technique of choice for determining the absolute configuration of flavonoids[112] and proanthocyanidins[113,114,115], this method is hampered by several ambiguities, like a host of empirical rules, dependence of the relative configuration as well as CE on a single phenomenon, i.e. the conformation of the C-ring, only two major transitions ($\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$) for a number of chromophores to work with, etc. (*cf*. Chapter 4). While the system of correlating the absolute configuration at all the chiral centres of a molecule to the observed CE is not ideal for monomeric compounds, the situation becomes even more

[111] De Bruyne, T.; Pieters, L.; Deelstra, H.; Vlietinck, A. *Biochem. System. Ecol*. **1999**, *27*, 445.
[112] Lévai, A. Acta Chimica Slovenica **1998**, *45*, 267.
[113] Barrett, M.W.; Klyne, W.; Scopes, P.M.; Fletcher, A.C.; Porter, L.J.; Haslam, E. *J Chem Soc.* Perkin Transactions 1 **1979**, 2375.
[114] Botha, J.J.; Ferreira, D.; Roux, D.G. *J Chem Soc.* Perkin Transactions 1 **1981a**, 1235.
[115] Calzada, F.; Cerda-Garcá-Rojas, C.M.; Meckes, M.; Cedillo-Rivera, R.; Bye, R.; Mata, R. *Journal of Natural Products* **1999**, *62*, 705.

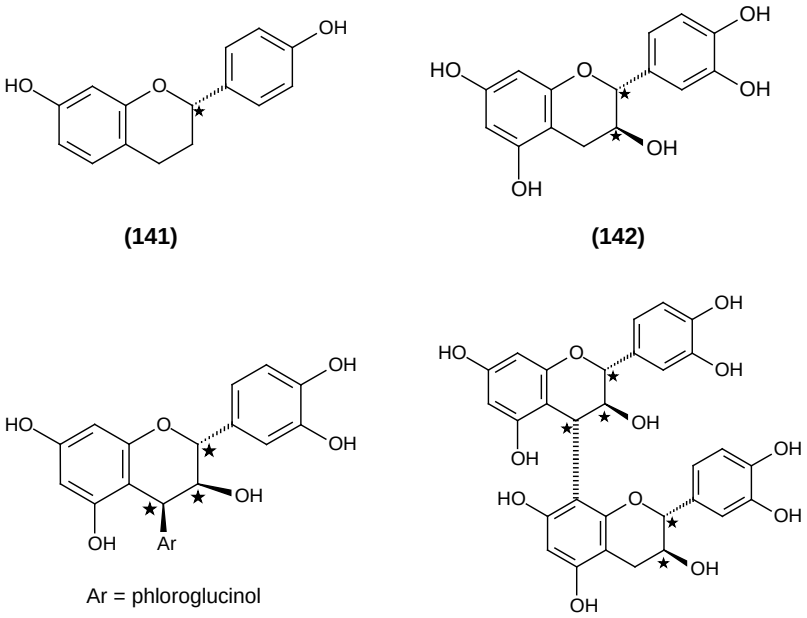
complicated when the absolute configuration of oligomeric compounds, having 4 or more stereo centres as well as more than one heterocyclic ring, is to be determined.

In an effort to find an alternative method of determining the absolute configuration at all the stereo centres of for instance proanthocyanidins, it was decided to investigate the application of the modern technique of vibrational circular dichroism (VCD) to this problem.[116] In this approach, Molecular Modelling would be used to calculate the relative energies of the different conformations which would then be used to determine the contribution of each conformation to the 'real' conformation of the molecule. This result can then be used to calculate the Infrared (IR) spectrum of the molecule, which can be related to the absolute configuration by VCD. Since IR, which is based on vibrational frequencies of functional groups in the molecule, is more sensitive to the specific environment of the functional group, it is envisaged that similar, but not the same chromophores would lead to different absorption frequencies in the IR and thus result in more information regarding the absolute configuration at the different stereo centres.

In order to generate a data base and eventually apply the technique of VCD to the stereochemistry of proanthocyanidins, it was decided to start the investigation from a flavonoid with only one chiral centre **(141)** and systematically increase the number of stereo centres until the level of oligomeric compounds is reached **(Figure 46)**. In this regard a range of optically active flavonoids with an increasing number of stereo centres had to be synthesised, preferentially in free phenolic form. Since (+)-catechin [(2*R*,3*S*)-(+)-3,3',4',5,7-penta-hydroxyflavan, **(145)**] and (-)-epicatechin [(2*R*,3*R*)-(-)-3,3',4',5,7-penta-hydroxyflavan, **(146)**] are freely available in optical active form from nature and can be transformed into their respective enantiomers, the whole synthetic endeavour was based on these compounds. The synthetic effort which forms the major part of this chapter was therefore mainly aimed at the functionalization of (+)-catechin and (-)-epicatechin in the

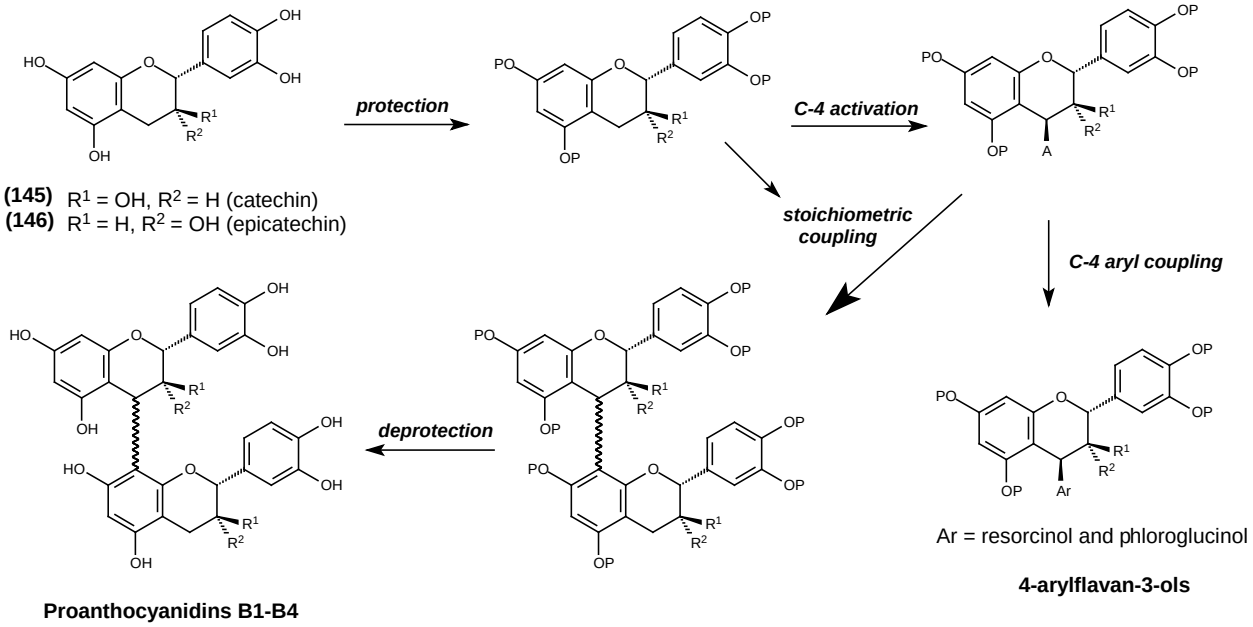
[116] Stephens, P.J. *J. Phys. Chem.* **1985**, *89*, 748.

4-position followed by the synthesis of 4-arylflavan-3-ols and the proanthocyanidins B1 to B4 **(Scheme 18)**, with the preparation of the flavan and the other oligomeric proanthocyanidins to receive attention at a later stage.



(141) **(142)** **(143)** **(144)**

Figure 46: Examples of different chiral centres molecules.

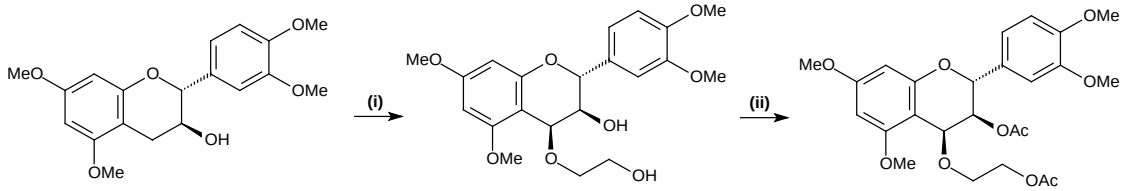


Scheme 18: Synthesis of 4-arylflavan-3-ols and the proanthocyanidins B1 to B4.

7.2 Synthesis of 4-arylflavan-3-ols

7.2.1 Functionalisation of catechin and epicatechin

In order to be able to synthesise the required compounds with three and five stereo centres by coupling the two catechin epimers **(145** and **146)** to with different nucleophiles, it was required to functionalise these compounds in the 4- position in such a way that a carbocation could be generated at this position when it is to be reacted with the nucleophiles. Since DDQ in conjunction with several alcohols[117] have been used in this regard, the methodology as described by Tückmantel *et al*.[118] and Saito *et al*.[119] was utilised in the activation reaction. Thus (+)-per-*O*-methylcatechin **(147)** was treated with DDQ and ethylene glycol in DCM **(Scheme 19)**[120] and the 4-hydroxyethoxy compound **(148)** obtained in 46 % yield.



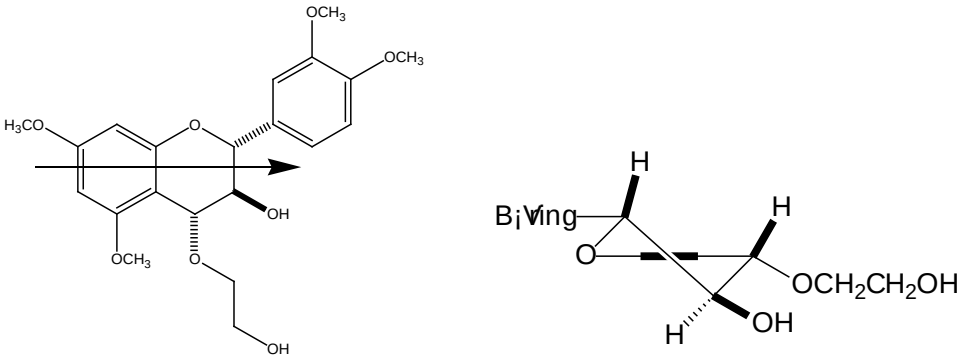
(147) **(148)** **(149)**

Scheme 19: C-4 functionalization of methylated catechin (i) DDQ, ethylene glycol, DCM, DMAP (ii) Acetic anhydride, pyridine, 60 ^{0}C, overnight.

In agreement with literature values, the ^{1}NMR spectrum **(Plate 17a)** of **(148)** showed two methylene groups at δ 3.98 (m) and δ 3.80 (m), thus confirming the presence of the hydroxyethoxy group. Coupling constants of $^3J_{2,3}$ = 10.4 Hz and $^3J_{3,4}$ = 3.4 Hz displayed by the heterocyclic protons indicated the product to have a 2,3-*trans*-3,4-*cis* relative configuration. Acetylation of the product **(148)** from the oxidation reaction finally confirmed the presence of the

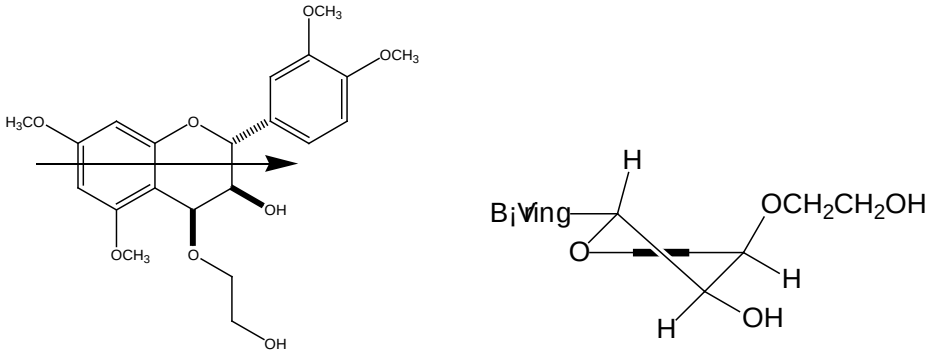
[117] Steenkamp, J.A.; Ferreira, D.; Roux, G. *Tetrahedron Lett.* **1985**, *26*, 3045.
[118] Tückmantel, W.; Kozikowski, A.P.; Romanczyk, Jr.L.J. *J. Am. Chem. Soc.* **1999**, *121*, 12073.
[119] Saito, A.; Nakajima, N.; Tanaka, A.; Ubukata, M. *Tetrahedron* **2002**, *58*, 7829.
[120] Tarascou, I.; Barathieu, K.; André, Y.; Pianet, I.; Dufourc, E.J.; Fouquet, E. *Eur. J. Org. Chem.* **2006**, 5367.

hydroxyethoxy group by displaying two acetoxy resonances at δ 2.08 and 1.91 (each s) in the ^{1}H-NMR spectrum **[(149)** and **Plate 17b]**.



Scheme 20: C-ring conformation of the 4α isomer of the hydroxyethoxy product (148).

On assuming half-chair E-conformations **(Scheme 20** and **21)** for both orientations (α and β) of the hydroxyethoxy group in the product, Molecular Modelling indicated $J_{2,3}$ and $J_{3,4}$ coupling constants of 10 and 4 Hz for the 2,3-*trans*-3,4-*cis*- isomer and both these J-values to be 10 Hz for the 2,3-*trans*-3,4-*trans* product.[121] Based on the E-conformer assumption, this strongly suggests the product to have a 4β substituent making it a rare example of a 2,3-*trans*-3,4-*cis* procyanidin equivalent.

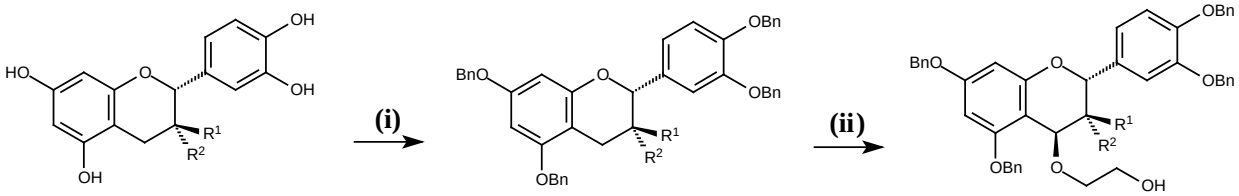


Scheme 21: C-ring conformation of the 4β isomer of the hydroxyethoxy product (148).

In order to have the free phenolic compounds available when required, it was decided to use benzylation as protection for the aromatic hydroxyl functions since it is fairly stable towards basic-

[121] Baig, M.I.; Clark-Lewis, J.W.; Thompson, M.J. *Australian Journal of Chemistry* **1969a**, *22*, 2645.

and acidic conditions and can easily be removed when required by hydrogenolysis. Thus catechin and epicatechin were subjected to the standard benzylation conditions **(Scheme 22)** and the products **(150)** and **(151) (Plates 15** and **16)** obtained in 65 and 72 % yields respectively. Subsequent C-4 functionalization of the benzylated flavan-3-ols **(150)** and **(151)** under conditions similar to that used of the methylated catechin afforded 60 and 50 % yields of the 4-ethoxy catechin **(152)** and epicatechin **(153)** analogues respectively.



(145) R^1 = OH, R^2 = H (catechin)
(146) R^1 = H, R^2 = OH (epicatechin)

(150) R^1 = OH, R^2 = H
(151) R^1 = H, R^2 = OH

(152) R^1 = OH, R^2 = H
(153) R^1 = H, R^2 = OH

Scheme 22: Benzylation and oxidation of catechin and epicatechin: (i) K_2CO_3, BnBr, DMF (ii) Ethylene glycol, DDQ, DMAP, DCM.

The ^{1}H-NMR spectra **(Plates 18** and **19)** of **(152)** and **(153)** displayed two sets of methylene protons at δ 3.98 - 3.78 (m) and 3.72 - 3.64 (m) for the catechin derivative and a single multiplet integrating for four protons at δ 3.84 - 3.60 in the case of the epicatechin analogue. In addition to the presence of the hydroxyethoxy group the spectra displayed coupling constants of δ 4.83 (d, *J* 3.6 Hz, H-4) and δ 5.05 (d, *J* 11.3 Hz, H-4) respectively, indicative of 3,4-*cis* stereochemistry for the catechin derivative and 3,4-*trans* relative configuration for the epicatechin derivative. Interestingly the orientation of the 3-OH group seems to have no influence on the stereochemical outcome of the reaction, since both isomers of the starting material gave the same 4*S* product. The complete diastereoselectivity of the reaction is probably explicable in term of selective DDQ abstraction of the 4-α hydrogen as the hydride, which can be explained by assuming steric interaction between the C-2 hydrogen having a β-axial orientation (the 2-phenyl group preferring to be in the equatorial position) for both catechin and epicatechin. The incoming oxidant (DDQ) will thus be directed towards the α-face of the C-ring, leaving the β-face open for attack by the nucleophile.

7.2.2 Coupling of functionalized catechin and epicatechin derivatives with phloroglucinol

With both the C-4 functionalized catechin isomers in hand, attention was subsequently turned towards preparing the substrates containing three stereogenic centres, i.e. the 4-arylflavan-3-ols **[(143)**; **(Figure 46)]** and **[(154)**; **(Scheme 23)]**. While both Lewis - and protic acids,[120,122,123] as well as clay catalysts[124,125] have been utilised in this type of reaction, it was decided to start the process for the synthesis of the required 4-arylflavan-3-ols with protic acid catalysis.

Since 0.1 M HCl catalysed reactions of flavan-3,4-diols and their 4-methoxy analogues with free phenolic phloroglucinol and resorcinol represents the standard methodology for the formation of 4-arylflavan-3-ols and proanthocyanidins[126], this was the reagent of choice for the first coupling reactions. In order to avoid any complexities in the NMR spectra of the products and not run the risk of partial debenzylation occurring during the reaction, the methylated catechin derivative **(148)** was utilised in the first coupling reaction. Since the methylated catechin would not be that soluble in water, the standard solvent for these reactions, a non-nucleophilic solvent, 2,2,2-trifluoroethanol (TFE), together with p-toluene-sulphonic acid as catalyst, was employed in the first coupling reaction with phloroglucinol **(Scheme 23)**. Although the product, 2,3-*trans*-3,4-*trans*-3',4',5,7-tetra-*O*-methyl-4-(2",4",6"-trihydroxyphenyl)-catechin **(154)** was formed, the yield was disappointingly low (6 %). The ^{1}H-NMR spectrum of the product **(154)** in acetone-d6 **(Plate 20a)** displayed severe line broadening in the aromatic region as well as duplication of methoxy signals, but the heterocyclic protons could clearly be identified at δ 5.23 (dd, $^{3}J_{2,3}$ = 8 Hz, $^{3}J_{3,4}$ = 6 Hz, H-3), 5.08 (d, $^{3}J_{3,4}$ = 6 Hz, H-4), and 4.71 (d, $^{3}J_{2,3}$ = 8 Hz, H-2); the

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[122] Kawamoto, H.; Nakatsubo, F.; Murakami, K. *Mokuzai Gakkaishi* **1991**, *37*, 488.

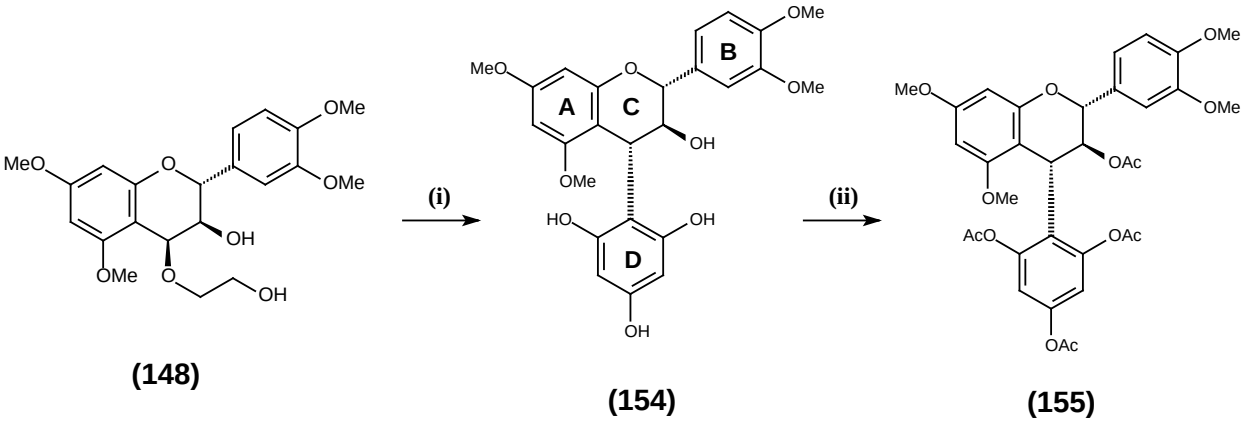
[123] Saito, A.; Emoto, M.; Tanaka, A.; Doi, Y.; Shoji, K.; Mizushina, Y.; Ikawa, H.; Yoshida, H.; Matsuura, N.; Nakajima, N. *Tetrahedron* **2004**, *60*, 12043.

[124] Kozikowski, A.P.; Tückmantel, W.; Böttcher, G.; Romanczyk, L.J. *J. Org. Chem*. **2003**, *68*, 1641.

[125] Sharma, P.K.; Kolchinski, A.; Shea, H.A.; Nair, J.J.; Gou, Y.; Romanczyk, L.J.; Jr.; Schmitz, H.H. *Organic Process Research & Development.*

[126] Botha, J.J.; Ferreira, D.; Roux, D.G. *J. Chem. Soc., Chem. Commun.* **1978**, 700.

coupling constants of H-2 and H-4 being indicative of a 2,3-*trans*-3,4-*trans* configuration.[127,128] The aromatic region of the ^{1}H NMR spectrum **(Plate 20b)** in $CDCl_3$ solution, however, displayed less line broadening and the appropriate signals, i.e. a set of *meta*-doublets [δ 6.00 (d, 1 H, *J* 2 Hz, H-8) and δ 5.96 (d, 1 H, *J* 2 Hz, H-6)] for the A-ring and a broad singlet [δ 6.28 (br, 2 H, H-3" and H-5")] for the free phenolic D-ring could clearly be identified. In addition to these signals, the four methoxy resonances [δ 4.03 - 3.78 (each s)] were also clearly visible as singlets, but the resonances from H-2 and H-4 were overlapping and appeared as a multiplet (δ 5.00 – 4.90). The structure of the product **(154)** was finally confirmed by acetylation with the NMR spectrum **(Plate 20c)** of the methylether acetate **(155)** displaying four acetoxy signals as singlets at δ 2.30 - 1.25 ppm.



(148) **(154)** **(155)**

Scheme 23: (i) phloroglucinol, pTSA, CF_3CH_2OH, rt (ii) Acetic anhydride, pyridine. 60 °C.

Since the reaction between phloroglucinol and the functionalised permethyl ether of catechin **(148)** using PTSA as catalyst in 2,2,2-trifluoroethanol gave very low yields, the catalyst was changed to $TiCl_4$ that was described in the paper by Fouquet *et al*.[120] when the reaction with the benzylated catechin was attempted. No visible reaction (TLC) could however be detected, so the catalyst was changed back to PTSA (in TFE), which led to the formation of some product (yield: 28 %) **(Scheme 24)**.

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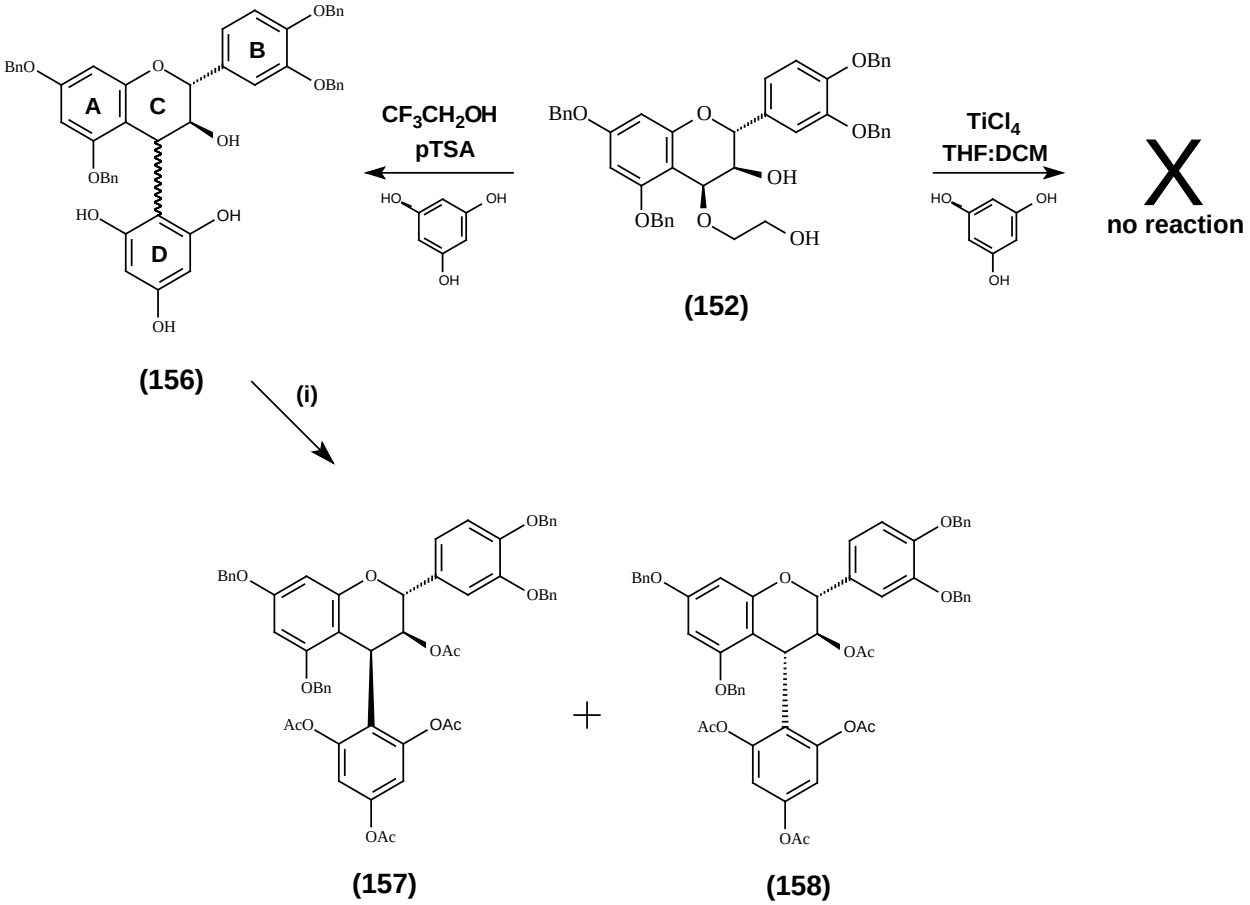
[127] Baig, M.I.; Clark-Lewis, J.W.; Thompson, M.J. *Aust. J. Chem.* **1969a**, *22*, 2645.
[128] Baig, M.I.; Clark-Lewis, J.W.; Jemison, R.W.; Thompson, M.J. *J. Chem. Soc*. **1969b**, *14*, 820.



Scheme 24: (i) Acetic anhydride, pyridine, 60 °C, overnight.

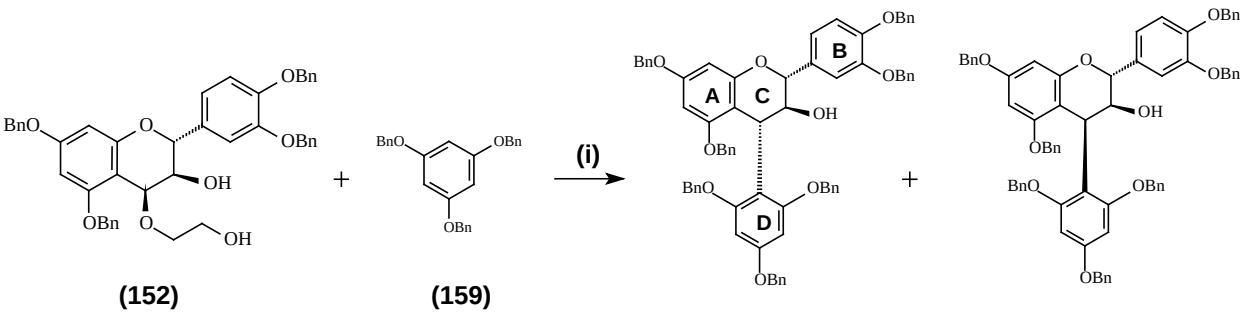
Although the ^{1}H NMR spectrum **(Plate 21a)** of the product showed considerable broadening and duplication of resonances, signals from the A- and D-rings could be identified in the aromatic region [δ 6.30 (m, 2 H, H-6, H-8) and δ 6.01 (m, 2 H, H-3'', H-5'')] thus leading to the confidence that the right product was indeed formed during the reaction. Acetylation and subsequent further purification of the initial product led to the isolation of two products R*f* 0.45 and 0.27 (T:EA, 9:1) in 12 and 10 % yield respectively. The ^{1}H-NMR spectrum **(Plate 21b)** of the first product (R*f* 0.45) clearly showed the two sets of *meta*-coupled resonances associated with the A- and D-rings respectively [δ 6.20 (d, J 2.0 Hz, H-8); and 6.15 (d, J 2.0 Hz, H-6) and δ 6.89 (d, J 2.0 Hz, H-3''); and 6.79 (d, J 2.0 Hz, H-5'') respectively] as well as the heterocyclic protons at δ 5.42 (dd, J 10.0, and 6.0 Hz, H-3); 5.27 (d, J 10.0 Hz, H-2), and 4.92 (d, J 6.0 Hz, H-4) indicating it to be the 2,3-*trans*; 3,4-*cis*-product **(157)**. Apart from the required *m*-doublets for the A- and D-rings [δ 6.19 (d, J 2.0 Hz, H-8); and 6.16 (d, J 2.0 Hz, H-6) and δ 6.80 (d, J 2.0 Hz, H-3''); 6.58 (d, J 2.0 Hz,

H-5")], the ^{1}H-NMR spectrum **(Plate 21c)** of the second acetate **(158)** indicated the coupling constants of both H-2 and H-4 on the C-ring protons [δ 4.75 (H-2) and 4.69 (H-4)] to be 10.0 Hz, thus defining this product as the 2,3-*trans*-3,4-*trans*-isomer from the reaction.[128] Since $TiCl_4$ is known to be very oxophilic and reacts violently with water and alcohols, the failure of the $TiCl_4$ catalysed reaction is probably explicable in term of the catalyst showing preference for reaction with the phloroglucinol and therefore did not activate the functionised catechin to the required extent for the reaction to proceed.

Since Fouquet *et al*.[120] and Kozikowski *et al.*[124] used $TiCl_4$ and Bentonite K-10 clay respectively together with benzylated nucleophiles in their coupling reactions, it was decided to abandon the free phenolic phloroglucinol approach in favour of the benzylated equivalent **(159)** in subsequent coupling reactions. Thus treatment of the functionalised perbenzylated catechin **(152)** and benzylated phloroglucinol **(159)** with $TiCl_4$ in THF:DCM gave the 2,3-*trans*-3,4-*trans* product **[(160)** and **(Plate 22)]**, in 65 % yield, which was accompanied by trace amounts of a second compound, probably the 2,3-*trans*-3,4-*cis* isomer **(161)**, that could not be fully characterised due to lack of material. Since Bentonite K-10 clay was not available at this stage of the work, various other catalysts were evaluated for the reaction **(Table 21** and **Scheme 25)**. Only Bentonite K-10, when it eventually arrived, however, showed some reaction and led, in agreement with the results of Kozikowski, to almost exclusive formation of the 2,3-*trans*-3,4-*trans* isomer of the product **(160)** in 60 % yield.

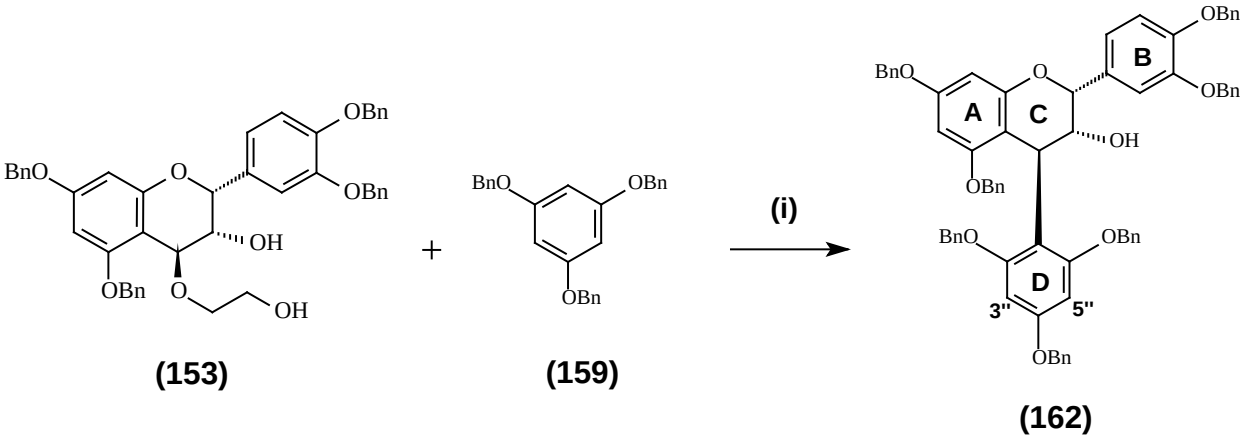
Table 21: Various catalysts evaluated for the reaction.

(i)	Catalyst	Result	Yield
1	$TiCl_4$, (THF:DCM)	2,3-*trans*-3,4-*trans* isomer **(160)**	65 %
2	Bentonite, DCM	No reaction observed	
3	Montmorillonite K-10, DCM		
4	Amberlyst 15, DCM		
5	pTSA, TFE	Several products in small quantities	
6	Bentonite K-10, DCM	2,3-*trans*-3,4-*trans* isomer **(160)**	60 %



Scheme 25: Catalyst evaluation for coupling of functionalised perbenzylated catechin (152) with benzylated phloroglucinol.

With the reaction conditions sorted out, attention was subsequently turned towards synthesising the other compound for completing the series of products with three stereo centres. Functionalized perbenzylated epicatechin **(153)** was therefore reacted with benzylated phloroglucinol **(159)** under Bentonite K-10 catalysis resulting in the formation of 2,3-*cis*-3,4-*trans*-3',4',5,7-tetra-*O*-benzyl-4-(2",4",6"-tribenzyloxyphenyl)-epicatechin **(162)** in a 55% yield **(Scheme 26)**. The *cis*- *trans*-isomer was accompanied by another product, probably the *cis*-*cis*-isomer, but due to insufficient material it could not be fully characterised.



Scheme 26: Coupling of functionalised perbenzylated epicatechin (153) with benzylated phloroglucinol (i) Bentonite K-10, DCM.

Although the ^{1}H NMR spectrum **(Plate 23)** of compound **(162)** showed considerable line broadening and duplication of signals due to restricted rotation around the interflavanyl bond, two sets of peaks originating from the *meta*-coupled protons were clearly visible at δ 6.35 (d, 1 H, J 2.0 Hz, H-8); 6.23 (d, 1 H, J 2.0 Hz, H-5"); 6.20 (d, 1 H, J 2.0 Hz, H-3") and 6.03 (d, 1 H, J 2.0 Hz, H-6) in the aromatic region, while the heterocyclic protons could be identified by resonances at δ 5.35 (d, 1 H, J 1.0 Hz, H-2); 4.45 (d, 1 H, J 12.0 Hz, H-4) and 4.02 (m, 1 H, H-3), thus confirming the product to be the 2,3-*cis*-3,4-*trans*-3',4',5,7-tetra-*O*-benzyl-4-(2",4",6"-tribenzyloxyphenyl)-epicatechin **(162)**. The formation of only the 2,3-*cis*-3,4-*trans*-isomer of the product can be explained in terms of steric hindrance where the α-orientation of both the substituents at C-2 and C-3 hampers attack of the incoming nucleophile towards this face of the molecule and is in agreement with literature reports[129] for reactions of epicatechin with several nucleophiles.

The relative low yield obtained in this reaction, when compared to literature values (72 – 76 %), is probably explicable in terms of possible oligomer formation due to a lower nucleophile to electrophile ratio (only 4 : 1) than that reported (between 5 and 10 to 1). This could lead to the D-ring, through its unsubstituted C-3 or C-5 positions, acting as a competing nucleophile during the reaction.

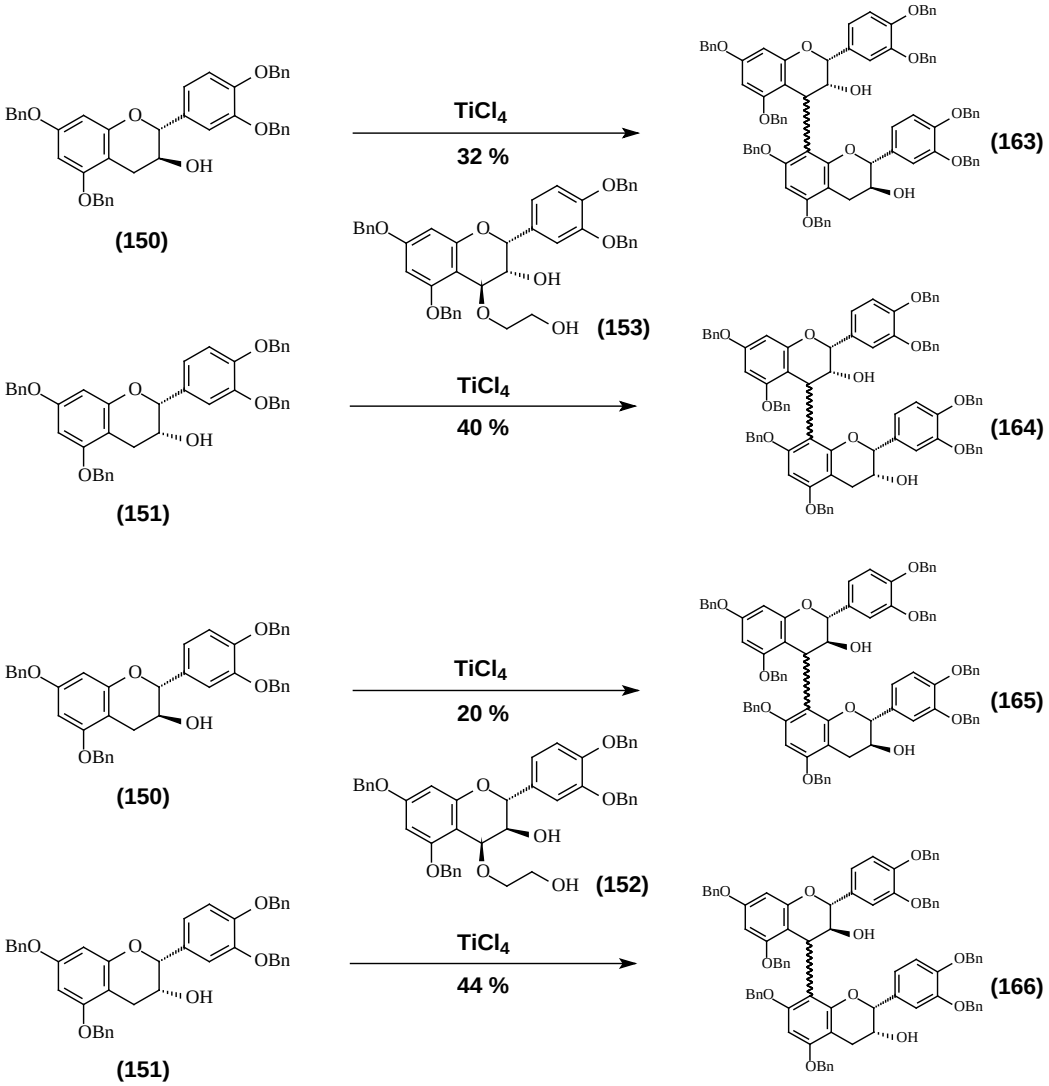
7.3 Synthesis of perbenzyl-procyanidin B1, B2, B3, and B4

Finally the synthesis of the compounds containing five stereogenic centres could be attempted and functionalised perbenzylated epicatechin **(153)** was reacted with benzylated catechin **(150)** and benzylated epicatechin **(151)** under $TiCl_4$ catalysis* to give the benzylated analogues of procyanidins B1 **(163)** and B2 **(164)** in 32 and 40 % yield respectively. This reaction was followed by the reaction of 3',4',5,7-tetra-*O*-benzyl-4β-(2-hydroxyethoxy)-catechin **(152)** with benzylated

[129] Kozikowski, A.P.; Tückmantel, W.; Hu, Y. *J. Org. Chem*. **2001**, *66*, 1287.

* Since $TiCl_4$ gave a slightly better yield for the reaction with phloroglucinol, this catalyst was used in subsequent reactions.

catechin **(150)** and benzylated epicatechin **(151)** under the same conditions leading to the benzylated procyanidins B3 **(165)** (20 % yield) and B4 **(166)** analogues (44 % yield) **(Scheme 27)**. The ^{1}H-NMR **(Plates 24-27)** of the synthesized perbenzyl-procyanidins B1 to B4 analogues **(163-166)** were complicated by rotational restriction to the extent that only the signals from the 4-methylene group of the 'lower unit' {δ 2.67 (dd, 1 H, *J* 16.3 and 8.8 Hz, H-4'') and 2.60 (dd, 1 H, *J* 16.8 and 9.8 Hz, H-4'')-[B1]; δ 3.00 (m, 2 H, 2 x H-4'')-[B2]; δ 2.72 (dd, 1 H, *J* 16.5 and 8.1 Hz, H-4'') and 2.41 (dd, 1 H, *J* 16.5 and 9.1 Hz, H-4'')-[B3]; δ 2.69 (m, 2 H, 2 x H-4'')-[B4]} could clearly be identified, thus confirming that reaction between the nucleophile and electrophile actually occurred. Full characterisation of the products will be done through proper derivatisation (on the methylether acetates), after removal of the benzyl protecting groups during the candidate's PhD study (*vide infra*).



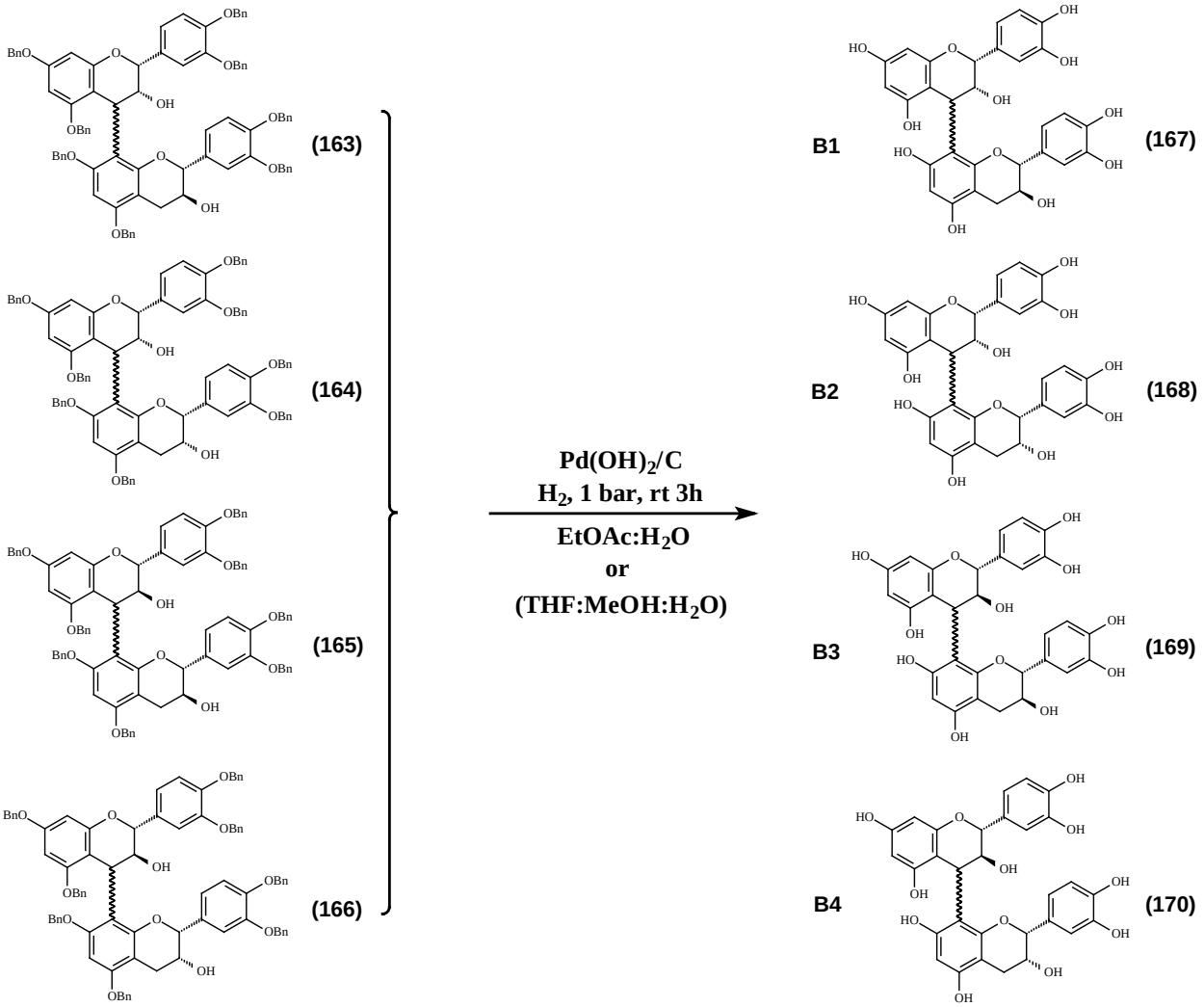
Scheme 27: Synthesis of perbenzyl-procyanidins B1-B4 *via* $TiCl_4$-catalysis.

7.4 Reductive removal of benzyl protecting groups

With all of the target compounds available as perbenzyl ethers, the only remaining step towards obtaining these compounds in the required in free phenolic form that would allow correlation of their VCD spectra with those of natural products, would be removal of the benzyl groups. After evaluating various conditions for removal of the benzyl groups, Sharma *et al*.[130] concluded that quantitative debenzylation was indeed possible through utilization of 20% $Pd(OH)_2/C$ as catalyst. In order to improve on the yields, these workers also found that the best results could be achieved if EtOAc:H_2O (1:1) is used as solvent. In this instance the benzylated starting material would be completely soluble in the ethyl acetate phase, while the free phenolic procyanidins would be insoluble in the organic phase, but fully soluble in the water, thus eliminating a separation step at the end of the reaction to get rid of any remaining partially benzylated products **(Scheme 28)**.

Utilisation of this methodology will therefore form the basis of the hydrogenation of the 4-arylderivatives as well as B1 to B4 in order to prepare these compounds as free phenols in a subsequent PhD investigation. Once available in free phenolic form the VCD spectra of all the target molecules could be obtained, while full characterisation (as methylether acetates) would also be possible for the prepared B1 to B4. In order to have the full series of procyanidins (B1 to B8) available for VCD analysis the remaining members of the series (B5 to B8) will also be synthesised according to the methodology utilised thus far as part of the candidate's PhD studies.

[130] Sharma, P.K.; Kolchinski, A.; Shea, H.A.; Nair, J.J.; Gou, Y.; Romanczyk, L.J.; Jr.; Schmitz, H.H. *Organic Process Research & Development.*



Scheme 28: Hydrogenation of perbenzyl-procyanidins B1-B4.

CHAPTER 8

8. Standard experimental techniques

8.1 Chromatographic techniques

8.1.1 Paper chromatography

Two-dimensional paper chromatograms were conducted on Whatman no 1 paper (28.5 X 46 cm) in two directions, using water-saturated butan-2-ol in the first direction and 2% (v/v) acetic acid in the second direction. After development, the chromatograms were air dried and investigated by UV-light and spraying reagents.

8.1.2 Column chromatography

Separations on Sephadex LH-20 were carried out on various column sizes at differing flow rates as specified. The Sephadex LH-20 was prepared by soaking it in the eluant [ethanol or methanol/water (50/50)] for 24-hours. The crude extract in a minimum amount of the eluant was applied to the packed Sephadex LH-20 column. Column fractions were eluted at a flow rate of 1 ml/min, and fractions of 20 ml were collected with an ISCO (model 273) automatic fraction collector.

Merck Kieselgel 60 column chromatography separations were performed on various column sizes at different flow rates under gravity. The glass column was packed with this suspension of Kieselgel 60 and applied the crude extract which already dissolved in a minimum of the appropriate solvents to the column. Columns were eluted at a flow rate of approximately 1

ml/min and fractions of 20 ml were collected with an ISCO (model 273) automatic fraction collector.

Flash column chromatography (FCC) was performed on a glass column (5 cm diameter) charged with 100g of Merck Kieselgel 60 (230-400 mesh) for every 1 g of the crude material. Air was displaced by elution with the appropriate solvent under N_2-pressure (*ca.* ~ 40 kPa). The crude product was dissolved in a minimum of the appropriate solvent and applied to the column. The purified product was recovered by elution under N_2-pressure in 15 ml fractions.

8.1.3 Thin layer chromatography

Qualitative thin layer chromatography (TLC) was performed on pre-coated Merck plastic sheets (silica gel $PF_{254,}$ 0.25 mm). After development, the plates were sprayed with vanillin-H_2SO_4. R_f values reported are those observed in these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 X 20 cm) coated with Kieselgel PF254 (1.0 mm), which were air-dried overnight at room temperature. The plates (loaded with 10-15 mg of material per plate) were developed in an appropriate eluent and dried in a stream of air. The bands were distinguished by UV (254 nm) light and scraped off. Compounds were eluted from the adsorbent with acetone, which was removed on a rotary evaporator under reduced pressure at 40^{o}C. Small-scale separations were conducted on Merck pre-coated (0.25) TLC plates, silica gel 60 F_{254} with each plate charged with 3-5 mg of the crude product.

8.2 Spraying reagents

8.2.1 Vanillin-sulphuric acid

The TLC plates were sprayed lightly with a solution of vanillin (1 g) in concentrated sulphuric acid (100 ml) and subsequently heated (*ca* 100 ^{o}C) to ensure optimum colour development.

8.2.2 Anisaldehyde

Thin-layer chromatograms were sprayed with a solution of anisaldehyde (5 ml) in concentrated sulphuric acid (5 ml) and ethanol (90 ml) and gently heated to 100 ^{o}C to ensure optimum colour development.

8.2.3 Formaldehyde-sulphuric acid[131]

TLC plates were quickly sprayed with 2% (v/v) solution of formaldehyde (37 wt % solution in water) in concentrated sulphuric acid and heated to 120 ^{o}C to ensure maximum development of colour.

8.2.4 Bis-diazotized benzidine[132]

Benzidine (5 g) dissolved in concentrated hydrochloric acid (14 ml) was added to distilled water (980 ml). The mixture (30 ml) was dissolved in sodium nitrite (10 % m/v, 20 ml). Paper chromatograms were gently sprayed with the freshly prepared mixture of this solution and subsequently washed with for 1 hour under running tap water.

8.3 Chemical methods

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131 Saayman, H.M.; Roux, D.G. *Biochem. J.* **1965**, *96*, 36.
132 Roux, D.G.; Maihs, E.A. *J. Chromatogr.* **1960**, *4*, 65.

8.3.1 Acetylation[133]

Dry phenolic material was dissolved in a minimum volume of pyridine and twice the amount of acetic anhydride was added. After 8-12 hours at ambient temperatures, crushed ice was added to precipitate the acetylated material which was filtered and excess pyridine washed out with cold water.

8.4 Drying solvents and reagents

THF was predried with sodium metal, and was refluxed over sodium/benzophenone under N_2 until a dark blue colour persisted with subsequent fresh distillation under N_2 prior to use.

DCM was predried with calcium chloride and was refluxed over calcium hydride under N_2 for 24 hours with subsequent distillation under N_2 prior to use.

DMF was purchased from chemical industry as an anhydrous solvent.

Acetone was left over molecular sieves and was refluxed under N_2 with subsequent distillation under N_2 prior to use.

8.5 Spectroscopical methods

8.5.1 Nuclear magnetic resonance spectrometry (NMR)

NMR spectra were recorded on a Bruker AVANCE DPX_{600} spectrometer with tetramethylsilane an internal standard. The solvents used were deuteriochloroform ($CDCl_3$, δ_H 7.24) and

[133] Kametani, T.; Kano, S. *J. Pharmac. Soc. Japan* **1962**, *82,* 1059.

deuterioacetone [$(CD_3)_2CO$/acetone d_6, δ_H 2.04]. Chemical shifts are reported in parts per million (ppm) on the δ-scale and coupling constants were measured in Hz. Abbreviations are used as follows:

Table 22: Abbreviations used in describing ^1H NMR signal multiplicities.

Abbreviation	Signal multiplicity
s	singlet
d	doublet
t	triplet
dd	doublet of doublets
br	broadened
m	multiplet
ddd	doublet of doublet of doublets

8.6 Abbreviations

The following abbreviations for solvents are used through out the experimental section:

A = acetone

C = chloroform

DCM = dichloromethane

DMF = dimethylformamide

E = diethyl ether

EtOH = ethanol

EA = ethyl acetate

H = hexane

MeO = methanol

THF = tetrahydrofuran

T = toluene

8.7 Infrared spectrometry (IR)

Compounds were prepared with KBr or KI (in the solid state) and examined with an IR spectrometer.

8.8 Finnigan LCQ trap mass spectrometry (MS)

All mass spectra were done by Thermo-Finnigan with reodine injector using electro-spray ionization technique at positive mode.

8.9 Circular dichroism (CD)

CD spectra were recorded on a Jasco J-710 spectropolarimeter with methanol as solvent.

8.10 Freeze-drying

Phenolic material in aqueous solution was freeze-dried using a Virtis Freezemobil 12SL at 40 millitorr.

CHAPTER 9

9. Isolation of compounds from *Scilla natalensis* (Hyacinthaceae)

9.1 Extractions

Samples of the methanol extract of above soil growth (1-AS) and root & bulb parts (2-RB) of *Scilla natalensis* were obtained from Prof. Pretorius at the Department of Oil, Crop and Climate Science at the University of the Free State. While the phytochemical investigation of 2-RB will be addressed in a PhD study, compounds form 1-AS were isolated as follows:

9.2 Compounds from fraction 1-AS

Fraction 1-AS (20 g) was dissolved in ethanol and subjected to column chromatography on Sephadex LH-20 (4 x 150 cm column, flow rate of 10 ml/30 min) with EtOH:H_2O = 9:1 as eluant to give fractions A-J (Table) (TLC analysis EA:M:H_2O, 7:2:1, v/v/v).

Table 23: Fractions obtained from the column separation of AS.

Fraction	Test tubes	Mass (g)
AS-A	1~93	10
AS-B	94~107	2
AS-C	108~130	1.5
AS-D	131~162	1.7
AS-E	163~363	1.1
AS-F	364~553	0.05

Acetylation of fraction AS-E (1.1 g) followed by PLC purification (T:EA:A, 9:0.5:0.5) yielded bands

H-B1 (0.07 g, R*f* 0.48), H-B2 (0.12 g, R*f* 0.62), H-B3 (0.03 g, R*f* 0.55) and H-B9 (0.06 g, R*f* 0.42).

9.2.1 Fractionation of band H-B1

Band H-B1 was purified twice by PLC (T:EA, 9.5:0.5) to give two bands, R*f* 0.56 and R*f* 0.42.

9.2.1.1 3',4'-Di-*O*-acetylchavicol[134] (91)

Band H-B1.5.1 (R*f* 0.56) afforded the title compound as a light yellow amorphous solid (3.6 mg).

^{1}H NMR: Plate 1a

^{13}C NMR: Plate 1b

HMBC: Plate 1c

COSY: Plate 1d

Table 12

9.2.1.2 4',4''-Di-*O*-acetyl-3''-methoxynyasol[135,136,137] (92)

Band H-B1.5.2 (R*f* 0.42) afforded the title compound as a light yellow amorphous solid (5.8 mg).

^{1}H NMR: Plate 2a

^{13}C NMR: Plate 2b

HMBC: Plate 2c

HMQC: Plate 2d

COSY: Plate 2e

NOE: Plate 2f

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134 Shenoy, N.R.; Choughuley, A.S.U. *J. Agric. Food Chem.* **1989**, *37*, 721.
135 Fujita, N.; Yoshimoto, T.; Samejima, M. *Mokuzai Gakkaishi* **1984**, *30*, 264.
136 Tsui, W.Y.; Brown, G.D. *Pergamon* PII: S0031-9422 **(1996**) 00442-6.
137 Zhang, H.J.; Sydara, K.; Tan, G.T.; Ma, C.; Southavong, B.; Soejarto, D.D.; Pezzuto, J.M.; Fong, H.H.S. *J. Nat. Prod*. **2004**, *67*, 194.

Table 13

9.2.2 Fractionation of band H-B2

Band H-B2 was purified twice by PLC (T:EA:A, 9:0.5:0.5) to give three compounds, R*f* 0.58, R*f* 0.43 and R*f* 0.53.

9.2.2.1 5,7-Diacetoxy-3-(4'-methoxybenzyl)-6-hydroxychroman-4-one (93a) or 5,6-diacetoxy-3-(4'-methoxybenzyl)-7-hydroxychroman-4-one (93b)

Band H-B2.10.2 (R*f* 0.58) afforded the chromanone (number) as a *light yellow amorphous solid* (2.6 mg).

^{1}H NMR: Plate 3a

^{13}C NMR: Plate 3b

NOE: Plate 3c

HMBC: Plate 3d

HMQC: Plate 3e

COSY: Plate 3f

Table 14

^{1}H NMR: Plate 3g

Table 15

IR (KBr) 3735, 2932, 2359, 1744, 1664, 1368, 1181, 1008, 902, 666 cm^{-1}

MS (CI) *m/z* 400.38 (M^{+}), (EI) 279 (10%), 193 (5%), 179 (5%), 167 (20%), 149 (60%), 125 (20%)

9.2.2.2 5,7-Diacetoxy-3-(3'-acetoxy-4'-methoxybenzyl)-chroman-4-one[138] (95)

[138] Silayo, A.; Ngadjui, B.T.; Abegaz, B.M. *Phytochemistry* **1999**, *52*, 947.

Band H-2.10.4 (R*f* 0.43) afforded the title compound as a white amorphous solid (4.5 mg).

^{1}H NMR: Plate 4a

NOE: Plate 4b

COSY: Plate 4c

Table 16

9.2.2.3 ***5,6,7-Triacetoxy-3-(3',4'-dimethoxybenzyl)-chroman-4-one*** **(96)**

Band H-2.7.2 (R*f* 0.53) afforded the title compound as a *light yellow amorphous solid* (3.7 mg).

^{1}H NMR: Plate 5a

NOE: Plate 5b

COSY: Plate 5c

^{13}C NMR: Plate 5d

Table 17

IR (KI) 2924, 2853, 2359, 1767, 1692, 1610, 1514, 1464, 1371, 1273, 1197, 1125, 1017 cm^{-1}

MS (CI) *m/z* 472.44 (Na^{+}), (EI) 511.5 (5%), 495.5 (100%), 490.6 (45%), 453.6 (15%), 431.5 (40%), 389.5 (35%)

9.2.3 **4'-*O*-acetyl-5,7-di-*O*-methylnaringenin[139] (97)**

Band H-B3 was purified twice by PLC (T:A:M, 9:0.5:0.5) to give the title compound as a white amorphous solid (R*f* 0.65, 1.5 mg).

^{1}H NMR: Plate 6a

NOE: Plate 6b

Table 18

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[139] Shoja, M.; Kristallogr, *Z. New cryst. Struct.* **1997**, *212*, 127.

9.2.4 2”,3”,4”,5,6”-Penta-*O*-acetyl-4’-*O*-methylapigenin-7-*O*-*β*-D-glucopyranoside[140] (98)

Band H-B9 was purified by PLC (T:A:M, 9:0.5:0.5) to give the title compound as a white amorphous solid (R*f* 0.54, 4.8 mg).

^{1}H NMR: Plate 7a ($CDCl_3$)

NOE: Plate 7b

COSY: Plate 7c

^{1}H NMR: Plate 7d (C_6D_6)

Table 19

[140] Kaloshina, N.A. *Khimiya Prirodnykh Soedinenii* **1988**, *3*, 453.

CHAPTER 10

10. Synthesis of 3-(3',4'-dimethoxybenzyl)-chroman-4-one (137)

The assignment of carbon peaks was based on spectra modelled with CS ChemDraw Office software unless specified.

10.1 2'-Hydroxy-3,4-dimethoxychalcone (125)

To a solution of 2-hydroxy acetophenone **(123)** (3.000 g, 22.06 mmol) in ethanol (50 ml) was added 60 % (m/v) *aq*. KOH (75 ml), whereafter the mixture was stirred at room temperature for 30 min. 3,4-Dimethoxybenzaldehyde **(124)** (4.400 g, 26.47 mmol, 1.200 equiv.) was added to the mixture and the reaction was monitored by TLC. After disappearance of the acetophenone (3 h), the reaction mixture was acidified with 3 M HCl, water (100 ml) was added and the product extracted with EA (3 x 100 ml). The organic phase was washed with water (100 ml) and *aq*. $NaHCO_3$ (100 ml). Drying of the EA extracts over Na_2SO_4 followed by evaporation of the solvent and FCC (toluene:EA, 9:1) gave the pure 2'-hydroxy-3,4-dimethoxychalcone **(125)** (4.285 g, 15.07 mmol, 68.34 % yield, Rf = 0.33) as a yellow amorphous solid[141].

^{1}H NMR **Plate 8** δ_H (600 MHz, $CDCl_3$, 25 ^oC) 7.94 (dd, 1 H, *J* 9 and 1 Hz, H-6'); 7.89 (d, 1 H, *J* 16 Hz, β-H); 7.52 (d, 1 H, *J* 16 Hz, α-H); 7.50 (m, 1 H, H-5'); 7.27 (dd, 1 H, *J* 8 and 2 Hz, H-6); 7.18 (d, 1 H, *J* 2 Hz, H-2); 7.03 (dd, 1 H, *J* 9 and 1 Hz, H-3'); 6.95 (m, 1 H, H-4'); 6.92 (d, 1 H, *J* 8 Hz, H-5); 3.98 and 3.95 (2 x s, 6 H, 2 x $OC\underline{H}_3$).

10.2 2'-Hydroxy-3,4-dimethoxydihydrochalcone (126)

[141] Kayser, O.; Kiderlen, A.F. *Phytother. Res*. **2001**, *15*, 148.

5 % Pd/C (0.100 g, 0.100 equiv.) was added to a solution of 2'-hydroxy-3,4-dimethoxychalcone **(125)** (1.000 g, 3.517 mmol) in acetone (250 ml). The reaction mixture was thus hydrogenated at atmospheric pressure for 2 h at room temperature with constant stirring. The catalyst was filtered off using silica gel and the filtrate concentrated in vacuo to give the pure 2'-hydroxy-3,4-dimethoxydihydrochalcone **(126)** (1.012 g, 3.530 mmol, 99.90 %) as a colourless amorphous solid.

^{1}H NMR **Plate 9** δ_H (600 MHz, $CDCl_3$, 25 °C) 7.76 (dd, 1 H, *J* 8 and 2 Hz, H-6'); 7.48 (m, 1 H, H-5'); 7.00 (dd, 1 H, *J* 8 and 1 Hz, H-3'); 6.89 (m, 1 H, H-4'); 6.82 (d, 1 H, *J* 8 Hz, H-5); 6.80 (d, 1 H, *J* 2 Hz, H-2); 6.78 (dd, 1 H, *J* 8 and 2 Hz, H-6); 3.88 and 3.87 (2 x s, 6 H, 2 x $OC\underline{H}_3$); 3.32 (t, 2 H, *J* 8 Hz, α-$C\underline{H}_2$); 3.04 (t, 2 H, *J* 8 Hz, β-$C\underline{H}_2$).

10.3 2'-Ethoxycarbonyloxy-3,4-dimethoxydihydrochalcone (127)

Pyridine (0.283 ml, 3.50 mmol, 2.00 equiv.), followed by ethyl chloroformate (0.283 ml, 3.50 mmol, 2.00 equiv.), was added to a solution of 2'-hydroxy-3,4-dimethoxydihydrochalcone **(126)** (0.452 g, 1.75 mmol) in dry DCM (15 ml) at 0 ℃. The temperature was increased to room temperature after 2 h and the reaction continued for a further 4h. DCM (20 ml) was added and the organic phase washed with brine (3 x 20 ml). Drying of the organic phases over Na_2SO_4 followed by in vacuo concentration and PLC (hexane:EA, 8:2) gave the title compound **(127)** (0.396 g, 1.16 mmol, 66.1 % yield, Rf = 0.48) as a white amorphous solid.

^{1}H NMR **Plate 10a** δ_H (600 MHz, $CDCl_3$, 25 °C) 7.74 (dd, 1 H, *J* 8 and 1 Hz, H-6'); 7.52 (m, 1 H, H-5'); 7.31 (m, 1 H, H-4'); 7.21 (dd, 1 H, *J* 8 and 1 Hz, H-3'); 6.79 (d, 1 H, *J* 9 Hz, H-5); 6.75 (br, 2 H, H-2 and H-6); 4.31 (q, 2 H, $OC\underline{H}_2CH_3$); 3.86 and 3.84 (2 x s, 6 H, 2 x $OC\underline{H}_3$); 3.21 (t, 2 H, *J* 8 Hz, α-$C\underline{H}_2$); 2.97 (t, 2 H, *J* 8 Hz, β-$C\underline{H}_2$); 1.37 (t, 3 H, *J* 7 Hz, $OCH_2C\underline{H}_3$).

^{13}C NMR **Plate 10b** δ_C (600 MHz, $CDCl_3$, 25 ^oC) C=O (199.25); C2' (153.26); O-(C=O)-O (149.27); C3 (148.95); C4 (147.45); C1 (133.73); C4' (133.26); C1' (130.87); C6' (129.85); C5' (126.30); C3' (123.40); C6 (120.18); C5 (111.89); C2 (111.40); O-$\underline{C}H_2CH_3$ (65.19); 2 x O$\underline{C}H_3$ (55.97 and 55.84); α-C (43.60); β-C (29.73); O-$CH_2\underline{C}H_3$ (14.19).

10.4 *3-(3',4'-Dimethoxybenzyl)-4-hydroxycoumarin* **(133)**

NaH (17.52 mg, 0.7250 mmol, 2.500 equiv.) was added to a stirred solution of 2'-ethoxycarbonyloxy-3,4-dimethoxydihydrochalcone **(127)** (0.100 g, 0.292 mol) in THF (1.5 ml) and the reaction mixture refluxed for 3 h. The excess NaH was neutralised with 3 M HCl solution (10 ml). The product was thus extracted with EA (3 x 10 ml) and the organic phase washed with aq. $NaHCO_3$ (20 ml). Drying of the organic phase over Na_2SO_4 followed by in vacuo concentration and PLC (hexane:EA, 8:2) gave the title compound **(133)** (0.033 g, 0.11 mmol, 33 % yield, Rf = 0.17) as a white amorphous solid.

^{1}H NMR **Plate 11a** δ_H (600 MHz, $CDCl_3$, 25 ^oC) 7.76 (dd, 1 H, *J* 8 and 2 Hz, H-5); 7.55 (m, 1 H, H-6); 7.35 (dd, 1 H, *J* 8 and 2 Hz, H-8); 7.28 (m, 1 H, H-7); 6.93 (br dd, 1 H, *J* 8 and 2 Hz, H-6'); 6.88 (br d, 1 H, *J* 2 Hz, H-2'); 6.84 (d, 1 H, *J* 8 Hz, H-5'); 4.00 (s, 2 H, 2 x H-9); 3.88 and 3.86 (2 x s, 6 H, 2 x O$\underline{C}H_3$).

^{13}C NMR **Plate 11b** δ_C (600 MHz, $CDCl_3$, 25 ^oC) C9 (30.06); 2 x O$\underline{C}H_3$ (55.95); C3 (104.24); C2', C5' (111.65); C5a (115.76); C8 (116.54); C6' (120.20); C6 (122.78); C5 (123.94); C1' (129.80); C7 (131.95); C3' (148.56); C4' (149.95); C8a (152.55); C2 (160.72); C4 (163.80).

IR (KBr) 3454, 2932, 2617, 1669, 1646, 1624, 1515, 1383, 1265, 1138, 1030, 763 cm^{-1}

MS (CI) *m/z* 312.32 (Na^+), (EI) 409.8 (8%), 376.7 (20%), 357.6 (5%), 335.7 (100%), 313.7 (9%), 301.7 (5%), 229.7 (6%).

10.5 *3-(3',4'-Dimethoxybenzyl)-4-O-(tert-butyldiphenylsilyl)coumarin* (134)

To a stirred solution of 2'-ethoxycarbonyloxy-3,4-dimethoxydihydrochalcone **(127)** (0.100 g, 0.292 mmol) in THF (3.11 ml), NaH (0.020 mg, 0.83 mmol, 2.9 equiv.) was added. The reaction mixture was refluxed for 5 h and divided into 2 portions of 1.5 ml each. TBDPSiCl (0.091 ml, 0.35 mmol, 1.2 equiv.) was added to one portion and the reaction mixture stirred at room temperature for 3 h. The excess NaH was neutralised with 3 M HCl solution (10 ml). The organic phase was thus extracted with EA (3 x 10 ml) and washed with aq. $NaHCO_3$ (20 ml). The combined EA extracts were dried over Na_2SO_4, concentrated in vacuo and purified by PLC (hexane:EA, 7:3) to give the title compound **(134)** (0.010 g, 0.018 mmol, 20 % yield, Rf = 0.28) as a light yellow amorphous solid.

^{1}H NMR **Plate 12a** δ_H (600 MHz, $(CD_3)_2CO$, 25 ^oC) 7.94 (dd, 1 H, *J* 8 and 2 Hz, H-5); 7.65 (m, 4 H, Ph); 7.40 (m, 1 H, H-6); 7.25 (m, 6 H, Ph); 7.12 (m, 2 H, H-7 and H-8); 6.89 (d, 1 H, *J* 2 Hz, H-2'); 6.73 (dd, 1 H, *J* 8 and 2 Hz, H-6'); 6.61 (d, 1 H, *J* 8 Hz, H-5'); 3.79 (s, 2 H, 2 x H-9); 3.58 and 3.57 (2 x s, 6 H, 2 x $OC\underline{H}_3$); 0.90 (s, 9 H, 3 x $C\underline{H}_3$).

^{13}C NMR **Plate 12b** δ_C (600 MHz, $(CD_3)_2CO$, 25 ^oC) C10 (13.44); C9 (18.74); 3 x $\underline{C}H_3$ (26.14); 2 x $O\underline{C}H_3$ (55.2); C3 (104.46); C5' (112.07); C2' (113.10); C8 (115.96); C6' (120.37); C6 (123.26); C5 (123.62); Ph (127.44 x 6); C7, C1' (129.18); C5a (131.03); Ph (134.73 x 6); C8a (136.43); C3' (147.80); C4' (149.24); C4 (152.83); C2 (163.42).

IR (KBr) 2931, 2857, 1620, 1513, 1458, 1379, 1265, 1231, 1191, 1142, 1112, 1028, 815 cm^{-1}

MS (CI) *m/z* 550.72 (Na^+), (EI) 573.7 (20%), 335.6 (100%), 333.6 (50%), 313.6 (30%).

10.6 *3-(3',4'-Dimethoxybenzyl)-chromen-4-one* (135)

A mixture of 2'-hydroxy-3,4-dimethoxydihydrochalcone **(126)** (0.100 g, 0.350 mmol) and $BF_3.Et_2O$ (0.133 ml, 1.05 mmol, 3.00 equiv.) was cooled to 10 °C, whereafter DMF (0.54 ml, 0.54 mmol, 1.5 equiv.) was added dropwise. In a second flask, DMF (0.94 ml, 0.94 mmol, 2.7 equiv.) was cooled to 10 °C and PCl_5 (0.180 g, 0.530 mmol, 1.50 equiv.) was added in small portions. The latter mixture was thus stirred at 55 °C until a light yellow colour indicative of (*N*,*N*'-dimethyl(chloromethylene)ammonium chloride) was observed (20 min.) and then slowly added to the first flask at 20 – 25 °C. The reaction mixture was stirred at room temperature for 2 h, slowly added to boiling diluted HCl (3 M, 15 ml) and cooled. The solution was extracted with EA (3 x 10 ml), the combined EA layers washed with water (20 ml) and brine (20 ml) and the combined organic phases dried over Na_2SO_4. The residue after in vacuo concentration was purified by PLC (hexane:EA, 8:2) to afford the title compound **(135)** (0.042 g, 0.35 mmol, 41 % yield, Rf = 0.40) as a light yellow amorphous solid.

^{1}H NMR **Plate 13a** δ_H (600 MHz, $CDCl_3$, 25 °C) 8.26 (dd, 1 H, *J* 8 and 2 Hz, H-5); 7.66 (m, 1 H, H-6); 7.61 (s, 1 H, H-2); 7.42 (m, 2 H, H-7 and H-8); 6.84 (m, 3 H, H-2', H-5' and H-6'); 3.88 and 3.87 (2 x s, 6 H, 2 x OC$\underline{H}_3$); 3.78 (s, 2 H, 2 x H-9).

^{13}C NMR **Plate 13b** δ_C (600 MHz, $CDCl_3$, 25 °C) C9 (31.30); 2 x O$\underline{C}H_3$ (55.89); C8 (111.39); C3(112.40); C6' (118.02); C6 (121.00); C5a (123.87); C5' (124.88); C2' (124.93); C5 (125.98); C1' (131.13); C7 (133.41); C4' (147.74); C3' (149.09); C2 (153.10); C8a (156.45); C4 (177.50).

IR (KI) 2958, 2403, 1638, 1515, 1467, 1400, 1355, 1260, 1239, 1140, 1024, 799, 755 cm^{-1}

MS (CI) *m/z* 296.32 (H^+), (EI) 361.5 (10%), 360.5 (40%), 298.5 (20%), 297.5 (100%), 200.4 (4%).

10.7 Hydrogenation of 3-(3',4'-dimethoxybenzyl)-chromen-4-one (135)

10 % Pd/C (0.020 g) was added to a solution of 3-(3',4'-dimethoxybenzyl)-chromen-4-one **(135)** (0.030 g, 0.10 mmol) in acetone (10 ml). The reaction mixture was hydrogenated (1.7 atm) at room temperature for 90 min. while stirring. The catalyst was filtered off through silica gel, followed by concentration and purification of the filtrate by PLC (hexane:EA, 8:2) to give *3-(3',4'-dimethoxybenzyl)-chroman-4-one* **(137)** (0.001 g, 3 % yield, Rf = 0.36), *3',4'-dimethoxyhomoisoflavan* **(136)** (0.013 g, 43 % yield, Rf = 0.42), and the two diastereomers of *cis-4-hydroxy-3',4'-dimethoxyhomoisoflavan* **(138)** (0.001 g, 3 % yield, Rf = 0.26) and *trans-4-hydroxy-3',4'-dimethoxyhomoisoflavan* **(139)** (0.001 g, 3 % yield, Rf = 0.22) as colourless amorphous solids.

10.7.1 *3-(3',4'-Dimethoxybenzyl)-chroman-4-one* (137)

^{1}H NMR **Plate 14a** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.95 (dd, 1 H, *J* 8 and 2 Hz, H-5); 7.50 (m, 1 H, H-6); 7.05 (m, 1 H, H-7); 6.99 (dd, 1 H, *J* 9 and 1 Hz, H-8); 6.84 (d, 1 H, H-5'); 6.79 (dd, 2 H, *J* 8 and 2 Hz, H-2' and H-6'); 4.40 (dd, 1 H, *J* 12 and 4 Hz, H-2); 4.21 (dd, 1 H, *J* 12 and 8 Hz, H-2); 3.90 (2 x s, 6 H, 2 x O$C\underline{H}_3$); 3.22 (dd, 1 H, *J* 14 and 5 Hz, H-9); 2.91 (m, 1 H, H-3); 2.71 (dd, 1 H, *J* 14 and 10 Hz, H-9).

^{13}C NMR **Plate 14b** δ_C (600 MHz, $CDCl_3$, 25 ^{o}C) C9 (31.92); C3 (47.89); 2 x O$\underline{C}H_3$ (60.38); C2 (69.43); C8 (111.36); C6' (112.18); C2' (113.78); C5' (117.80); C5a (120.59); C6 (121.19); C5 (121.48); C4' (127.48); C3' (130.69); C7 (135.92); C1' (147.93); C8a (149.12); C4 (193.92).

10.7.2 *3',4'-Dimethoxyhomoisoflavan* (136)

^{1}H NMR **Plate 14c** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.08 (br, 1 H, H-7); 7.00 (br d, 1 H, H-8); 6.84 (m, 1 H, H-6); 6.83 (d, 1 H, *J* 8 Hz, H-5'); 6.81 (br d, 1 H, H-5); 6.73 (dd, 1 H, *J* 8 and 2 Hz, H-6'); 6.71 (d, 1 H, *J* 2 Hz, H-2'); 4.19 (ddd, 1 H, *J* 11 and 3 and 2 Hz, H-2); 3.87 (s, 6 H, 2 x OC$\underline{H}_3$); 3.83 (dd, 1 H, *J* 11 and 9 Hz, H-2); 2.81 (dd, 1 H, *J* 16 and 5 Hz, H-9); 2.66 (dd, 1 H, J 14 and 8 Hz, H-4); 2.57 (dd, 1 H, J 14 and 8 Hz, H-4); 2.52 (dd, 1 H, *J* 16 and 8 Hz, H-9); 2.31 (m, 1 H, H-3).

10.7.3 *cis-4-Hydroxy-3',4'-dimethoxyhomoisoflavan* (138)

^{1}H NMR **Plate 14d** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.23 (m, 2 H, H-5 and H-6); 6.91 (m, 1 H, H-7); 6.84 (m, 4 H, H-8, H-2', H-5' and H-6'); 4.54 (d, 1 H, *J* 3 Hz, H-4); 4.12 (m, 2 H, 2 x H-2); 4.11 (s, 1 H, O$\underline{H}$); 3.89 (s, 6 H, 2 x OC$\underline{H}_3$); 2.85 (dd, 1 H, *J* 14 and 8 Hz, H-9); 2.65 (dd, 1 H, *J* 14 and 8 Hz, H-9); 2.33 (m, 1 H, H-3).

10.7.4 *trans-4-Hydroxy-3',4'-dimethoxyhomoisoflavan* (139)

^{1}H NMR **Plate 14e** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.34 (dd, 1 H, *J* 8 and 2 Hz, H-5); 7.26 (m, 1 H, H-6); 6.97 (m, 1 H, H-7); 6.90 (dd, 1 H, *J* 8 and 1 Hz, H-8); 6.82 (d, 1 H, *J* 8 Hz, H-5'); 6.74 (dd, 1 H, *J* 8 and 2 Hz, H-6'); 6.71 (d, 1 H, *J* 2 Hz, H-2'); 4.52 (d, 1 H, *J* 4 Hz, H-4); 4.25 (dd, 1 H, *J* 12 and 3 Hz, H-2); 4.01 (dd, 1 H, *J* 12 and 4 Hz, H-2); 3.88 (s, 6 H, 2 x OC$\underline{H}_3$); 2.67 (dd, 1 H, *J* 14 and 7 Hz, H-9); 2.51 (dd, 1 H, *J* 14 and 9 Hz, H-9); 2.23 (m, 1 H, H-3).

CHAPTER 11

11. Synthesis of Selected Procyanidins through the C-4 Functionalization of Flavan-3-ols

11.1 Benzylation of starting materials

11.1.1 3',4',5,7-Tetra-*O*-benzyl-(+)-catechin (150)

Benzyl bromide (0.185 ml, 4.50 equiv.) and K_2CO_3 (0.208 g, 6.00 equiv.) were added to a solution of catechin **(145)** (0.100 g, 0.347 mmol) in dry DMF (10 ml) at 0^{o}C. The reaction was stirred at 0^{o}C for 2 h and then at room temperature for 48 h. The reaction mixture was then diluted with EA (3 x 10 ml) and washed with water (20 ml) and brine (20 ml). The organic layer was dried with $MgSO_4$ and the solvent was removed *in vacuo* to give a viscous brown residue which was purified by PLC (hexane:EA, 8:2) to give 3',4',5,7-tetra-*O*-benzyl-(+)-catechin **(150)** as a white amorphous solid[120] (0.106 g, 0.163 mmol, 65.0 % yield, Rf = 0.45).

^{1}H NMR **Plate 15** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.51-7.30 (m, 20 H, Ar-H); 7.05 (br, 1 H, H-2'); 6.98 (br, 2 H, H-5' and H-6'); 6.29 (d, 1 H, *J* 2.3 Hz, H-8); 6.23 (d, 1 H, *J* 2.3 Hz, H-6); 5.20 (br, 4 H, CH_2Ph); 5.05 (s, 2 H, CH_2Ph); 5.02 (s, 2 H, CH_2Ph); 4.65 (d, 1 H, *J* 8.2 Hz, H-2); 4.02 (m, 1 H, H-3), 3.14 (dd, 1 H, *J* 16 and 6 Hz, 4α-H); 2.67 (dd, 1 H, *J* 16 and 9 Hz, 4β-H).

11.1.2 3',4',5,7-Tetra-*O*-benzyl-(-)-epicatechin (151)

Benzyl bromide (0.231 ml, 4.50 equiv.) and K_2CO_3 (0.358 g, 6.00 equiv.) were added to a solution of epicatechin **(146)** (125.3 mg, 0.4320 mmol) in dry DMF (10 ml) at 0^{o}C. The reaction was stirred at 0^{o}C for 2 h and then at room temperature for 48 h. The reaction mixture was then

diluted with EA (3 x 10 ml) and washed with water (20 ml) and brine (20 ml). The organic layer was dried with $MgSO_4$ and the solvent was removed *in vacuo* to give a viscous brown residue which was purified by PLC (hexane:EA, 8:2) to give 3',4',5,7-tetra-*O*-benzyl-(-)-epicatechin **(151)** as a white amorphous solid[120] (0.202 g, 0.310 mmol, 71.7 % yield, Rf = 0.45).

^{1}H NMR **Plate 16** δ_H (600 MHz, $CDCl_3$, 25 ^oC) 7.52-7.31 (m, 20 H, Ar-H); 7.17 (d, 1 H, *J* 1.5 Hz, H-2'); 7.02 (dd, 1 H, *J* 1.5 and 8.5 Hz, H-6'); 7.00 (d, 1 H, *J* 8.5 Hz, H-5'); 6.29 (br, 2 H, H-6 and H-8); 5.22 (s, 4 H, CH_2Ph); 5.05 (s, 4 H, CH_2Ph); 4.94 (br, 1 H, H-2); 4.24 (br, 1 H, H-3); 3.03 (dd, 1 H, *J* 17 and 2 Hz, 4α-H); 2.95 (dd, 1 H, *J* 17 and 4 Hz, 4β-H).

11.2 C-4 Functionalisation of flavan-3-ols

11.2.1 3',4',5,7-Tetra-*O*-methyl-4β-(2-hydroxyethoxy)-catechin (148)

Ethylene glycol (0.17 ml, 6.0 equiv.) and DDQ (0.13 g, 2.0 equiv.) were added to a stirred solution of 3',4',5,7-tetra-*O*-methylcatechin **(147)** (0.100 g, 0.290 mmol) in dry dichloromethane (10 ml) at room temperature while stirring under argon atmosphere. A black-green colour appeared instantaneously. After 3 h of vigorous stirring, 4-(Dimethylamino)pyridine (0.070 g, 2.0 equiv.) was added and stirring continued for another 10 min. The solvent was evaporated *in vacuo* and the resulting crude material purified by PLC (hexane:EA:methanol, 6:3:1) to give 3',4',5,7-tetra-*O*-methyl-4β-(2-hydroxyethoxy)-catechin **(148)** (53.3 mg, 0.131 mmol, 46.0 % yield, Rf = 0.5).

^{1}H NMR **Plate 17a** δ_H (600 MHz, $CDCl_3$, 25 ^oC) 7.07 (dd, 1 H, *J* 8.1 and 1.9 Hz, H-6'); 6.92 (d, 1 H, *J* 8.1 Hz, H-5'); 7.03 (d, 1 H, *J* 1.9 Hz, H-2'); 6.14 (d, 1 H, *J* 2.2 Hz, H-8); 6.10 (d, 1 H, *J* 2.2 Hz, H-6); 5.01 (d, 1 H, *J* 10.4 Hz, H-2); 4.82 (d, 1 H, *J* 3.4 Hz, H-4); 3.98 (m, 3 H, 2 x H-9 and H-3); 3.80 (m, 2 H, 2 x H-10); 3.94-3.75 (s, 12 H, 4 x OCH_3).

^{1}H NMR **Plate 17b** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.03 (dd, 1 H, *J* 8 and 2 Hz, H-6'); 6.97 (d, 1 H, *J* 2 Hz, H-2'); 6.88 (d, 1 H, *J* 8 Hz, H-5'); 6.11 (d, 1 H, *J* 2 Hz, H-8); 6.09 (d, 1 H, *J* 2 Hz, H-6); 5.31 (br, 2 H, H-2 and H-3); 4.90 (d, 1 H, *J* 2 Hz, H-4); 4.28 (m, 2 H, 2 x H-10); 3.92 (m, 2 H, 2 x H-9); 3.91-3.75 (4 x s, 12 H, 4 x OC$\underline{H}_3$); 2.08 and 1.91 (2 x s, 6 H, 2 x OAc).

11.2.2 3',4',5,7-Tetra-*O*-benzyl-4β-(2-hydroxyethoxy)-catechin (152)

In a procedure similar to that described for methylated catechin, ethylene glycol (0.055 ml, 6.0 equiv.), DDQ (0.074 g, 2.0 equiv.), 3',4',5,7-tetra-*O*-benzylcatechin **(150)** (0.106 g, 0.163 mmol), and 4-(Dimethylamino)pyridine (0.040 g, 2.0 equiv.) were reacted in dry DCM (5 ml) at room temperature under argon for 3 hours. The resulting crude material was purified by PLC (hexane:EA:methanol, 6:3:1) to obtain 3',4',5,7-tetra-*O*-benzyl-4β-(2-hydroxyethoxy)-catechin **(152)** as a beige amorphous solid[120] (0.067 g, 0.094 mmol, 60 % yield, Rf = 0.5).

^{1}H NMR **Plate 18** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.53-7.29 (m, 20 H, Ar-$\underline{H}$); 7.11 (d, 1 H, *J* 1.7 Hz, H-2'); 7.03 (dd, 1 H, *J* 8.3 and 1.7 Hz, H-6'); 6.97 (d, 1 H, *J* 8.3 Hz, H-5'); 6.29 (d, 1 H, *J* 2.2 Hz, H-8); 6.18 (d, 1 H, *J* 2.2 Hz, H-6); 5.20-5.00 (m, 8 H, 4 x C$\underline{H}_2$Ph); 4.96 (d, 1 H, *J* 11.5 Hz, H-2); 4.83 (d, 1 H, *J* 3.6 Hz, H-4); 3.98-3.78 (m, 3 H, 2 x H-9 and H-3); 3.72-3.64 (m, 2 H, 2 x H-10).

11.2.3 3',4',5,7-Tetra-*O*-benzyl-4β-(2-hydroxyethoxy)-epicatechin (153)

In a procedure similar to that described for methylated catechin, ethylene glycol (0.302 ml, 20.0 equiv.), DDQ (0.123 g, 2.00 equiv.), 3',4',5,7-tetra-*O*-benzylepicatechin **(151)** (0.176 g, 0.271 mmol), and 4-(Dimethylamino)pyridine (0.132 g, 4.00 equiv.) were reacted in dry dichloromethane (5 ml) at room temperature under argon for 3.5 hours. The resulting crude material was purified by PLC (hexane:EA:methanol, 6:3:1) and the product, 3',4',5,7-tetra-*O*-benzyl-4β- (2-hydroxyethoxy)-epicatechin **(153)** obtained as a pale pink solid[120]

(0.092 g, 0.13 mmol, 50 % yield, Rf = 0.5).

^{1}H NMR **Plate 19** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.50-7.30 (m, 20 H, Ar-H); 7.16 (d, 1 H, *J* 1.6 Hz, H-2'); 7.04 (dd, 1 H, *J* 8.3 and 1.6 Hz, H-6'); 7.00 (d, 1 H, *J* 8.3 Hz, H-5'); 6.32 (d, 1 H, *J* 2.1 Hz, H-8); 6.29 (d, 1 H, *J* 2.1 Hz, H-6); 5.22 (br, 4 H, 2 x CH_2Ph); 5.11 (br, 2 H, CH_2Ph); 5.05 (d, 1 H, *J* 11.3 Hz, H-4); 5.03 (br, 2 H, CH_2Ph); 4.63 (d, 1 H, *J* 2.8 Hz, H-2); 3.99 (dd, 1 H, *J* 11.3 and 2.8 Hz, H-3); 3.84-3.60 (m, 4 H, 2 x H-9 and 2 x H-10).

11.3 Coupling of nucleophiles to flavan-3-ols

11.3.1 2,3-*trans*-3,4-*trans*-3',4',5,7-Tetra-*O*-methyl-4-(2",4",6"-trihydroxyphenyl)-catechin (154)

para-Toluenesulfonic acid (0.10 g, 1.5 equiv.) was added to a solution of 3',4',5,7-tetra-*O*-methyl-4β-(2-hydroxyethoxy)-catechin **(148)** (22.3 mg, 0.0550 mmol) and phloroglucinol (10.42 mg, 0.08200 mmol, 1.500 equiv.) in CF_3CH_2OH (1 ml). The reaction mixture was stirred at room temperature for 5 h before the products were extracted into EA (3 x 20 ml) and washed with *aq* NaCl (10 ml). The extract was dried (Na_2SO_4) before the solvent was removed and the crude material purified by PLC (toluene:EA:,methanol, 6:3:1) to give 2,3-*trans*-3,4-*trans*-3',4',5,7-tetra-*O*-methyl-4-(2",4",6"-trihydroxyphenyl)-catechin **(154)** (1.6 mg, 0.0034 mmol, 6.0 % yield, Rf = 0.6).

^{1}H NMR **Plate 20a** δ_H (600 MHz, $(CD_3)_2CO$, 25 ^{o}C) 8.30 (br, 1 H, OH); 8.03 (s, 1 H, OH); 7.065 (d, 1 H, *J* 2 Hz, H-2'); 6.97 (dd, 1 H, *J* 8 and 2 Hz, H-6'); 6.95 (d, 1 H, *J* 8 Hz, H-5'); 6.38 (d, 1 H, *J* 2 Hz, H-5"); 6.23 (d, 1 H, *J* 2 Hz, H-3"); 6.03 (s, 1 H, OH); 5.87 (br, 2 H, H-6 and H-8); 5.23 (dd, 1 H, *J* 6 and 8 Hz, H-3); 5.08 (d, 1 H, *J* 6 Hz, H-4); 4.71 (d, 1 H, *J* 8 Hz, H-2); 4.06-3.72 (m, 12 H, 4 x OCH_3).

^{1}H NMR **Plate 20b** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 8.20 (s, 1 H, O<u>H</u>); 6.98-6.90 (m, 3 H, H-2', H-5', H-6'); 6.28 (br, 2 H, H-3'' and H-5''); 6.00 (d, 1 H, *J* 2 Hz, H-8); 5.96 (d, 1 H, *J* 2 Hz, H-6); 5.00-4.90 (m, 2 H, H-2 and H-4); 4.75 (br, 1 H, H-3); 4.03-3.78 (4 x s, 12 H, 4 x OC<u>H</u>$_3$).

^{1}H NMR **Plate 20c** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 6.96 (dd, 1 H, *J* 8.0 and 2.0 Hz, H-6'); 6.93 (d, 1 H, *J* 2.0 Hz, H-2'); 6.90 (d, 1 H, *J* 8.0 Hz, H-5'); 6.56 (d, 1 H, *J* 2.0 Hz, H-5''); 6.41 (d, 1 H, *J* 2.0 Hz, H-3''); 6.18 (d, 1 H, *J* 2.0 Hz, H-8); 6.16 (d, 1 H, *J* 2.0 Hz, H-6); 4.95-4.85 (m, 3 H, H-2, H-3, H-4); 3.95-3.75 (s, 12 H, 4 x OC<u>H</u>$_3$); 2.30-1.25 (s, 12 H, 4 x OAc).

11.3.2 3',4',5,7-Tetra-*O*-benzyl-4-(2'',4'',6''-trihydroxyphenyl)-catechin (156)

para-Toluenesulfonic acid (0.10 g, 1.5 equiv.) was added to a solution of 3',4',5,7-tetra-*O*-benzyl-4β-(2-hydroxyethoxy)-catechin **(152)** (0.050 g, 0.071 mmol) and phloroglucinol (13.31 mg, 0.1060 mmol, 1.500 equiv.) in CF_3CH_2OH (1 ml). The reaction mixture was stirred at room temperature for 7 h before the products were extracted into EA (3 x 20 ml) and the mixture washed with *aq* NaCl (10 ml). The ethyl acetate extract was dried over Na_2SO_4, the solvent removed by evaporation under vacuum and the crude material purified by PLC (toluene:acetone:methanol, 7:2:1) to give 3',4',5,7-tetra-*O*-benzyl-4-(2'',4'',6''-trihydroxyphenyl)-catechin **(156)** (15.23 mg, 0.01962 mmol, 27.89 % yield, Rf = 0.55).

^{1}H NMR **Plate 21a** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.48-7.18 (m, 20 H, 4 x OBn), 7.03 (br, 1 H, H-5'), 6.97 (m, 3 H, H-2', H-6', O<u>H</u>), 6.30 (br, 2 H, H-6 and H-8), 6.01 (br, 2 H, H-3'' and H-5''), 5.22-4.88 (br, 8 H, 4 x OC<u>H</u>$_2$Ph), 4.86 (m, 1 H, H-2), 4.53 (br, 1 H, H-3), 4.21 (m, 1 H, H-4).

^{1}H NMR **Plate 21b** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.50-7.25 (m, 20 H, 4 x OBn); 7.05 (d, 1 H, *J* 2.0 Hz, H-2'); 6.95 (dd, 1 H, *J* 8.0 and 2.0 Hz, H-6'); 6.92 (d, 1 H, *J* 8.0 Hz, H-5'); 6.89 (d, 1 H, *J* 2.0

Hz, H-3”); 6.79 (d, 1 H, *J* 2.0 Hz, H-5”); 6.20 (d, 1 H, *J* 2.0 Hz, H-8); 6.15 (d, 1 H, *J* 2.0 Hz, H-6); 5.42 (dd, 1 H, *J* 10.0 and 6.0 Hz, H-3); 5.27 (d, 1 H, *J* 10.0 Hz, H-2); 5.18-4.72 (s, 8 H, 4 x OC<u>H</u>-$_2$Ph); 4.92 (d, 1 H, *J* 6.0 Hz, H-4); 2.33-1.61 (s, 12 H, 4 x OAc).

^{1}H NMR **Plate 21c** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.48-7.28 (m, 20 H, 4 x OBn); 7.05 (d, 1 H, *J* 2.0 Hz, H-2’); 6.98 (dd, 1 H, *J* 8.0 and 2.0 Hz, H-6’); 6.92 (d, 1 H, *J* 8.0 Hz, H-5’); 6.80 (d, 1 H, *J* 2.0 Hz, H-3”); 6.58 (d, 1 H, *J* 2.0 Hz, H-5”); 6.19 (d, 1 H, *J* 2.0 Hz, H-8); 6.16 (d, 1 H, *J* 2.0 Hz, H-6); 5.72 (t, 1 H, *J* 10.0 Hz, H-3); 5.21-4.53 (s, 8 H, 4 x OC<u>H</u>$_2$Ph); 4.75 (d, 1 H, *J* 10.0 Hz, H-2); 4.69 (d, 1 H, *J* 10.0 Hz, H-4); 2.30-1.50 (s, 12 H, 4 x OAc)

11.3.3 2,3-*trans*-3,4-*trans*-3’,4’,5,7-Tetra-*O*-benzyl-4-(2”,4”,6”-tribenzyloxyphenyl)-catechin (160)

$TiCl_4$ (1 M in CH_2Cl_2) (0.548 ml, 2.00 equiv.) was added dropwise with ice cooling to 3’,4’,5,7-tetra-*O*-benzyl-4β-(2-hydroxyethoxy)-catechin **(152)** (0.195 g, 0.274 mmol) and per *O*-benzylated phloroglucinol **(159)** (0.4350 g, 1.096 mmol, 4.000 equiv.) in dry THF (11 ml) and DCM (9 ml). The resulting dark-red solution was subsequently stirred at room temperature for 3 h, before it was treated with saturated aqueous $NaHCO_3$ (10 ml) and water (10 ml). The $TiO_2.xH_2O$ precipitate was removed by filtration and washed with DCM (20 ml) and water (10 ml). The organic layer was washed with brine (10 ml) and the aqueous phases were extracted with DCM (3 x 10 ml). The combined organic phases were dried over $MgSO_4$, before the solvents were removed *in vacuo* and the resulting dark-red oil purified by PLC (hexane:EA, 7:3) to obtain 2,3-*trans*-3,4-*trans*-3’,4’,5,7-tetra-*O*-benzyl-4-(2”,4”,6”-tribenzyloxyphenyl)-catechin **(160)** (180.8 mg, 0.1730 mmol, 63.13 % yield, Rf = 0.5).

^{1}H NMR **Plate 22** δ_H (600 MHz, $CDCl_3$, 25 ^{o}C) 7.49-6.87 (m, 35 H, 7 x OBn); 7.12 (m, 2 H, H-5’ and H-6’); 7.02 (d, 1 H, *J* 1.0, H-2’); 6.28 (d, 1 H, *J* 2.0 Hz, H-8); 6.20 (d, 1 H, *J* 2.0 Hz, H-6); 6.12

(d, 1 H, *J* 2.0 Hz, H-5''); 6.07 (d, 1 H, *J* 2.0 Hz, H-3''); 5.17-4.55 (s, 14 H, 7 x OC$\underline{H}_2$Ph); 4.70 (d, 1 H, *J* 12.0 Hz, H-4); 4.49 (d, 1 H, *J* 12.0 Hz, H-2); 4.33 (m, 1 H, H-3).

11.3.4 2,3-*cis*-3,4-*trans*-3',4',5,7-Tetra-*O*-benzyl-4-(2'',4'',6''-tribenzyloxyphenyl)-epicatechin (162)

Bentonite K-10 clay (0.275 g) and per-*O*-benzylated phloroglucinol **(159)** (0.516 g, 1.30 mmol, 4.00 equiv.) were mixed in dry DCM (10 ml) at 0°C. A solution of 3',4',5,7-tetra-*O*-benzyl-4β-(2-hydroxyethoxy)-epicatechin **(153)** (0.231 g, 0.325 mmol) in DCM (3 ml) was added and the mixture stirred at 0 °C for 1 hour before the temperature was allowed to rise up 10 °C and stirring continued for another 3 hours. The clay was filtered off (through celite) and after evaporation of the solvents, the resulting purple oil was purified by PLC (hexane:EA, 7:3) to obtain 2,3-*cis*-3,4-*trans*-3',4',5,7-tetra-*O*-benzyl-4-(2'',4'',6''-tribenzyloxyphenyl)-epicatechin **(162)** (187.8 mg, 0.1797 mmol, 55.28 % yield, Rf = 0.56).

^{1}H NMR **Plate 23** δ_H (600 MHz, $CDCl_3$, 25 °C) 7.52-7.09 (m, 35 H, 7 x OBn); 7.03-6.90 (m, 3 H, H-2', H-5', H-6'); 6.35 (d, 1 H, *J* 2.0 Hz, H-8); 6.23 (d, 1 H, *J* 2.0 Hz, H-5''); 6.20 (d, 1 H, *J* 2.0 Hz, H-3''); 6.03 (d, 1 H, *J* 2.0 Hz, H-6); 5.35 (d, 1 H, *J* 1.0 Hz, H-2); 5.21-4.63 (s, 14 H, 7 x OC$\underline{H}_2$Ph); 4.45 (d, 1 H, *J* 12.0 Hz, H-4); 4.02 (m, 1 H, H-3).

11.4 General procedure for the preparation of perbenzyl-procyanidin B1-B4

$TiCl_4$ (1 M in CH_2Cl_2) (0.282 ml, 2.00 equiv.) was added dropwise with ice cooling to a solution of C-4 functionalized per-*O*-benzylated catechin **(152)** or -epicatechin **(153)** (0.100 g, 0.141 mmol) and per-*O*-benzylated catechin **(150)** or -epicatechin **(151)** (0.366 g, 0.563 mmol, 4.00 equiv.) in dry THF (11 ml) and DCM (9 ml) at 0 °C. The resulting dark-red solution was stirred at room temperature for 3 h, before it was treated with saturated aqueous $NaHCO_3$ (10 ml) and water (10

ml). The $TiO_2.xH_2O$ precipitate was removed by filtration and rinsed with DCM (20 ml) and water (10 ml). The organic layer was washed with brine and the aqueous phases extracted with DCM (3 x 10 ml). The combined organic phases were then dried with $MgSO_4$, before the solvents were removed *in vacuo* and the resulting dark-red oil purified by PLC (hexane:EA, 7:3) to obtain the desired product.

Perbenzyl-procyanidin B1 **(163)**: (0.060 g, 0.046 mmol, 32 % yield, Rf = 0.42), **Plate 24**

Perbenzyl-procyanidin B2 **(164)**: (0.073 g, 0.056 mmol, 40 % yield, Rf = 0.38), **Plate 25**

Perbenzyl-procyanidin B3 **(165)**: (0.036 g, 0.027 mmol, 20 % yield, Rf = 0.35), **Plate 26**

Perbenzyl-procyanidin B4 **(166)**: (0.080 g, 0.061 mmol, 43 % yield, Rf = 0.40), **Plate 27**