CHAPTER 1

THE STEREOCHEMISTRY OF FLAVONOIDS

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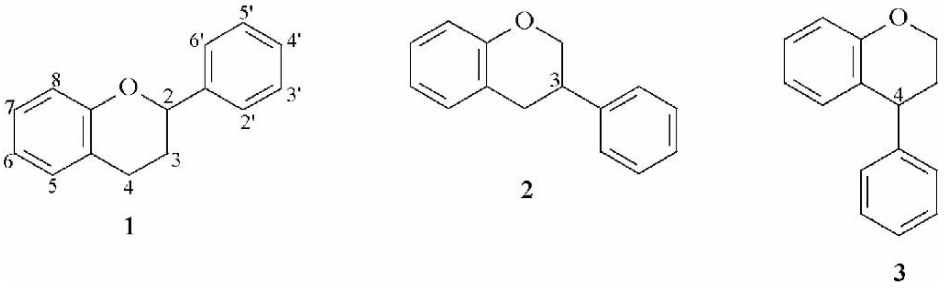
1. INTRODUCTION

The study of flavonoid chemistry has emerged, like that of most natural products, from the search for new compounds with useful physiological properties. Semisynthetic endeavors of oligoflavonoids are in most instances confined to those substitution patterns exhibited by monomeric natural products that are available in quantities sufficient for preparative purposes. In order to alleviate these restrictions, several programs focusing on synthesis of enantiomeric pure flavonoid monomers have been undertaken. However, synthesis of the desired enantiomer in optically pure forms remains a daunting objective and is limited to only a few types of compounds. Chalcone epoxides, α- and β-hydroxydihydrochalcones, dihydroflavonols, flavan-3-ols, flavan-3,4-diols, isoflavans, isoflavanones, and pterocarpans thus far have been synthesized in reasonable yields and purity.

2. NOMENCLATURE

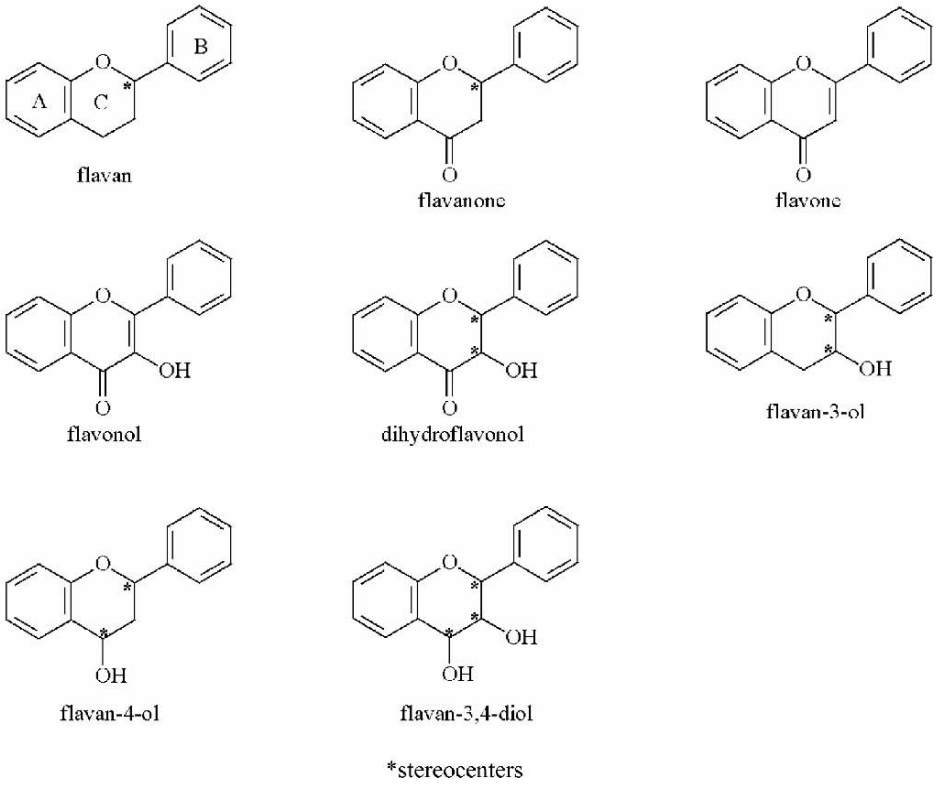
The term "flavonoid" is generally used to describe a broad collection of natural products that include a C_6-C_3-C_6 carbon framework, or more specifically a phenylbenzopyran functionality. Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans) **1**, isoflavonoids (3-benzopyrans) **2**, and the neoflavonoids (4-benzopyrans) **3**. These

groups usually share a common chalcone precursor, and therefore are biogenetically and structurally related.



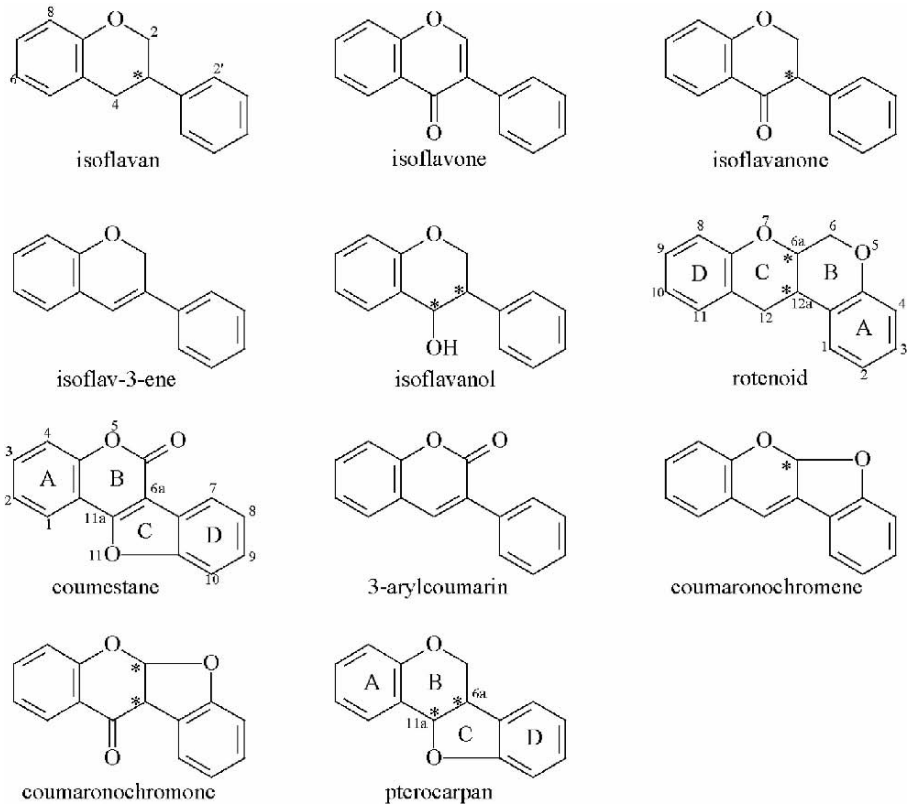
2.1. 2-Phenylbenzopyrans (C_6-C_3-C_6 Backbone)

Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into the following groups:



2.2. Isoflavonoids

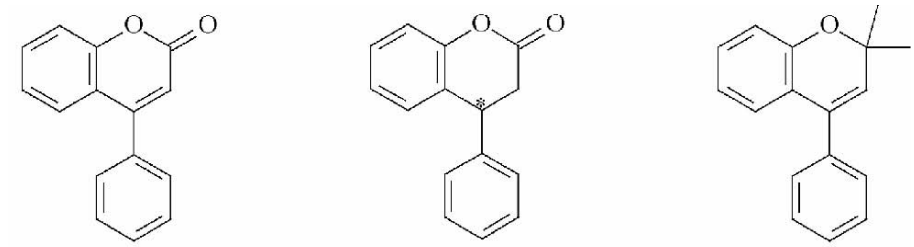
The isoflavonoids are a distinctive subclass of the flavonoids. These compounds possess a 3-phenylchroman skeleton that is biogenetically derived by 1,2-aryl migration in a 2-phenylchroman precursor. Despite their limited distribution in the plant kingdom, isoflavonoids are remarkably diverse as far as structural variations are concerned. This arises not only from the number and complexity of substituents on the basic 3-phenylchroman system, but also from the different oxidation levels and presence of additional heterocyclic rings. Isoflavonoids are subdivided into the following groups:



*stereocenters

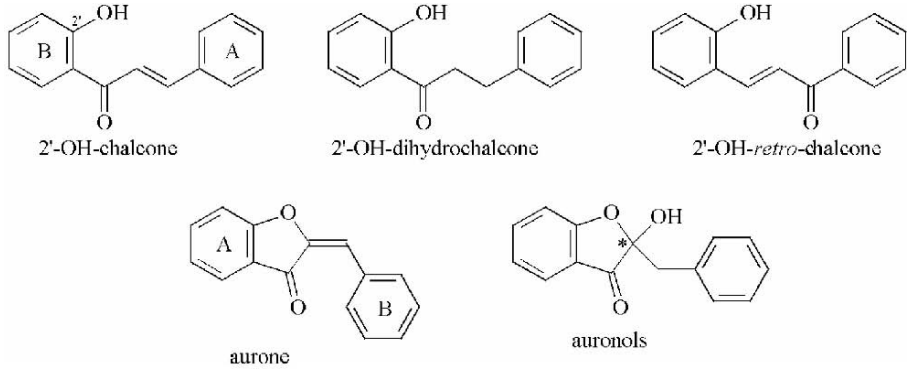
2.3. Neoflavonoids

The neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins (4-aryl-2*H*-1-benzopyran-2-ones), 3,4-dihydro-4-arylcoumarins, and neoflavenes.



2.4. Minor Flavonoids

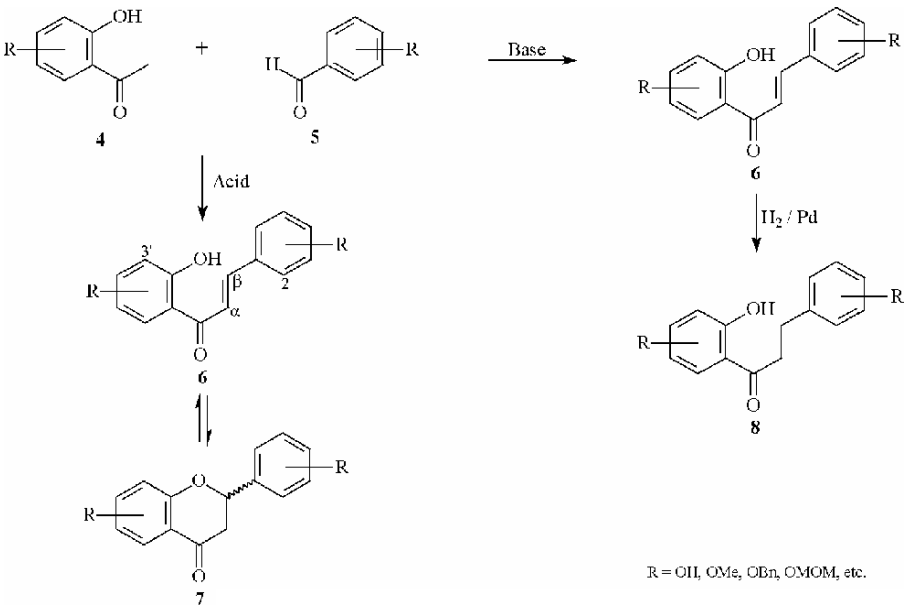
Natural products such as chalcones and aurones also contain a C_6-C_3-C_6 backbone and are considered to be minor flavonoids. These groups of compounds include the 2′-hydroxychalcones, 2′-OH-dihydrochalcones, 2′-OH-*retro*-chalcone, aurones (2-benzylidenecoumaranone), and auronols.



3. SYNTHESIS OF FLAVONOIDS

3.1. Chalcones, Dihydrochalcones, and Racemic Flavonoids

Chalcones and dihydrochalcones are considered to be the primary C_6-C_3-C_6 precursors and constitute important intermediates in the synthesis of flavonoids. Chalcones are readily accessible via two well-established routes comprising a base-catalyzed aldol condensation or acid-mediated aldolization of 2-hydroxy-acetophenones **4** and benzaldehydes **5** (Von Konstanecki and Rossbach, 1896; Augustyn et al., 1990a) (Scheme 1.1). The base-catalyzed aldol condensation is usually the preferred route toward chalcone **6** formation, since under acidic conditions cyclization of the ensuing chalcone leads to formation of corresponding racemic flavanones **7** (Claisen and Claparède, 1881). Dihydrochalcones **8** are generally obtained via reduction (H_2/Pd) of the preceding chalcones (Scheme 1.1).

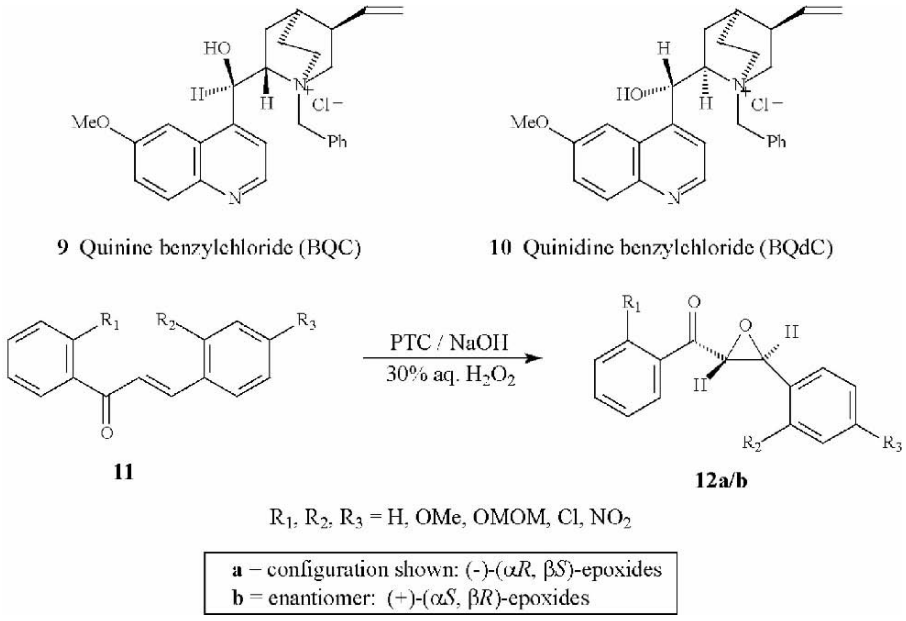


Scheme 1.1 *Acid- and base-catalyzed synthesis of chalcones, racemic flavanones, and dihydrochalcones.*

3.2. Asymmetric Epoxidation of Chalcones

Asymmetric epoxidation of olefinic bonds plays a crucial role in introducing chirality in the synthesis of several classes of optically active natural compounds. Sharpless (Katsuki and Sharpless, 1980; Johnson and Sharpless 1993) and Jacobson (1993) developed viable protocols for the enantioselective epoxidation of allylic alcohols and unfunctionalized olefins. However, attempts regarding the enantioselective epoxidation of α,β-unsaturated ketones, in particular chalcones, have met with limited success.

Wynberg and Greijdanus (1978) first reported the utilization of quinine benzylchloride **9** (BQC) and quinidine benzylchloride (BQdC) **10** as chiral phase-transfer catalysts (PTC). Since then, the use of PTC has emerged as one of the preferred methods for the asymmetric epoxidation of α,β-unsaturated ketones and led to the first stereoselective synthesis of (-)- and (+)-*trans*-chalcone epoxides **12a/b** [yield: 38–92%; enantiomeric excess (ee): 25–48%] (Helder et al., 1976; Wynberg and Greijdanus, 1978) (Scheme 1.2).



Scheme 1.2 *Epoxidation of chalcones **11** with BQC **9** and BQdC **10** as PTC.*

Except for the poor ee, this protocol demonstrated the preferential formation of (-)-(α*R*,β*S*)-**12a** and (+)-(α*S*,β*R*)-**12b** epoxides, with BQC **9** and BQdC **10** used, respectively, as PTC. This resulted in several investigations of alternative catalysts and reaction conditions to enhance the enantioselectivity of the epoxidation of

enones (Table 1.1). However, these attempts were limited to nonchalcone enones and a few non- and monooxygenated chalcone substrates, which lacked natural product oxygenation patterns.

Table 1.1 *Asymmetric epoxidation of electron-deficient olefins*

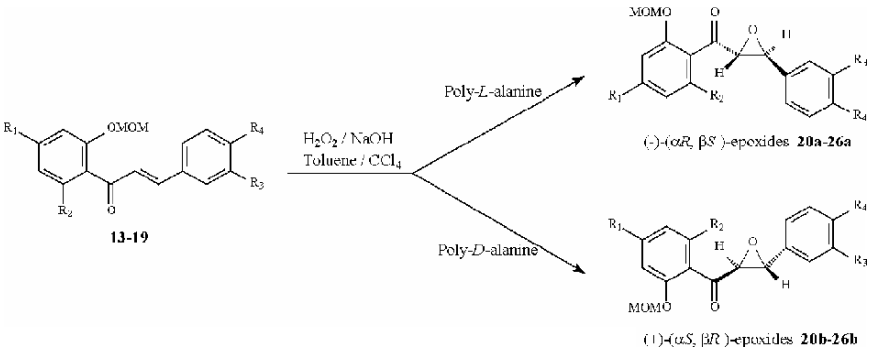
Type of reaction and reaction conditions	*References*
1. Bovine serum albumin catalyzed epoxidation:	
Bovine serum albumin (BSA) under Weitz–Scheffer conditions and *aq* NaOCl with α- and β-cyclodextrins as catalysts.	Colonna et al., 1985; Colonna and Manfredi, 1986
2. Zinc-mediated asymmetric epoxidation:	
Metal-based catalytic systems: Epoxidation of α,β-unsaturated ketones with O_2 in the presence of Et_2Zn and (*R,R*)-*N*-methylpseudoephedrine.	Enders et al., 1996, 1997
Metal-based polymeric catalytic systems: Polybinaphthyl zinc catalyst for the asymmetric epoxidation of enones in the presence of Bu^tOOH (TBPH).	Yu et al., 1999
3. Lanthanide–BINOL systems:	
Asymmetric epoxidation of enones using lanthanoid complexes: Several kinds of heterobimetallic chiral catalysts [La- and Yb–BINOL and La- and Yb-3-hydroxymethyl–BINOL complexes] are useful for this procedure, using TBHP and cumene hydroperoxide (CMHP).	Bougauchi et al., 1997; Daikai et al., 1998
Enantioselective epoxidation of α,β-enones by utilizing chiral La(*O-i*-Pr)$_3$-(*S*)-6,6′-dibromo-BINOL and Gd(*O-i*-Pr)$_3$-(*S*)-6,6′-diphenyl–BINOL catalysts and CMHP.	Chen et al., 2001
4. Diethyl tartrate–metal peroxides:	
Modified Sharpless protocol, with chiral metal alkyl peroxides as nucleophilic oxidants: Using (+)-diethyl tartrate [(+)-DET] as chiral modifier in the presence of Li-TBHP and *n*-BuLi, yielded the (+)-chalcone epoxide. The (-)-chalcone epoxide was obtained simply via replacing *n*-BuLi with *n*-Bu_2Mg.	Elston et al., 1997
5. Phase-tranfer catalyst:	
Enantioselective epoxidation of chalcones utilizing Cinchona alkaloid-derived quaternary ammonium phase-transfer catalysts bearing an *N*-anthracenylmethyl function with sodium hypochlorite as oxidant.	Lygo and Wainwright, 1998, 1999
Use of chiral quaternary cinchonidinium and dihydrocinchonidinium cations for the nucleophilic epoxidation of various α,β-enones, utilizing KOCl in stoichiometric amounts as oxidant, at –40°C.	Lygo and To, 2001; Corey and Zhang, 1999

Table 1.1 (continued)

Type of reaction and reaction conditions	*References*
Catalytic asymmetric epoxidation of enones promoted by *aq*. H_2O_2 with chiral ammonium salts (cinchonine or quinidine derivatives).	Arai et al., 2002
Epoxidation of enones under mild reaction conditions, using a new chiral quaternary ammonium bromide with dual functions as phase transfer catalyst.	Ooi et al., 2004;
Asymmetric epoxidation with optically active hydroperoxides (cumyl hydroperoxide) and mediated by cinchonine- and cinchonidine-derived phase-transfer catalyst	Adam et al., 2001
Epoxidation of chalcones using the phase-transfer catalyst, chiral monoaza-15-crown-5 lariat ethers, synthesized from D-glucose, galactose, and mannitol, with TBHP as oxidant.	Bakó et al., 1999, 2004
Use of optically active solvents [2-(*N,N*-diethylamino)-1-butanol or 2-(*N,N*-di-*n*-butylamino)-1-butanol], *n*-Bu_4NBr as PTC and alkaline H_2O_2.	Singh and Arora, 1987
6. Epoxidation with chiral dioxiranes:	
Involving dimethyldioxirane (DMDO) type of epoxidation utilizing chiral dioxiranes generated *in situ* from potassium peroxomonosulfate (oxone) and asymmetric ketones.	Wang and Shi, 1997; Wang et al., 1997, 1999
2-Substituted-2,4-endo-dimethyl-8-oxabicyclo[3.2.1]octan-3-ones as catalyst for the asymmetric epoxidation of alkenes with oxone.	Klein and Roberts, 2002
7. Polyamino acid-catalyzed epoxidation:	
Julia–Colonna asymmetric epoxidation, originally employs a three-phase system comprising alkaline H_2O_2, an organic solvent (hexane or toluene) and an insoluble polymer (poly-L-/-D-alanine or -leucine).	Julia et al., 1980; Colonna et al., 1983;
Asymmetric epoxidation using a nonaqueous two-phase system of urea hydrogen peroxide (UHP) in THF or tert-butyl methyl ether, with immobilized poly-L-/-D-leucine.	Banfi et al., 1984
Julia–Colonna stereoselective epoxidation under nonaqueous conditions using polyamino acid (poly-L-/-D-alanine or β-leucine) on silica (PaaSiCat).	Adger et al., 1997; Bentley et al., 1997
β-Peptides as catalyst: poly-β-leucine in Julia–Colonna asymmetric epoxidation.	Geller and Roberts, 1999;
Polyethylene glycol (PEG)-bound poly-L-leucine acts as a THF-soluble catalyst for the Julia–Colonna asymmetric epoxidation of enones.	Carde et al., 1999 Coffey et al., 2001 Flood et al., 2001

As a feasible alternative to the utilization of enzymes as catalysts in organic reactions, Julia and Colonna (Julia et al., 1980, 1982; Colonna et al., 1983; Banfi et al., 1984) investigated the use of synthetic peptides in the epoxidation of chalcones. Because of the potential use of polyoxygenated chalcone epoxides as chirons in the enantiomeric synthesis of flavonoids and to determine the effect of different levels of oxygenation and substitution patterns on the poly-amino acid-catalyzed epoxidation, this protocol was extended to a series of chalcones exhibiting aromatic oxygenation patterns usually encountered in the naturally occurring flavonoids (Bezuidenhoudt et al., 1987; Augustyn et al., 1990a) (Table 1.2).

Table 1.2 *Asymmetric epoxidation of chalcones* ***20a/b–26a/b*** *using poly-L- and poly-D-alanine as catalysts*



Epoxides	R_1	R_2	R_3	R_4	*Alanine*	*% yield*	$[\alpha]^{278}$	*% ee*
(-)-20a	H	H	H	H	*L*	65	-50	38
(+)-20b	H	H	H	H	*D*	57	+75	53
(-)-21a	H	H	H	OMe	*L*	64	-76	66
(+)-21b	H	H	H	OMe	*D*	38	+52	46
(-)-22a	OMe	H	H	OMe	*L*	74	-122	84
(+)-22b	OMe	H	H	OMe	*D*	26	+77	53
(-)-23a	OMe	H	OMe	OMe	*L*	46	-79	62
(+)-23b	OMe	H	OMe	OMe	*D*	34	+31	25
(-)-24a	OMe	OMe	H	OMe	*L*	*	*	32
(+)-24b	OMe	OMe	H	OMe	*D*	*	*	20
(-)-25a	OMe	OMe	OMe	OMe	*L*	*	*	*
(+)-25b	OMe	OMe	OMe	OMe	*D*	*	*	*
(-)-26a	OMOM	H	H	OMe	*L*	43	*	70
(+)-26b	OMOM	H	H	OMe	*D*	36	*	36

*Not reported

The triphasic system comprising poly-L- or poly-D-alanine, alkaline H_2O_2, and organic solvent (CCl_4 or toluene) was utilized during the enantioselective epoxidation of chalcones **13–19**, to afford epoxides **20a/b-26a/b** in moderate yield and ee.

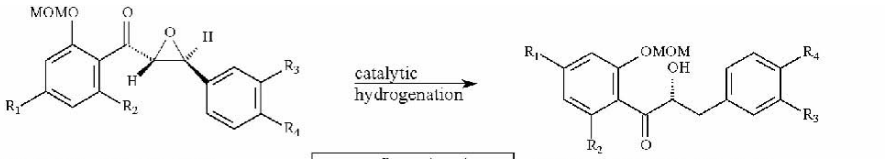
Although the Julia asymmetric epoxidation has proved to be a reliable reaction to afford polyoxygenated chalcone epoxides in good yield and moderate to high ee's, this protocol is not without limitations, since reaction times are often unacceptably long and require continuous addition of oxidant and base. Degradation of the poly-amino acid under such reaction conditions also poses difficulties. Bentley and Roberts found satisfactory solutions to many of these problems by conducting the asymmetric epoxidation in a two-phase non-aqueous system consisting of oxidant, a nonnucleophilic base, immobilized poly-amino acid, and an organic solvent (Itsuno et al., 1990; Lasterra-Sanchez et al., 1996; Bentley et al., 1997). This procedure afforded chiral enone epoxides in high yields and optical purity with a substantial reduction in reaction times and also was extended successfully to chalcone substrates (Nel et al., 1998, 1999a; Van Rensburg et al., 1996, 1997a) (See also Sections 3.3 and 3.4).

3.3. α- and β-Hydroxydihydrochalcones

α- and β-Hydroxydihydrochalcones constitute rare groups of C_6-C_3-C_6 metabolites presumably sharing a close biogenetic relationship with the α-methyldeoxybenzoins and isoflavonoids (Bhakuni et al., 1973; Shukla et al., 1973; Bezuidenhoudt et al., 1981; Beltrami et al., 1982; Ferrari et al., 1983; Thakkar and Cushman, 1995). Wynberg prepared an aromatic deoxy α-hydroxydihydrochalcone via catalytic hydrogenation of the corresponding chalcone (Marsman and Wynberg, 1979). However, by utilizing the versatile epoxidation methodology, Bezuidenhoudt et al. (1987) and Augustyn et al. (1990a, 1990b) extended this protocol to the enantioselective synthesis of a series of α-hydroxydihydrochalcones. Treatment of (-)-**20a-26a** and (+)-chalcone epoxides **20b-26b** with either Pd-$BaSO_4$/H_2 or Pd-C/H_2 afforded (+)-**27a-33a** and (-)-α-hydroxydihydrochalcones **27b-33b**, respectively, in moderate to high yields and moderate ee's (Table 1.3).

Although several procedures, comprising diverse reagents, such as benzeneselenolate ion, samarium diiodide, aluminium amalgam/ultrasound, and metallic lithium in liquid ammonia, have been used for the regioselective reductive ring opening of α,β-epoxyketones to form the β-hydroxyketone (Molander and Hahn, 1986; Otsubo et al., 1987; Moreno et al., 1993; Engman and Stern, 1994), the most general reagent for these conversions is tributyltin hydride (TBTH)/azobisisobutyronitrile (AIBN) (Hasegawa et al., 1992). This method was applied to a series of chalcone epoxides comprising the methyl ethers of substrates with natural hydroxylation patterns (Nel et al., 1998, 1999a).

Table 1.3 *Synthesis of α-hydroxydihydrochalcones* ***27a/b–33a/b***



20a/b $R_1 = R_2 = R_3 = R_4 =$ H
21a/b $R_1 - R_2 - R_3 -$ H, $R_4 -$ OMe
22a/b $R_2 = R_3 =$ H, $R_1 = R_4 =$ OMe
23a/b $R_2 =$ H, $R_1 = R_3 = R_4 =$ OMe
24a/b $R_3 =$ H, $R_1 = R_2 = R_4 =$ OMe
25a/b $R_1 - R_2 - R_3 - R_4 -$ OMe
26a/b $R_1 -$ OMOM, $R_2 - R_3 -$ H, $R_4 -$ OMe

a – configuration shown
b – enantiomer

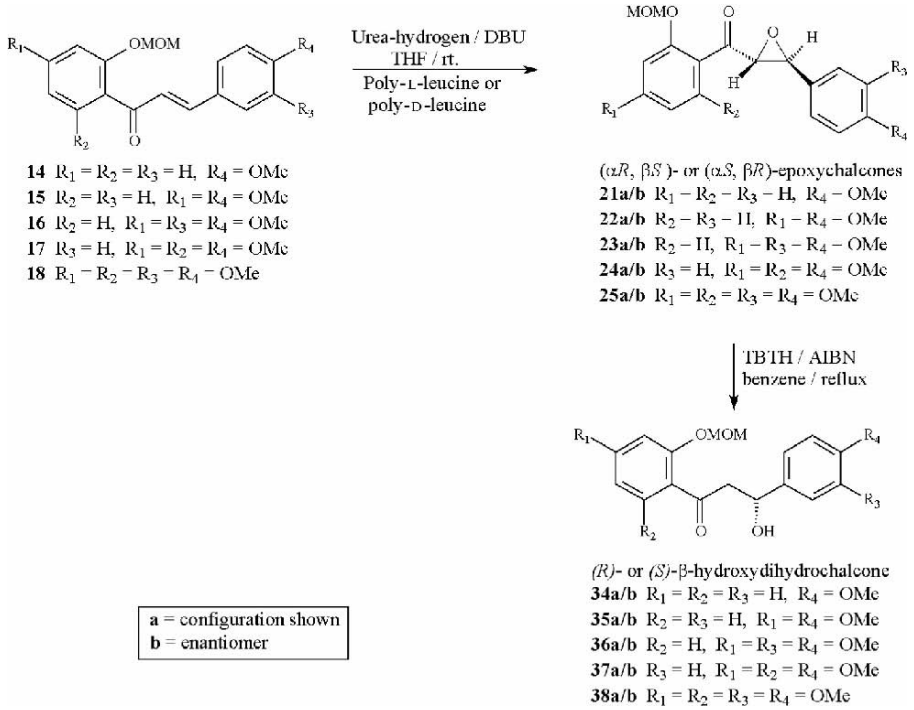
27a/b $R_1 = R_2 = R_3 = R_4 =$ H
28a/b $R_1 - R_2 - R_3 -$ H, $R_4 -$ OMe
29a/b $R_2 = R_3 =$ H, $R_1 = R_4 =$ OMe
30a/b $R_2 =$ H, $R_1 = R_3 = R_4 =$ OMe
31a/b $R_3 =$ H, $R_1 = R_2 = R_4 =$ OMe
32a/b $R_1 - R_2 - R_3 - R_4 -$ OMe
33a/b $R_1 -$ OMOM, $R_2 - R_3 -$ H, $R_4 -$ OMe

Substrate (% ee)	*Catalyst – H_2*	*Product*	*% yield*	*% ee*
(-)-20a (38)	Pd / $BaSO_4$	**(+)-27a**	92	27
(+)-20b (53)	Pd / $BaSO_4$	**(-)-27b**	61	54
(-)-21a (66)	Pd / $BaSO_4$	**(+)-28a**	51	61
(+)-21b (46)	Pd / $BaSO_4$	**(-)-28b**	72	48
(-)-22a (84)	Pd / $BaSO_4$	**(+)-29a**	88	76
(+)-22b (53)	Pd / $BaSO_4$	**(-)-29b**	70	52
(-)-23a (62)	10% Pd / C	**(+)-30a**	42	61
(+)-23b (25)	10% Pd / C	**(-)-30b**	40	16
(-)-24a (32)	5% Pd / C	**(+)-31a**	*	24
(+)-24b (20)	5% Pd / C	**(-)-31b**	*	19
(-)-25a (*)	10% Pd / C	**(+)-32a**	*	14
(+)-25b (*)	10% Pd / C	**(-)-32b**	*	16
(-)-26a (70)	Pd / $BaSO_4$	**(+)-33a**	50	65
(+)-26b (36)	Pd / $BaSO_4$	**(-)-33b**	46	32

*Not reported

Since the Julia asymmetric epoxidation of chalcones often gives disappointing stereoselectivity, Nel et al. (1998, 1999a) also used the improved two-phase nonaqueous system with poly-amino acids as asymmetric catalysts, recently developed by Bentley and Roberts (Lasterra-Sanchez et al., 1996; Bentley et al., 1997). Treatment of enones **14-18** with immobilized poly-L-leucine (PLL)/urea-hydrogen peroxide complex (UHP) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dry THF, afforded the (-)-(α*R*,β*S*)-*trans*-epoxychalcones **21a-25a** in moderate to high yields (21-80%) and improved optical purity (53-95% ee). The enantiomeric (+)-(α*S*,β*R*)-*trans*-epoxychalcones **21b-25b** were similarly obtained using immobilized poly-D-leucine (PDL) (yield, 19-76%; ee, 50-90%). The chalcone epoxides **21a/b-25a/b** were then treated with TBTH/AIBN in refluxing benzene to afford the (*R*)- **34a-38a** and (*S*)-2′-*O*-methoxymethyl-β-hydroxydihydrochalcones **34b-38b** in excellent yields (70-90%) and without loss of optically purity (Table 1.4).

Table 1.4 *β-Hydroxydihydrochalcone formation*

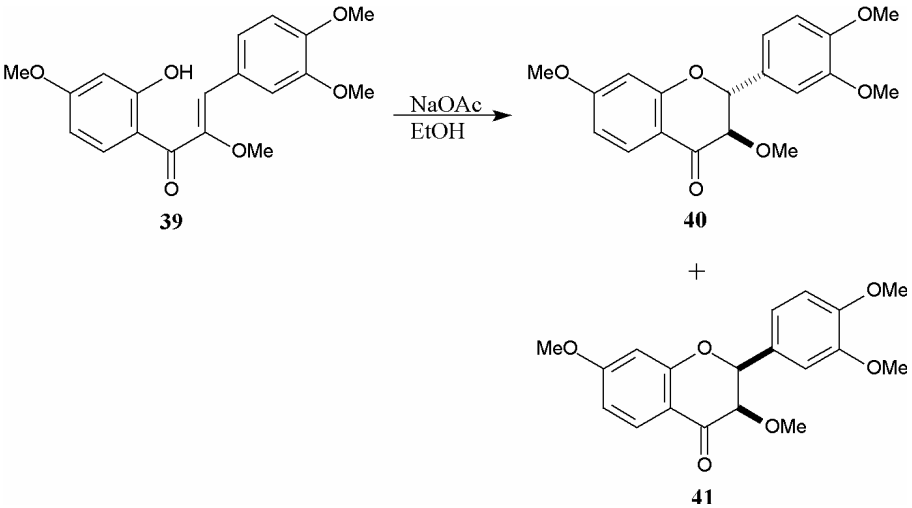


Chalcone	Poly amino acid	Chalcone-epoxide	% yield	% ee	β-hydroxy-dihydro-chalcone	% yield	% ee
14	PLL	**21a**	71	85	**34a**	73	85
14	PDL	**21b**	69	81	**34b**	70	80
15	PLL	**22a**	80	95	**35a**	83	91
15	PDL	**22b**	76	90	**35b**	90	88
16	PLL	**23a**	64	88	**36a**	78	84
16	PDL	**23b**	61	87	**36b**	81	85
17	PLL	**24a**	36	60	**37a**	79	55
17	PDL	**24b**	33	61	**37b**	76	61
18	PLL	**25a**	21	53	**38a**	83	48
18	PDL	**25b**	19	50	**38b**	78	47

3.4. Dihydroflavonols

Although the Algar-Flynn-Oyamada (AFO) protocol (Geissman and Fukushima, 1948; Dean and Podimuang, 1965) and the Weeler reaction were mainly used for the synthesis of aurones, it was demonstrated that these reactions can be adapted for the formation of racemic dihydroflavonols (Saxena et al., 1985; Patonay et al., 1993; Donnelly and Doran, 1975; Donnelly et al., 1979; Donnelly and Emerson, 1990; Donnelly and Higginbotham, 1990) in moderate to good yields.

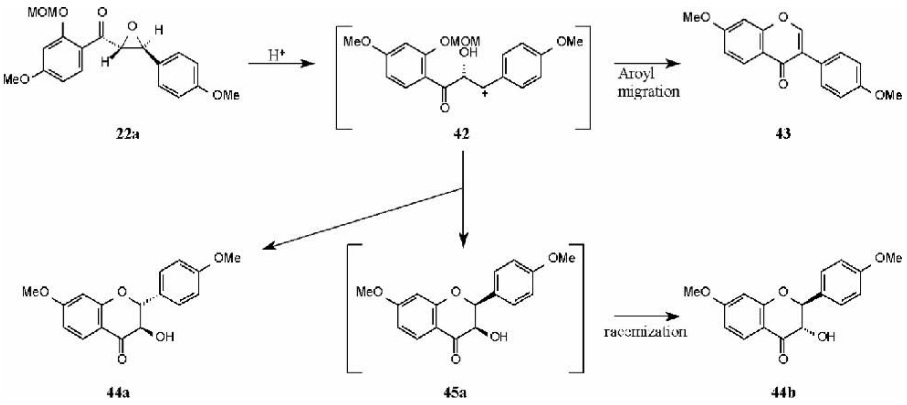
Cyclization of 2′-hydroxy-α,3,4,4′-tetramethoxychalcone **39** with sodium acetate in ethanol furnished both 3,3′,4′,7-*O*-tetramethyl-2,3-*trans*-**40** and 3,3',4',7-*O*-tetramethyl-2,3-*cis*-dihydroflavonols **41** in 22% and 11% yields, respectively (Scheme 1.3). However, this method was not applicable to cycli-zation of α-OH-chalcones (Van der Merwe et al., 1972; Ferreira et al., 1975).



39 **40** **41**

Scheme 1.3 *Chalcone cyclization with NaOAc in EtOH to yield trans- and cis-dihydroflavonols.*

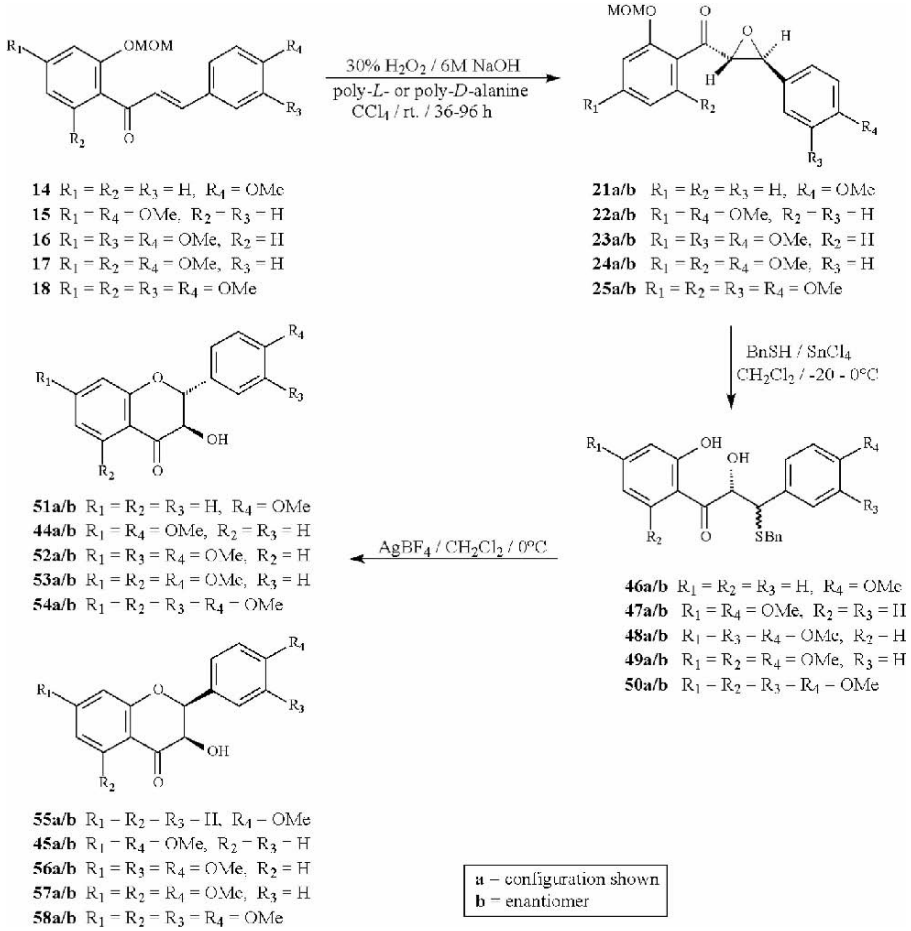
Initial attempts toward acid catalyzed cyclization of the chalcone epoxide to the corresponding (2*R*,3*R*)-2,3-*trans*- **44a** and (2*S*,3*R*)-2,3-*cis*-dihydroflavonols **45a** were hampered by two difficulties, i.e., aryl migration with formation of 4′,7-dimethoxyisoflavone **43** and the epimerization/racemization of the thermodynamically less stable (2*S*,3*R*)-2,3-*cis*-4′,7-dimethoxydihydroflavonol **45a** to yield (2*S*,3*S*)-2,3-*trans*-dihydroflavonol **44b** (Augustyn et al., 1990a) (Scheme 1.4). The "loss" of optical purity in the **22a** → **44a** conversion indicates competition between protonation of the heterocyclic oxygen and hydrolysis of the 2′-*O*-acetal functionality, hence leading to a considerable degree of S_N1 character for the cyclization step with concomitant racemization at C-β of a presumed carbocationic intermediate **42**, yielding dihydroflavonols **44a** and **45a**. The thermodynamically less stable (2*S*,3*R*)-2,3-*cis*-dihydroflavonol **45a** is rapidly racemized at C-3 to give a mixture of **45a** and **44b** under the prevailing acidic conditions. Formation of the isoflavone **43** is attributed to acid-catalyzed cleavage of the highly reactive oxirane functionality prior to deprotection.



Scheme 1.4 *Attempts toward synthesis of (2R,3R)-2,3-trans-* ***44a*** *and (2S,3R)-2,3-cis-dihydroflavonols 45****a*** *using acid-catalyzed cyclization.*

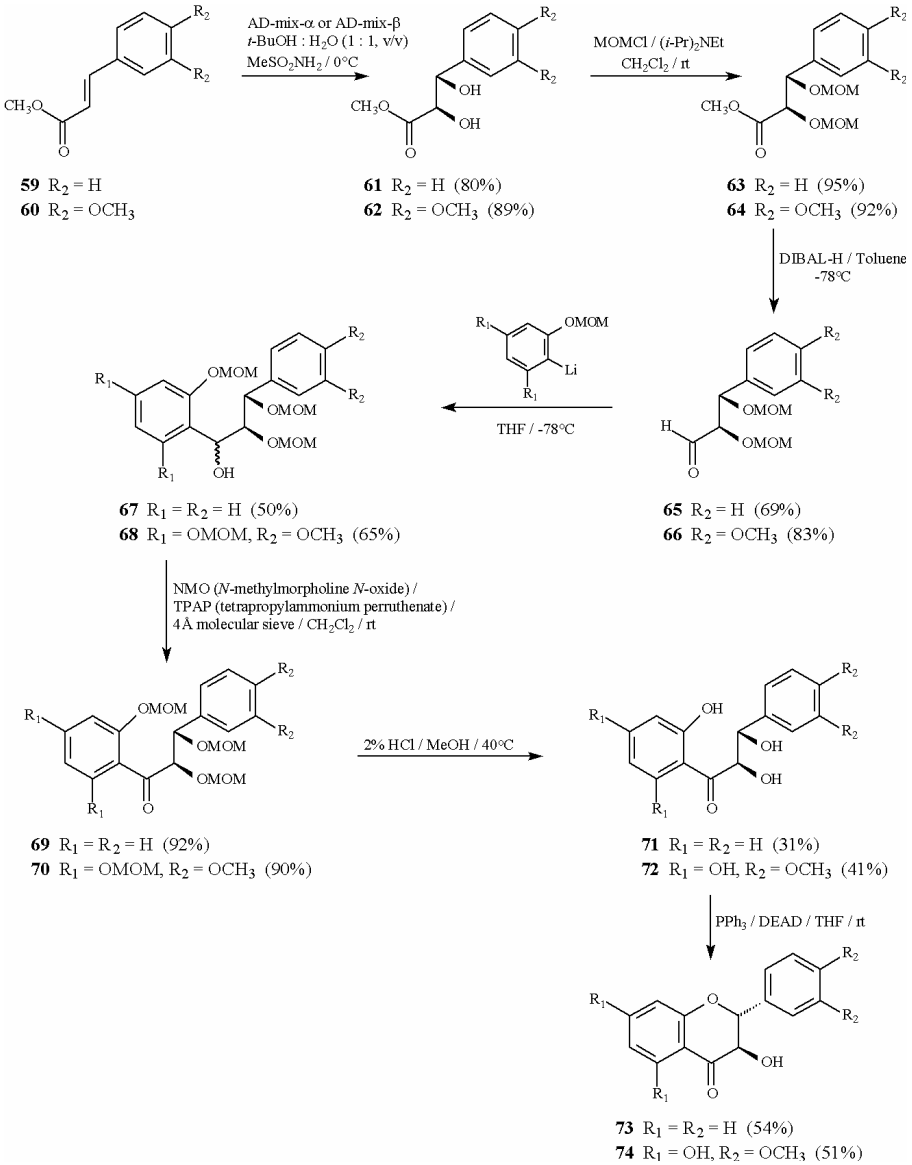
In order to enhance the S_N2 nature of the ring closure step, and thus the formation of **44a**, methods aimed at the selective removal of the 2'-*O*-methoxymethyl group under mild conditions were explored. It was anticipated that deprotection of the 2′-*O*-methoxymethyl group with concomitant cyclization would enhance the preservation of optical integrity. In order to circumvent the problem of isoflavone formation, Van Rensburg et al. (1996, 1997a) investigated methods aimed at the initial nucleophilic opening of the oxirane functionality, followed by deprotection and cyclization. The excellent nucleophilic and nucleofugic properties of mercaptans (Barrett et al., 1989) prompted evaluation of thiols in the presence of Lewis acids and resulted in the selection of the phenylmethanethiol–tin(IV) chloride ($BnSH/SnCl_4$) system as the reagent of choice for the oxirane cleavage (Chini et al., 1992). Treatment of the series of chalcone epoxides **21a/b-25a/b** with $BnSH/SnCl_4$ selectively cleaved the C_β-O bond of the oxirane functionality at –20°C and effectively deprotected the methoxymethyl group at 0°C to give the corresponding α,2′-dihydroxy-β-benzylsulfanyldihydrochalcones **46a/b-50a/b** as diastereomeric mixtures (*syn: anti, ca.* 2.3:1) in 86-93% yield. Treatment of these α-hydroxy-β-benzylsulfanyldihydrochalcones **46a/b-50a/b** with the thiophilic Lewis acid, silver tetrafluoroborate ($AgBF_4$) in CH_2Cl_2 at 0°C, gave the 2,3-*trans*-dihydroflavonols **44a/b, 51a/b-54a/b** in good yield and albeit in low proportions for the first time also the 2,3-*cis* analogues **45a/b, 55a/b-58a/b** (Table 1.5).

Table 1.5 *Asymmetric synthesis of dihydroflavonols*



Epoxide	% yield	% ee	Dihydro-chalcone	% yield	Dihydro-flavonol	% yield	% ee	*trans:cis*
21a	99	84	**46a**	86	**51a / 55a**	86	83	93 :7
21b	98	69	**46b**	90	**51b / 55b**	83	69	94 : 6
22a	98	86	**47a**	93	**44a / 45a**	71	84	79 :21
22b	98	74	**47b**	90	**44b / 45b**	72	75	83 : 17
23a	99	67	**48a**	89	**52a / 56a**	81	68	85 : 15
23b	98	58	**48b**	91	**52b / 56b**	79	58	86 : 14
24a	97	70	**49a**	89	**53a / 57a**	65	69	78 : 22
24b	97	53	**49b**	89	**53b / 57b**	64	53	84 : 16
25a	79	49	**50a**	91	**54a / 58a**	61	47	82 :18
25b	76	49	**50b**	88	**54b / 58b**	63	44	80 :20

A highly enantioselective synthetic method (99%, ee) was reported by Jew et al. (2000) for optically pure (2*R*,3*R*)-dihydroflavonols, by using catalytic asymmetric dihydroxylation and an intramolecular Mitsunobu reaction as key steps (Scheme 1.5).



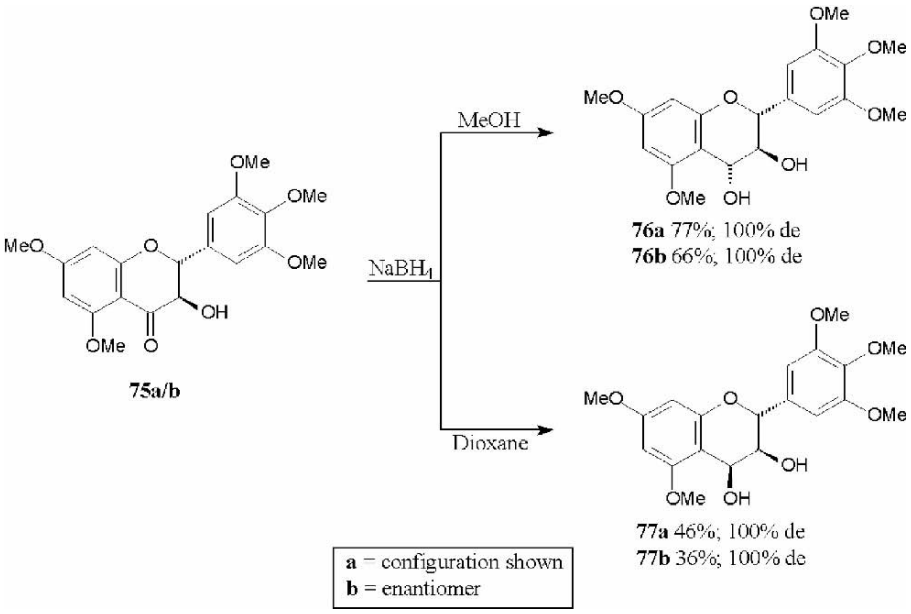
Scheme 1.5 *Synthesis of dihydroflavonol* ***73*** *and 3′,4′-di-O-methyltaxifolin* ***74****.*

Sharpless asymmetric dihydroxylation of **59** and **60** with AD-mix-α gave the 2*R*,3*S*-diols **61** and **62** in excellent yields (80% and 89%, respectively) and ee (99%). This was followed by protection of the C-2 and C-3 hydroxyl groups with MOMCl and reduction with diisobutylaluminium hydride to give the corresponding aldehydes **65** and **66**. Addition of aryllitium to aldehydes **65** and **66** afforded the secondary alcohols **67** and **68**. Oxidation of **67** and **68** produced the corresponding ketones **69** and **70**, which were deprotected under acidic conditions to give the pentahydroxyketones **71** and **72**. An intramolecular Mitsunobu (Mitsunobu, 1981) reaction afforded dihydroflavonol **73** and 3′,4'-di-*O*-methyltaxifolin **74**, respectively. The absolute configuration of the newly formed stereogenic center C-2 of **73** and **74** were assigned as 2*R*, consistent with the S_N2-mechanism of the Mitsunobu reaction.

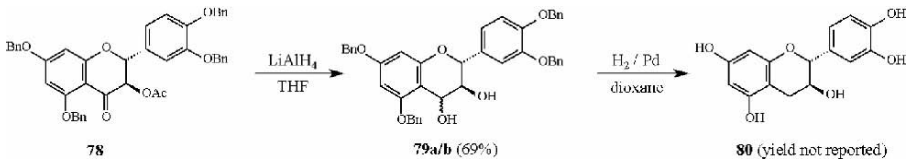
3.5. Flavan-3-ols and Flavan-3,4-diols

Flavan-3-ols, e.g., (+)-catechin and (-)-epicatechin, represent the largest class of naturally occurring C_6-C_3-C_6 monomeric flavonoids. Flavan-3-ols also have received considerable interest over the last few years because of their importance as the constituent units of proanthocyanidins (Porter, 1988, 1994; Ferreira and Bekker, 1996; Ferreira and Li, 2000; Ferreira and Slade, 2002; Ferreira et al., 2005). Progress in the study of these complex phenolics is often hampered by the limited availability of naturally occurring flavan-3-ol nucleophiles with 2,3-*trans,* and especially 2,3-*cis,* configuration. One of the most common ways for the synthesis of flavan-3-ols and the closely related flavan-3,4-diol analogues involves the reductive transformation of dihydroflavonols. Reduction of the dihydroflavonols **75a**/**b** with sodium borohydride in methanol affords the 2,3-*trans*-3,4-*trans*-flavan-3,4-diols **76a/b**, while reduction in an aprotic solvent like dioxane yielded the C_4-epimers **77a/b** exclusively (Scheme 1.6) (Takahashi et al., 1984; Onda et al., 1989). Such reversal in the direction of the hydride attack could probably be explained in terms of the presence of hydrogen bonding in aprotic solvents.

Catechin **80** represents the only flavan-3-ol synthesized from the corresponding dihydroflavonol (Weinges, 1958; Freudenberg and Weinges, 1958). Consecutive treatment of 2,3-*trans*-3-*O*-acetyldihydroquercetin tetra-*O*-benzyl ether **78** with $LiAlH_4$ and H_2/Pd gave the free phenolic flavan-3-ol **79** in optically pure form (Scheme 1.7). ^{13}C-Labeled (±)-catechin recently was synthesized by utilizing osmium-catalyzed dihydroxylation of a flav-3-ene intermediate as a key step to yield the 2,3-*trans*-3,4-*cis*-isomer with high diastereoselectivity. The first attempt included ten steps, starting from $K^{13}CN$ (Nay et al., 2000). A slightly different but improved approach was later developed by the same group (Arnaudinaud et al., 2001a, 2001b) for the formation of ^{13}C-labeled (-)-procyanidin B-3. Improved yields were reported and the number of steps to the pivotal intermediate flav-3-ene was reduced. A disadvantage using these protocols is that enantiomeric mixtures are formed that require more refined and usally more expensive separation methods.

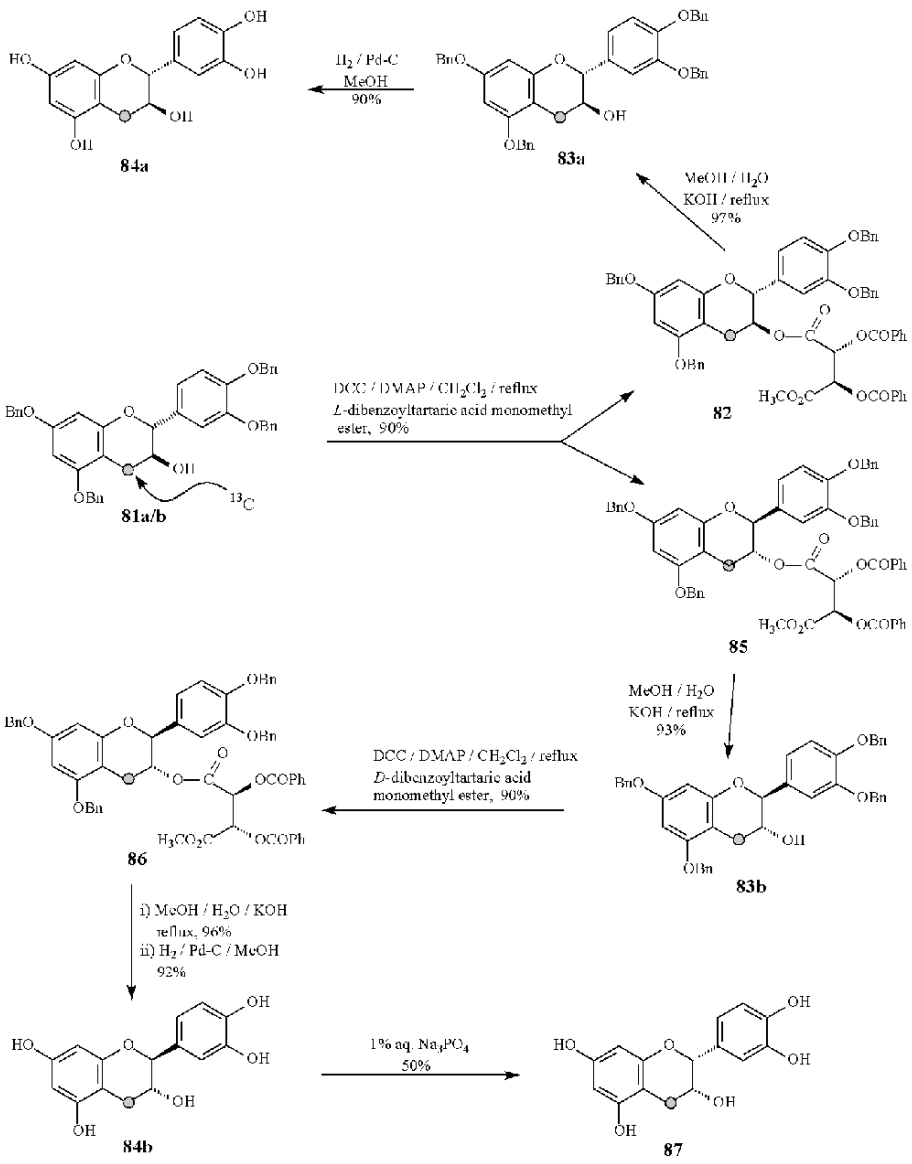


Scheme 1.6 *Reduction of dihydroflavonols with $NaBH_4$ to afford flavan-3,4-diols*



Scheme 1.7 *Reduction of 2,3-trans-3-O-acetyldihydroquercetin tetra-O-benzylether **78** to yield catechin 80.*

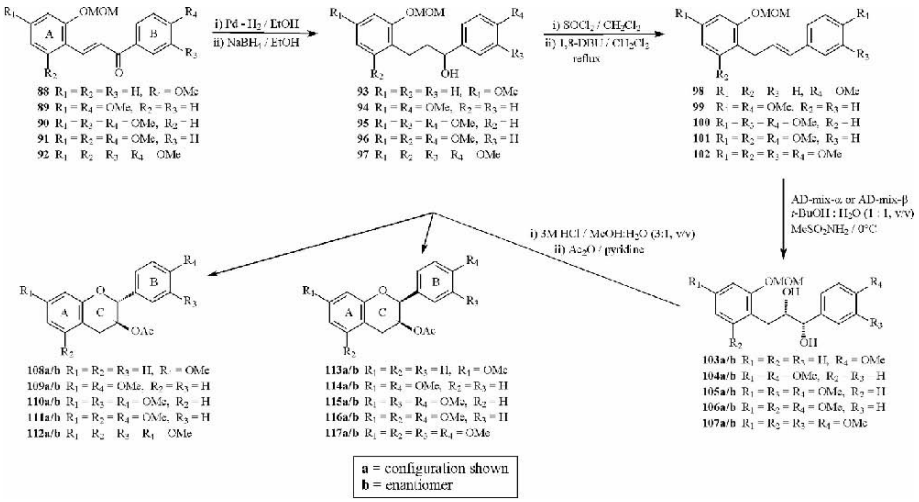
(+)-[^{13}C]-Catechin **84a** and (-)-[^{13}C]-epicatechin **87** were isolated in high ee, respectively, by the formation of their tartaric acid derivatives (Nay et al., 2001). The resolution process included the esterification of the 3-OH group of **81a/b** with L-dibenzoyltartaric acid monomethyl ester to give a mixture of diastereomers **82** and **85** (92%) (Scheme 1.8). The (+)-catechin derivative **82** was crystallized in hexane/dichloromethane (3:1) (diastereometic excess [de] > 99%), while the (-)-*ent*-catechin derivative **85** remained in solution. The diastereomeric pure (de = 99%) (-)-*ent*-catechin derivative **86** also was isolated by crystallization after hydrolysis ($MeOH/H_2O/KOH$) of **85**, following esterification with D-tartaric acid. (+)-[^{13}C]-catechin **84a** was isolated in a high yield and ee (99%) after hydrolysis and reduction/deprotection steps. Epimerization at C-2 of (-)-[^{13}C]-*ent*-catechin **84b**, using 1% (w/v) *aq.* Na_3PO_4, led to an equilibrium mixture of (-)-**84b** and (-)-[^{13}C]-epicatechin **87** in an approximate 3:1 ratio after 20 hr at 25°C (ee >99%).



Scheme 1.8 *Synthesis via resolutions of (+)-[^{13}C]-catechin* ***84a*** *and (-)-[^{13}C]-epicatechin* ***87****.*

In order to address the issue of stereocontrol at C-2 and C-3 of the flavan-3-ol molecular framework, Van Rensburg et al. (1997b, 1997c) designed a concise protocol based on the transformation of *retro*-chalcones into 1,3-diaryl-propenes (Table 1.6). These compounds are then subjected to asymmetric dihydroxylation to give polyoxygenated diarylpropan-1,2-diols, which are used as chirons for essentially enantiopure flavan-3-ols. This protocol included a base-catalyzed condensation of the appropriately oxygenated acetophenones and benzaldehydes to

Table 1.6 *Synthesis of flavan-3-ols* ***108a/b-117a/b***



Propan-1-ols	*% yield*	*Prop-enes*	*% yield*	*1,2-diols*	*% yield*	*% ee*	*Flavan-3-ols*	*% yield*	*Trans:cis*
93	99	**98**	73	**103a**	82	99	**108a/113a**	87	1:0.33
				103b	84	99	**108b/113b**	88	1:031
94	98	**99**	74	**104a**	86	99	**109a/114a**	88	1:036
				104b	82	99	**109b/114b**	90	1:0.33
95	99	**100**	70	**105a**	85	99	**110a/115a**	82	1:0.32
				105b	83	99	**110b/115b**	80	1:0.30
96	98	**101**	68	**106a**	80	99	**111a/116a**	71	1:0.32
				106b	83	99	**111b/116b**	70	1:0.33
97	99	**102**	66	**107a**	80	99	**112a/117a**	66	1:0.34
				107b	87	99	**112b/117b**	65	1;0.35

In all cases, the ee was 99%.

afford the (*E*)-*retro*-chalcones **88-92** ($J_{\alpha,\beta}$ 15.8–16.0 Hz). Consecutive reduction (Pd-H_2 and $NaBH_4$), followed by elimination {$SOCl_2$ and 1,8-diazabicyclo[5.4.0]undec-7-ene (1,8-DBU)} of the ensuing alcohols **93–97** afforded the (*E*)-1,3-diarylpropenes (deoxodihydrochalcones) **98–102** ($J_{1,2}$ 16 Hz) in resonable overall yield (65–73%). Owing to the excellent results obtained (Sharpless et al., 1977, 1992; Kwong et al., 1990; Jeong et al., 1992; Amberg et al., 1993; Gobel and Sharpless, 1993; Wang et al., 1993; Kolb et al., 1994a, 1994b; Norrby et al., 1994) during asymmetric dihydroxylation (AD reaction) of olefins with AD-mix-α or AD-mix-β, these stereoselective catalysts were utilized for the introduction of chirality at C-2 and C-3 of the flavan-3-ol framework. Thus, treatment of the protected (*E*)-propenes **98–102** at 0°C with AD-mix-α in the two phase system ^{t}BuOH: H_2O (1:1) afforded the (+)-(1*S*,2*S*)-*syn*-diols **103a–107a** ($J_{1,2}$ 5.8 -6.5 Hz) in high yields (80–86%) and optical purity (99% ee). The (-)-(1*R*,2*R*)-*syn*-diols **103b–107b** were similarly obtained by using AD-mix-β (yield: 82–87%, 99% ee). Application of the Lewis acid-catalyzed phenylmethanethiol ring-opening and cyclization of chalcone epoxides in the synthesis of dihydroflavonols (see Section 3.4) (Van Rensburg et al., 1996, 1997a) to cyclization of the diols, however, resulted in slow (24 hr) and low percentage conversion (10–20%) into flavan-3-ols.

In order to transform the diols more effectively into the corresponding flavan-3-ols, methods aimed at the selective removal of the 2'-*O*-methoxymethyl group and subsequent ring closure under mild acidic conditions were explored. Simultaneous deprotection and cyclization of diols **103a/b–107a/b** in the presence of 3M HCl in MeOH, followed by acetylation, yielded the 2,3-*trans*- (yield: 48-68%) **108a/b–112a/b** and for the first time 2,3-*cis*-flavan-3-ols (yield:17–22%) **113a/b–117a/b** in excellent enantiomeric excess (>99%). Assignment of the absolute configuration of the resulting flavan-3-ol derivatives **108a/b–117a/b** by ^{1}H-NMR and CD data confirmed the configuration of the diols as derived from the Sharpless model.

The potential of this protocol in the chemistry of the oligomeric proanthocyanidins is evident, especially in view of its aptitude to the synthesis of free phenolic analogues. The latter analogues are as conveniently accessible by simply using more labile protecting groups instead of *O*-methyl ethers. This was illustrated by Nel et al. (1999b) by synthesis of the 4′,7′-dihydroxyflavan-3-ol diastereomers to confirm (2*R*,3*S*)-guibourtinidol as a new natural product. Owing to the acid lability of methoxymethyl derivative, the MOM functionality was used as a protecting group. This method was extended to the synthesis of the full range of flavan-3-ols, comprising different oxygenated phenolic substitutions as found in nature (Nel et al., 1999c).

3.6. Isoflavonoids

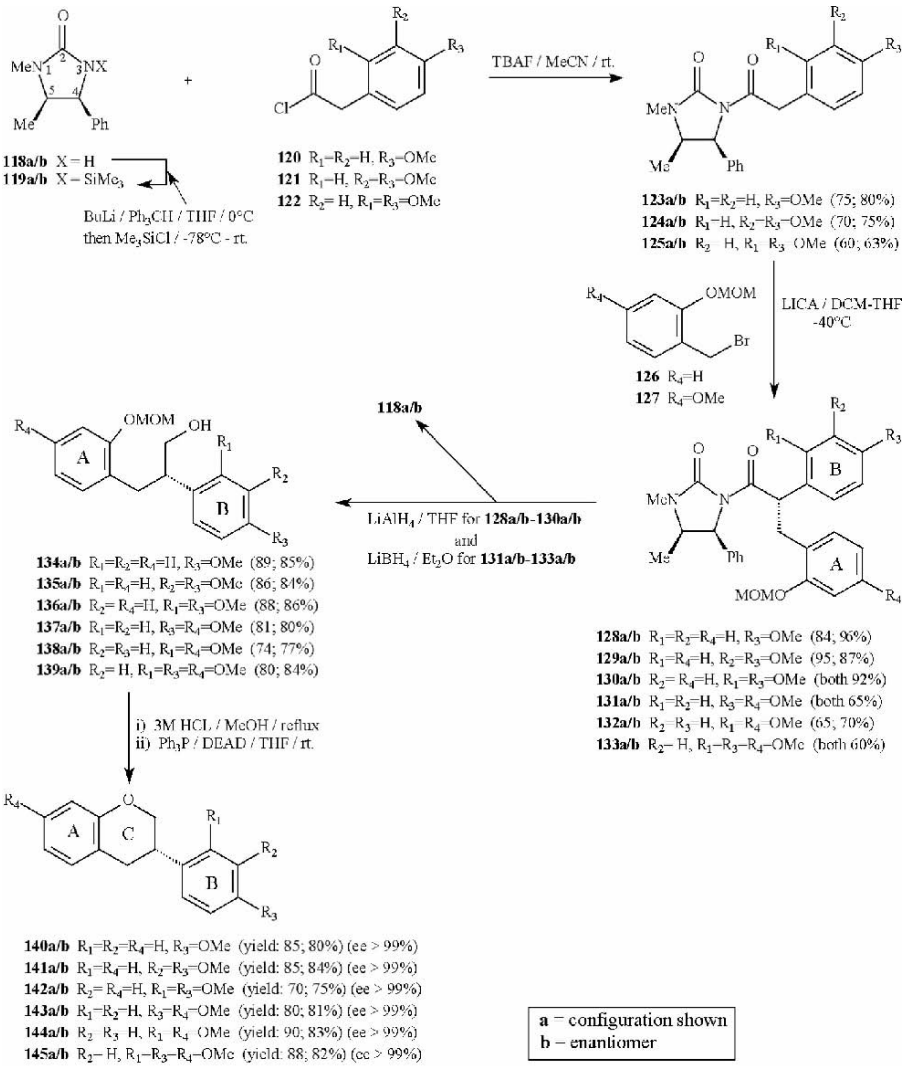
Synthetic routes to optically pure pterocarpans, exhibiting the aromatic oxygenation patterns of naturally occurring isoflavonoids, are limited by the lack of readily accessible starting materials. These restrictions and the challenge to form the tetracyclic ring system with stereocontrol led to the development of various synthetic approaches. Synthetic endeavors toward pterocarpans comprise Heck arylation (Ishiguro et al., 1982; Narkhede et al., 1990), the reduction and cyclization of the corresponding 2'-hydroxyisoflavanones (Krishna Prasad et al., 1986), cycloaddition reactions of 2*H*-chromenes with 2-alkoxy-1,4-benzoquinones (Engler et al., 1990; Subburaj et al., 1997), and 1,3-Michael–Claisen annulation (Ozaki et al., 1988, 1989). Only two methods, i.e., asymmetric dihydroxylation of an isoflav-3-ene (Pinard et al., 1998) and subsequent "hydrogenative cyclization" or 1,4-benzoquinone cyclo-addition reactions utilizing chiral Ti(IV) complexes (Engler et al., 1991, 1999), permitted enantioselective access to this class of compounds.

3.6.1. Isoflavans

Given the fact that the configuration at C-3 would dictate the configuration at C-2 or C-4 in the 3-phenylchroman framework, a series of isoflavans were synthesized, which would then afford stereoselective access to other classes of chiral isoflavonoids (Versteeg et al., 1995, 1998, 1999). The protocol involved the stereoselective α-benzylation of phenylacetic acid derivatives, subsequent reductive removal of the chiral auxiliary, and cyclization into the isoflavans (Scheme 1.9). Owing to the efficiency of the asymmetric alkylation reactions of chiral imide enolates, (4*S*,5*R*)-(+)- and (4*R*,5*S*)-(-)-1,5-dimethyl-4-phenyl-2-imidazolidinones **118a** and **118b** were used as chiral auxiliaries in the benzylation reactions (Close, 1950; Roder et al., 1984; Evans et al., 1987; Cardillo et al., 1988; Drewes et al., 1993). The basicity of the imidazolidinones was decreased by utilizing the trimethylsilyl ethers **119a** and **119b** in the acylation step using the phenylacetyl chlorides **120-122**. The ensuing *N*-acyl imidazolidinones **123a/b-125a/b** were then alkylated with the appropriate 2-*O*-methoxymethylbenzyl bromides **126** and **127** in good to excellent yields with only one diastereomer isolated (de > 99%). Removal of the chiral auxiliary was effected by reductive deamination using $LiAlH_4$ in THF for imides **128a/b-130a/b** and a saturated solution of $LiBH_4$ in ether for analogues **131a/b-133a/b** to give the 2,3-diarylpropan-1-ols **134a/b-139a/b** (Cardillo et al., 1989; Paderes et al., 1991). Acidic deprotection (3M HCl in MeOH), followed by cyclization under Mitsunobu conditions (Shih et al., 1987) afforded the target isoflavans **140a/b-145a/b** in excellent yields and in nearly enantiopure form (ee >96-99%).

The stereochemistry of the alkylation step is explicable in terms of the preferential formation of a *Z*-enolate (Evans at al., 1982). Attack of the electrophile is then directed to the face of the enolate opposite the phenyl moiety on the chiral auxiliary. The chiral auxiliary with 4*S*-configuration led to propanols exhibiting positive optical rotations and those from 4*R*-*N*-acyloxazolidinones showing negative values, in accordance with observations by Evans et al. (1982).

Alkylation of (4*S*,5*R*)-(+)-*N*-phenylacetylimidazolidinones resulted in (+)-propanols and (3*S*)-isoflavans and (4*R*,5*S*)-(-)-*N*-phenylacetylimidazolidinones in (-)-propanols and (3*R*)-isoflavans.



Scheme 1.9 *Stereoselective synthesis of (R)- and (S)-isoflavans.*

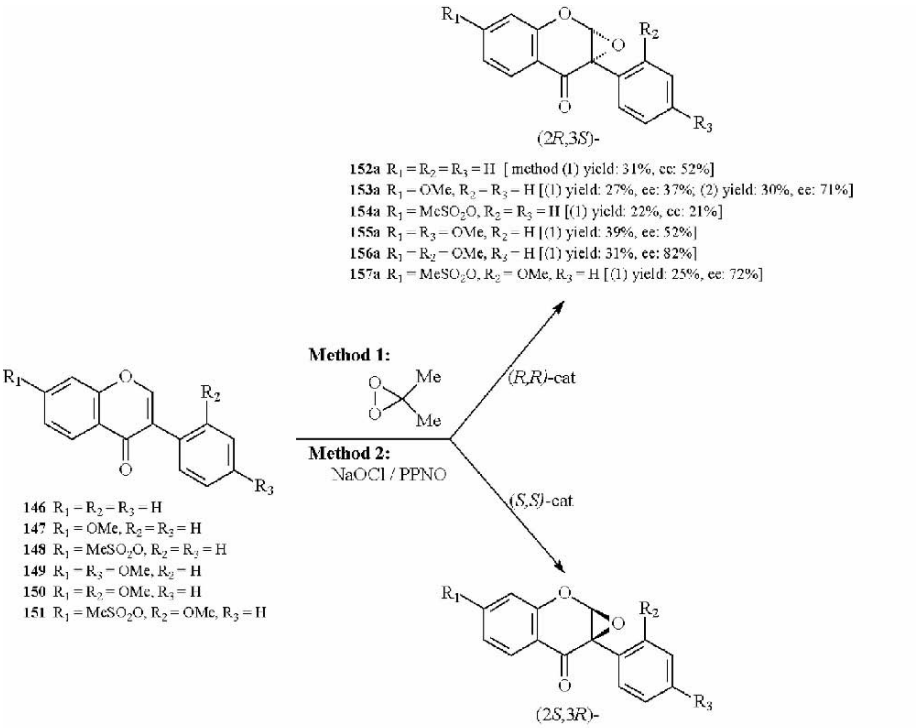
3.6.2. Isoflavone Epoxides

The first representatives of flavone epoxides were prepared either by alkaline hydrogen peroxide epoxidation of isoflavones or by an intramolecular Darzens reaction of α-bromo-*O*-acyloxyacetophenones. Lévai et al. (1998) demonstrated that dimethyldioxirane (DMDO) is a convenient and effective reagent for the epoxidation

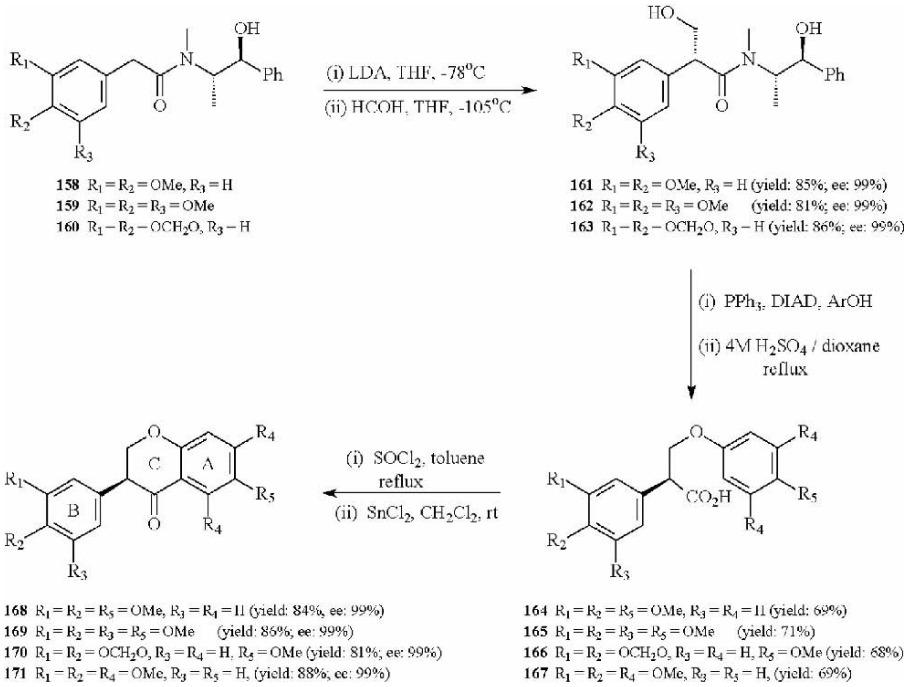
of various substituted isoflavones and subsequently prepared isoflavone glycoside epoxides in high yields by utilizing this versatile oxidizing agent. However, attempts to synthesize enantiomeric isoflavone epoxides with DMDO and a chiral auxiliary demonstrated that the sugar chiral auxiliary did not exercise enantiofacial selectivity and epoxides were isolated as 1:1 diasteromeric mixtures. The Jacobsen's Mn(III)salen complexes have proved to be highly efficient catalyst for the enantioselective epoxidation of olefins by using various oxygen donors. It was demonstrated that epoxidation of 2,2-dimethyl-2*H*-chromenes, in the presence of optically active Mn(III)salen complexes and DMDO, proceeded enantioselectively. Epoxidation of isoflavones **146–151**, utilizing the Mn(III)salen complexes (*R*,*R*)- and (*S*,*S*)-*N*,*N*´-*bis*(3,4-di-*t*-butylsalicylidene)-1,2-cyclohexanediaminomanganese chloride as catalysts and DMDO or NaOCl as oxygen donors, afforded for the first time the optically active isoflavone epoxides **152a/b–157a/b** (Scheme 1.10).

3.6.3. Isoflavanones

By employing a stereocontrolled aldol reaction as the key step, optically active isoflavones **168–171** were synthesized for the first time by Vicario et al. (2000) in good yields and excellent ee's (Scheme 1.11). This sequence included an asymmetric aldol reaction between (*S*,*S*)-(+)-pseudoephedrine arylacetamide and formaldehyde to introduce chirality in the isoflavanone carbon framework at C-3. This was followed by the introduction of the B-ring as a phenol ether under Mitsunobu conditions and subsequent removal of the chiral auxiliary. Acids **164–167** were then converted by an intramolecular Friedel–Crafts acylation, yielding the isoflavanones **168–171** in good yields and essentially enantiopure.



Scheme 1.10 *Enantioselective synthesis of isoflavone epoxides* ***152a/b–157a/b****.*



158 $R_1 = R_2 = OMe$, $R_3 = H$
159 $R_1 = R_2 = R_3 = OMe$
160 $R_1 - R_2 - OCH_2O$, $R_3 - H$

161 $R_1 = R_2 = OMe$, $R_3 = H$ (yield: 85%; ee: 99%)
162 $R_1 = R_2 = R_3 = OMe$ (yield: 81%; ee: 99%)
163 $R_1 - R_2 - OCH_2O$, $R_3 - H$ (yield: 86%; ee: 99%)

168 $R_1 = R_2 = R_5 = OMe$, $R_3 = R_4 = H$ (yield: 84%, ee: 99%)
169 $R_1 = R_2 = R_3 = R_5 = OMe$ (yield: 86%; ee: 99%)
170 $R_1 = R_2 = OCH_2O$, $R_3 = R_4 = H$, $R_5 = OMe$ (yield: 81%; ee: 99%)
171 $R_1 - R_2 - R_4 = OMe$, $R_3 - R_5 - H$, (yield: 88%, ee: 99%)

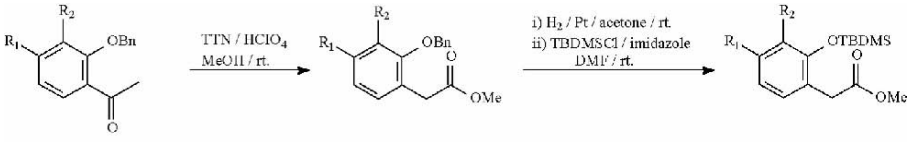
164 $R_1 = R_2 = R_5 = OMe$, $R_3 = R_4 = H$ (yield: 69%)
165 $R_1 = R_2 = R_3 = R_5 = OMe$ (yield: 71%)
166 $R_1 = R_2 = OCH_2O$, $R_3 = R_4 = H$, $R_5 = OMe$ (yield: 68%)
167 $R_1 - R_2 - R_4 = OMe$, $R_3 = R_5 = H$, (yield: 69%)

Scheme 1.11 *Stereoselective synthesis of isoflavanones* ***168–171****.*

3.6.4. Pterocarpans

Despite the identification of the first 6a-hydroxypterocarpan, (+)-pisatin, in 1960 (Cruickshank and Perrin), synthetic protocols to these potent phytoalexins are limited by lengthy multistep routes and a lack of diversity as far as phenolic hydroxylation patterns are concerned. These confinements are so restrictive that only two 6a-hydroxypterocarpans, i.e., pisatin and variabilin, have been synthesized (Bevan et al., 1964; Mansfield, 1982; Pinard et al., 1998).

The results reported for the stereoselective aldol condensation between methyl ketones and aldehydes employing diisopropylethylamine and chiral boron triflates (Paterson and Goodman, 1989) prompted the investigation for a more direct synthetic approach to address the issue of stereocontrol at C-6a and C-11a of the pterocarpan framework (Van Aardt et al., 1998, 1999, 2001). Depending on the lability and/or stability of protecting groups under certain reaction conditions, this protocol included methoxymethyl protection of the benzaldehydes **181** and **182** (labile in the presence of Lewis acids such as $SnCl_4$) and phenylacetates **178–180** as *t*-butyldimethylsilyl (TBDMS) ethers (stable under acidic conditions). Since 2-hydroxy-, 2-hydroxy-4-methoxy- and 2-hydroxy-3,4-dimethoxyphenylacetic acids are not commercially available, the required phenylacetates **175–177** were prepared via a thallium(III)nitrate (TTN) oxidative rearrangement (McKillop et al., 1973) of 2-benzyloxyacetophenones **172–174** (Scheme 1.12). Debenzylation and silylation afforded the requisite acetates **178–180** in high yields.



172 $R_1 = H, R_2 = H$
173 $R_1 = OMe, R_2 = H$
174 $R_1 = OMe, R_2 = OMe$

175 $R_1 = H, R_2 = H$
176 $R_1 = OMe, R_2 = H$
177 $R_1 = OMe, R_2 = OMe$

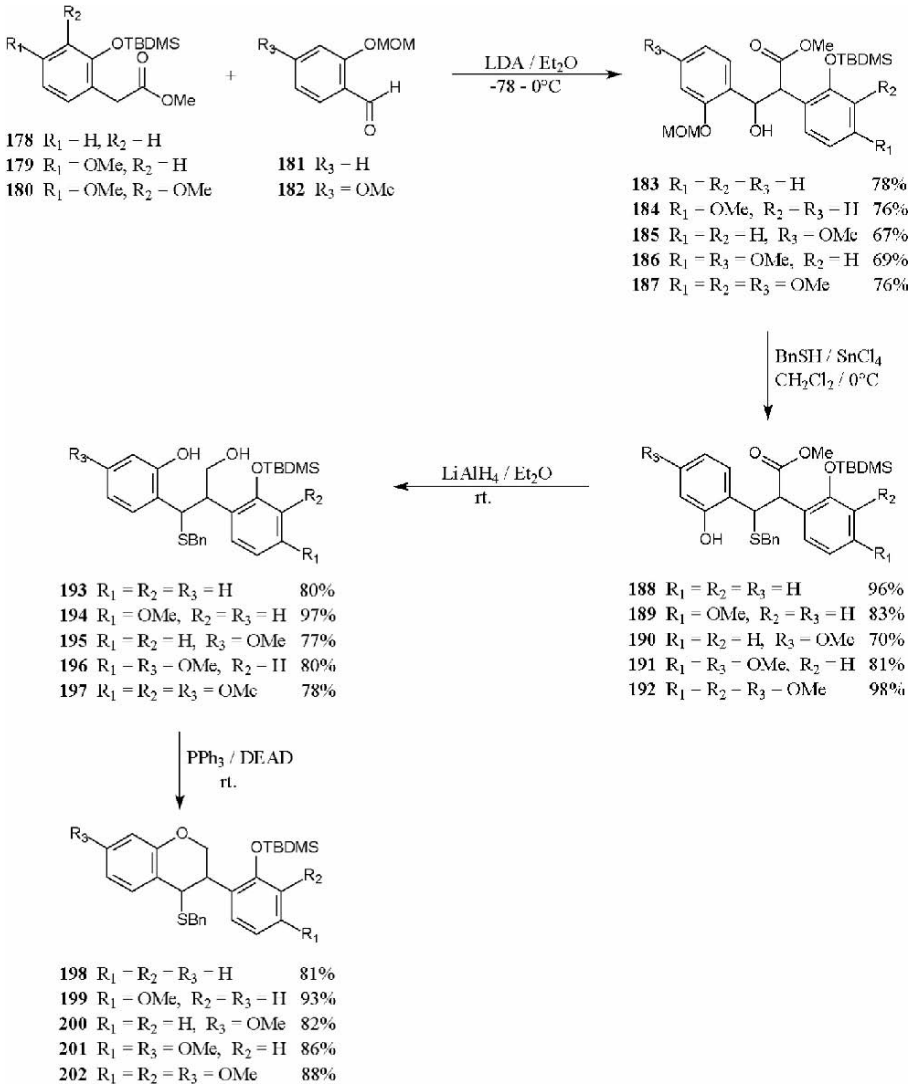
178 $R_1 = H, R_2 = H$
179 $R_1 = OMe, R_2 = H$
180 $R_1 = OMe, R_2 = OMe$

Scheme 1.12 *The synthesis of phenylacetates **178–180**.*

The subsequent condensation between the ester enolates and the benzaldehydes afforded the 2,3-diaryl-3-hydroxypropanoates **183-187** in moderate to good yields (67-78%) (Scheme 1.13). Since acid deprotection of the MOM group led to decomposition (Greene and Wuts, 1991), $SnCl_4$ in the presence of $PhCH_2SH$ as nucleophile was utilized as a selective deprotecting agent to afford the 2,3-diaryl-3-benzylsulfanylpropanoates **188-192** in 70-96% yield. Subsequent reduction of **188-292** with $LiAlH_4$ (yield: 77-97%) and ensuing cyclization under Mitsunobu conditions (Mitsunobu, 1981) [PPh_3/DEAD (diethylazodicarboxylate)] afforded the 4-benzylsulfanyliso-flavans **198-202** in good overall yields.

Cleavage of the silyl ethers using tetrabutylammonium fluoride (TBAF) on silica (Clark, 1978) gave 4-benzylsulfanyl-2′-hydroxyisoflavans **203–206**, which were converted to the 6a,11a-*cis*-pterocarpans **207–210** in yields of 39–82% using the thiophilic Lewis acids, dimethyl(methylthio)sulfonium tetra-fluoroborate (DMTSF) or silver trifluoromethanesulfonate (CF_3SO_3Ag) (Trost and Murayama, 1981; Williams et al., 1984; Trost and Sato, 1985) (Scheme 1.14).

Isoflav-3-enes **215** and **216** were obtained via periodate oxidation of the *cis*-and *trans*-4-benzyl-sulfanylisoflavans **201** and **202** followed by thermal elimination of the sulfoxides **213** and **214** (Emerson et al., 1967; Kice and Campbell, 1967; Trost et al., 1976) (Table 1.7). Owing to the instability of isoflav-3-enes **215** and **216**, swift transformation to the corresponding isoflavan-3,4-diols was essential. The commercially available AD-mix-α or -β was not reactive enough to effect asymmetric dihydroxylation. Therefore, treatment of isoflav-3-enes **215** and **216** in CH_2Cl_2 at −78°C with stoichiometric amounts of OsO_4 in the presence of the chiral catalyst dihydroquinine *p*-chlorobenzoate (DHQ-CLB) **211** afforded (-)-(3*R*,4*S*)-*syn*-diols **217a** and **218a** in acceptable yields (63–68%) and excellent enantiomeric excesses (>99%) (Kolb et al., 1994a; Pinard et al., 1998). The (+)-(3*S*,4*R*)-*syn*-diols **217b** and **218b** were similarly obtained by using dihydroquinidine *p*-chlorobenzoate (DHQD-CLB) **212** as chiral ligand.



178 $R_1 = H, R_2 = H$
179 $R_1 = OMe, R_2 = H$
180 $R_1 = OMe, R_2 = OMe$

181 $R_3 = H$
182 $R_3 = OMe$

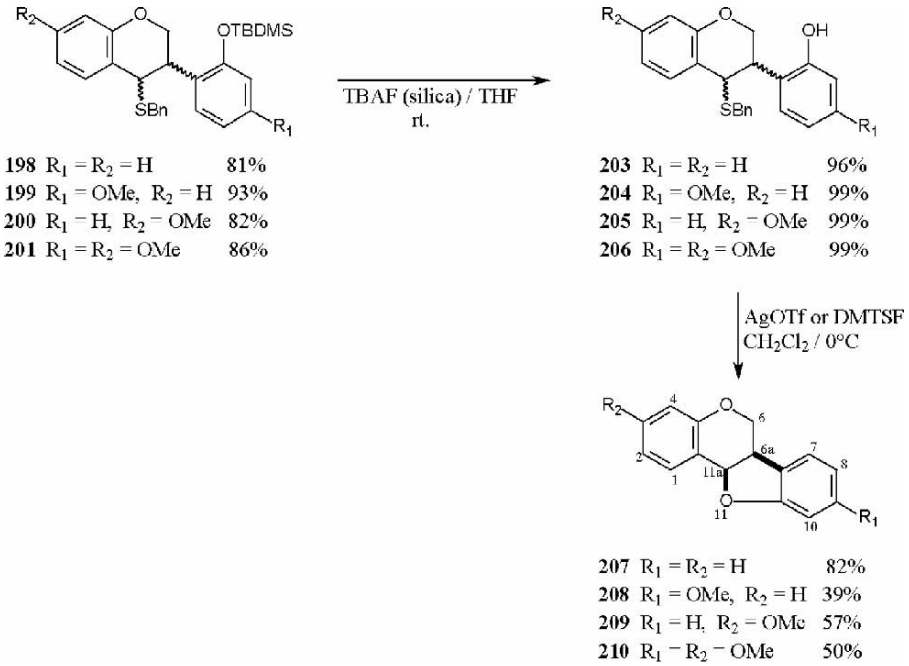
183 $R_1 = R_2 = R_3 = H$ 78%
184 $R_1 = OMe, R_2 = R_3 = H$ 76%
185 $R_1 = R_2 = H, R_3 = OMe$ 67%
186 $R_1 = R_3 = OMe, R_2 = H$ 69%
187 $R_1 = R_2 = R_3 = OMe$ 76%

188 $R_1 = R_2 = R_3 = H$ 96%
189 $R_1 = OMe, R_2 = R_3 = H$ 83%
190 $R_1 = R_2 = H, R_3 = OMe$ 70%
191 $R_1 = R_3 = OMe, R_2 = H$ 81%
192 $R_1 = R_2 = R_3 = OMe$ 98%

193 $R_1 = R_2 = R_3 = H$ 80%
194 $R_1 = OMe, R_2 = R_3 = H$ 97%
195 $R_1 = R_2 = H, R_3 = OMe$ 77%
196 $R_1 = R_3 = OMe, R_2 = H$ 80%
197 $R_1 = R_2 = R_3 = OMe$ 78%

198 $R_1 = R_2 = R_3 = H$ 81%
199 $R_1 = OMe, R_2 = R_3 = H$ 93%
200 $R_1 = R_2 = H, R_3 = OMe$ 82%
201 $R_1 = R_3 = OMe, R_2 = H$ 86%
202 $R_1 = R_2 = R_3 = OMe$ 88%

Scheme 1.13 *Direct synthesis of 4-benzylsulfanylisoflavans* ***198–202*** *via condensation of phenylacetates with bezaldehydes.*

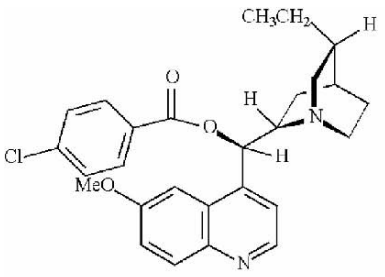


198 $R_1 = R_2 = H$ 81%
199 $R_1 = OMe$, $R_2 = H$ 93%
200 $R_1 = H$, $R_2 = OMe$ 82%
201 $R_1 = R_2 = OMe$ 86%

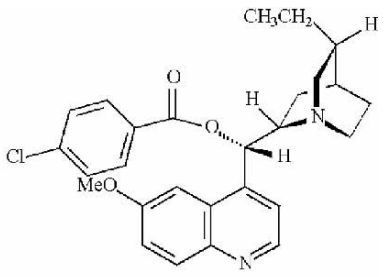
203 $R_1 = R_2 = H$ 96%
204 $R_1 = OMe$, $R_2 = H$ 99%
205 $R_1 = H$, $R_2 = OMe$ 99%
206 $R_1 = R_2 = OMe$ 99%

207 $R_1 = R_2 = H$ 82%
208 $R_1 = OMe$, $R_2 = H$ 39%
209 $R_1 = H$, $R_2 = OMe$ 57%
210 $R_1 = R_2 = OMe$ 50%

Scheme 1.14 *Synthesis of (6a,11a)-cis-pterocarpans* ***207-210****.*

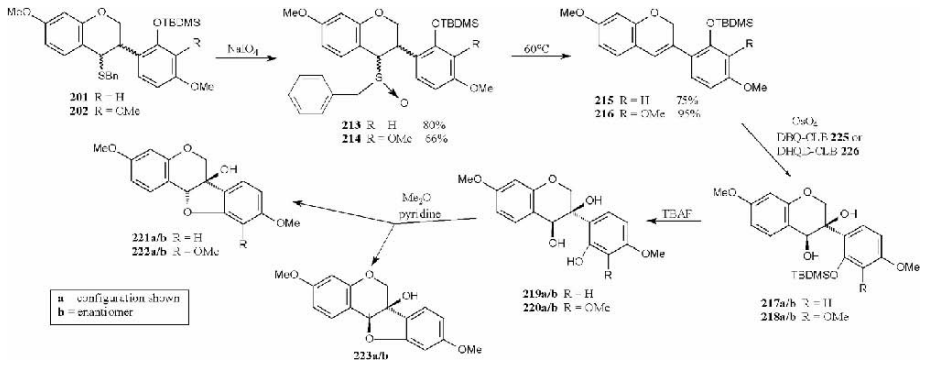


211 (DHQ-CLB)



212 (DHQD-CLB)

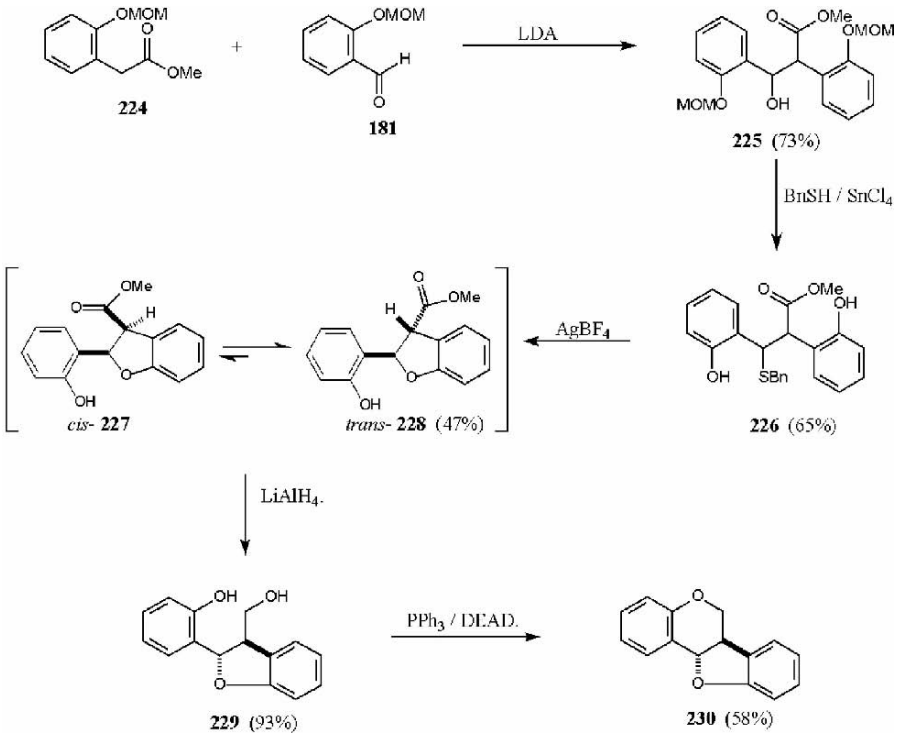
Table 1.7 *Formation of (6a,11a)-cis-* ***221a/b, 222a,b*** *and (6a,11b)-trans pterocarpans* ***223a***



Isoflav-3-ene	*Ligand*	*Diol*	*yield (%)*	*ee (%)*	*2′-OH*	*yield (%)*	*Pterocarpan*	*yield (%)*	*ee (%)*
215	**211**	**217a** (3*R*,4*S*)	65	>99	**219a**	100	**221a** (6a*R*,11a*R*)	70	>99
	212	**217b** (3*R*,4*S*)	68	>99	**219b**	100	**221b** (6a*S*,11a*S*)	75	>99
					219a	100	**223a** (6a*R*,11a*S*)	10	>99
					219b	100	**223b** (6a*S*,11a*R*)	9	>99
216	**211**	**218a** (3*R*,4*S*)	66	>99	**220a**	100	**222a** (6a*R*,11a*R*)	75	>99
	212	**218b** (3*R*,4*S*)	63	>99	**220b**	100	**222b** (6a*S*,11a*S*)	73	>99

Deprotection (TBAF suspended on silica) of diols **217a/b** and **218a/b** afforded 2′-hydroxyisoflavan-3,4-diols **219a/b** and **220a/b** in quantitative yields, which then served as precursors to the respective 6a-hydroxypterocarpans **221a/b** and **222a/b**. Attempted cyclization employing Mitsunobu conditions was unsuccessful. However, selective mesylation (Ms_2O, pyridine) activated the benzylic 4-hydroxyl group sufficiently to afford the requisite (6a,11a)-*cis*-6a-hydroxypterocarpans **221a/b** and **222a/b** in good yields and essentially optically pure form. It is interesting to note that cyclization of diols **219a** (3*R*,4*S*) and **219b** (3*S*,4*R*) also afforded the (6a*R*,11a*S*)- and (6a*S*,11a*R*)-*trans*-6a-hydroxyptercarpans **223a** and **223b**, respectively, as minor products (9–10% yield) and was the first report on the formation of the configurationally hindered 6a,11a-*trans*-analogues.

In all reported pterocarpan syntheses, formation of the six-membered B-ring invariably precedes closure of the five-membered C-ring. Once the B-ring is formed, Dreiding models indicate that it becomes virtually impossible to close the C-ring in a configuration other than the 6a,11a-*cis*-form. It was envisaged that the reversal of the order of cyclization, i.e., initial C-ring formation followed by B-ring closure, may provide synthetic access to the hitherto unknown 6a,11a-*trans*-pterocarpans. Thus, aldol condensation between the MOM-protected phenylacetate **224** and benzaldehyde **181**, using LDA for enolate generation, afforded the 2,3-diaryl-3-hydroxypropanoate **225** in 73% yield (Scheme 1.15).



Scheme 1.15 *Synthesis of (6a,11a)-trans-pterocarpan **230**.*

Deprotection of the acetal functionality of **225** using $SnCl_4/PhCH_2SH$ afforded 2,3-diaryl-3-benzylsulfanylpropanoate **226** in 65% yield. Cyclization ($AgBF_4$) of **226** to first form the pterocarpan C/D-ring system, afforded the thermodynamically more stable *trans*-fused 2,3-disubstituted dihydro-benzofuran **228** (47%; $J_{2,3}$ = 8.5 Hz). Subsequent reduction ($LiAlH_4$) gave the primary alcohol **229** (93%), which was converted under Mitsunobu cyclization conditions into the 6a,11a-*trans*-pterocarpan **230** ($J_{6a,11a}$ = 13.5 Hz in 58% yield).

4. ENZYMATIC STEREOSPECIFIC BIOSYNTHESIS OF FLAVONOIDS

Most enzymes of flavonoid biosynthesis are highly stereoselective and/or stereospecific; however, for many enzymes this claim rests on only one or a few published reports (Table 1.8) (Forkmann and Heller, 1999).

Flavonoids are synthesized via the phenylpropanoid pathway, beginning with the deamination of phenylalanine by the enzyme L-phenylalanine ammonia-lyase (PAL). PAL is specific for the naturally occurring L-isomer of phenylalanine; D-phenylalanine is not a substrate (Koukol and Conn, 1961).

Perhaps the most stereochemically important reaction of flavonoid biosynthesis is that catalyzed by chalcone–flavanone isomerase (CHI), which sets the stereochemistry at C-2 of the flavonoid heterocyclic ring. CHI specifically generates (2*S*)-flavanones from chalcones and is well characterized at the biochemical and structural levels (Bednar and Hadcock, 1988; Jez et al., 2000). The 2*S*-flavanone is a critical intermediate for formation of several flavonoid classes whose biosynthesis branches at this point, including flavones, flavonols, flavan-4-ols, anthocyanins, and isoflavonoids, and enzymes that use flavanone as substrate (including flavanone 2-hydroxylase/licodione synthase, flavone synthase II, flavone synthase I, flavanone 3-hydroxylase, flavonoid 3'-hydroxylase, flavanone 4-reductase, and isoflavone synthase) have been shown to be highly stereospecific for the 2*S*-enantiomer (Table 1.8). Other farther downstream enzymes, such as dihydroflavonol reductase, flavonol synthase, anthocyanidin reductase, and leucoanthocyanidin reductase, which do not directly use flavanone as substrate, also show a high degree of specificity for the naturally occurring stereochemistries at C-2 and C-3 (the latter generated by flavanone-3β-hydroxylase).

In contrast to most flavonoid enzymes, the 2-oxoglutarate-dependent dioxygenases flavonol synthase (FLS) and anthocyanidin synthase (ANS) show broad substrate and product selectivities *in vitro* (both accept flavanone, dihydroflavonol, and leucoanthocyanidin as substrates) (Lukacin et al., 2003; Martens et al., 2003; Turnbull et al., 2000, 2004; Welford et al., 2001). Detailed structural and *in vitro* studies, with particular attention to the stereochemistry of substrate and product, have shed light on how they catalyze reactions with their true substrates *in vivo* (Turnbull et al., 2000, 2004; Welford et al., 2001; Prescott et al., 2002; Wilmouth et al., 2002). For example, FLS and ANS show a preference for substrates with natural C-2 and C-3 stereochemistries [(i.e. (2*R*,3*R*)-dihydroquercetin for FLS and (2*R*,3*S*, 4*R/S*)- leucoanthocyanin for ANS], but hydroxylate both (2*R*)- and (2*S*)-naringenin equally well *in vitro*, which suggests that the C-3 hydroxyl group is important in biasing substrate selectivity (Turnbull et al., 2004).

Table 1.8 *Stereoselective and/or Specific Enzymes of Flavonoid Biosynthesis*

Enzyme	*Stereoselectivity*	*Stereospcificity*	*Key references*
Phenylalanine ammonia lyase	L-phenylalanine		Koukol and Conn, 1961
Chalcone isomerase		(2*S*)-flavanone	Bednar and Hadcock, 1988; Hahlbrock et al., 1970; Jez et al., 2000
Flavanone 2-hydroxylase (licodione synthase)	(2*S*)-flavanone		Akashi et al., 1998; Otani et al., 1994
Flavanone 4-reductase	(2*S*)-flavanone	(2*S*, 4*R*)-flavan-*4*-ol	Fischer et al., 1988; Stich and Forkmann, 1988
Flavone synthase	(2*S*)-flavanone		Britsch, 1990; Kochs et al., 1987; Martens et al., 2001; Sutter et al., 1975
Flavone 3β-hydroxylase	(2*S*)-flavanone	(2*R*,3*R*)-dihydroflavonol	Britsch and Grisebach, 1986; Britsch et al., 1992
Flavonoid 3'-hydroxylase	(2*S*)-flavanone, (2*R*,3*R*)-dihydroflavonol		Fritsch and Grisebach, 1975; Hagmann et al., 1983
Flavonol synthase	(2*R*,3*R*)-dihydroflavonol		Lukacin et al., 2003; Martens et al., 2003; Prescott et al., 2002; Turnbull et al., 2004
Dihydroflavonol 4-reductase	(2*R*,3*R*)-dihydroflavonol	(2*R*, 3*S*, 4*S*)-flavan-2,3-*trans*-3,4-*cis*-diol	Stafford and Lester, 1982, 1984, 1985
Leucoanthocyanidin 4-reductase	(2*R*, 3*S*, 4*S*)-flavan-2,3-*trans*-3,4-*cis*-diol	(2*R*, 3*S*)-flavan-3-ol	Stafford and Lester, 1984; Tanner et al., 2003
Anthocyanidin synthase	(2*R*, 3*S*, 4*S*)-flavan-2,3-*trans*-3,4-*cis*-diol		Turnbull et al., 2004; Wilmouth et al., 2002
Anthocyanidin reductase		(2*R*, 3*R*)-flavan-3-ol	Xie et al., 2003
Isoflavone synthase	(2*S*)-flavanone		Hagmann and Grisebach, 1984; Kochs and Grisebach, 1986

Table 1.8 *(continued)*

Enzyme	*Stereoselectivity*	*Stereospecificity*	*Key references*
Isoflavone reductase		(2*R*)-isoflavanone	Fischer et al., 1990a; Paiva et al., 1991, 1994
Vestitone reductase	(2*R*)-isoflavanone		Fischer et al., 1990b; Guo and Paiva, 1995
7,2'-Dihydroxy-4'-methoxyisoflavanol dehydratase		(-)-medicarpin	Guo et al., 1994
3,9-dihydroxypterocarpan 6a-hydroxylase	(6a*S*, 11a*S*)-3,9-dihydroxypterocarpan	(6a*S*, 11a*S*)-3,6a,9-trihydroxypterocarpan	Hagmann et al., 1984; Schopfer et al., 1998
6a-Hydroxymaackiain-3-*O*-methyltransferase	(+)-6a-hydroxymaackiain	(+)-pisatin	Preisig et al., 1989; Wu et al., 1997

The flavan-*3*-ols (+)-catechin and (-)-epicatechin (Figure 1.1) form the building blocks of proanthocyanidins (condensed tannins), a class of molecules of considerable interest in view of their impacts on animal health (Dixon at al., 2005). The C-2 and C-3 stereochemistries of (+)-catechin (2,3-*trans*) are identical to those of intermediates in the flavonoid pathway, and a pathway leading from (2*R*, 3*S*, 4*S*)-leucoanthocyanidin to (+)-catechin, catalyzed by leucoanthocyanidin reductase (LAR), has been demonstrated and confirmed by the cloning of a leucoanthocyanidin reductase from the tannin-rich forage legume *Desmodium uncinatum* (Stafford and Lester, 1984, 1985; Tanner et al., 2003). LAR is a member of the Reductase–Epimerase–Dehydrogenase family of proteins, whose members include isoflavone reductase and related homologues (Min et al., 2003).

For many years, the origin of the 2,3-*cis* (-)-epicatechin units in proanthocyanidins was a mystery. This problem was resolved by the demonstration that the pathway leading to (-)-epicatechin proceeds from leucocyanidin through cyanidin in reactions catalyzed by ANS and anthocyanidin reductase (ANR) (Xie et al., 2003). ANR, an enzyme with weak sequence homology to dihydroflavonol reductase, can introduce the 2,3-*cis* stereochemistry by acting on an achiral intermediate (anthocyanidin). Mechanisms have been proposed for this reaction, and it is possible that other ANR-like enzymes might exist with the ability to introduce alternate stereochemistries (Xie et al., 2004).

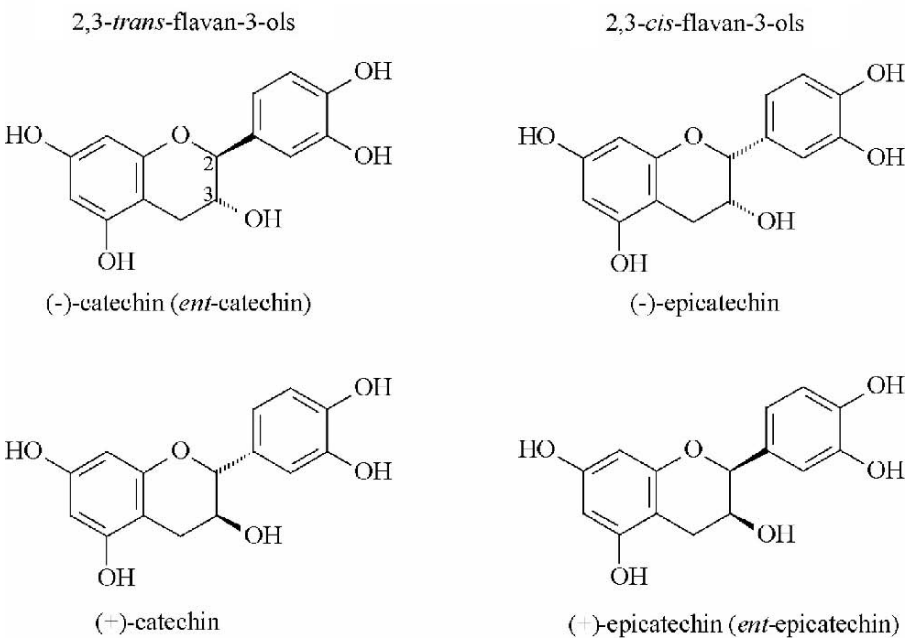


Figure 1.1 *Flavan-3-ol isomers.*

5. STEREOCHEMISTRY RELATED TO BIOLOGICAL ACTIVITY

5.1. Catechin

The Asian native *Centaurea maculosa* (spotted knapweed) has displaced native weeds and crops throughout the western United States. Contributing to the invasiveness of this exotic is the secretion of the phytotoxic *trans*-flavan-*3*-ol (-)-catechin from its roots (Bais et al., 2002) (Figure 1.1). Both enantiomers of catechin are present in root exudates of *C. maculosa*; however, only (-)-catechin had allelopathic (phytotoxic) activity. Interestingly, (+)-catechin (but not (-)-catechin) displayed antibacterial activity against several root pathogens, which suggests that secretion of a racemic mixture may simultaneously protect *C. maculosa* roots against microbial pathogens and weaken roots of neighboring plants (Bais et al., 2002). When the phytotoxicity of catechin was examined in more detail, only the (-)-enantiomer elicited generation of reactive oxygen species and calcium-signaling events in roots of susceptible species (Bais et al., 2003a). Additional studies with the *cis*-flavan-3-ols (+)-epicatechin and (-)-epicatechin showed that (+)-epicatechin, like (-)-catechin, inhibited root and shoot differentiation and seed germination of several of the plants examined, while (-)-epicatechin did not show inhibition (Bais et al., 2003b). Both (-)-catechin and (+)-epicatechin are of the 2*S* configuration, which suggests that the stereochemistry at C-2 is important for allelopathic activity. Interestingly, (+)-epicatechin also was

effective at inhibiting *C. maculosa*, which is resistant to (-)-catechin (Bais et al., 2003b).

Metabolic engineering of (-)-catechin biosynthesis to address the contribution of (-)-catechin to the invasiveness of *C. maculosa* by knockdown experiments and also to engineer (-)-catechin root secretion into nonallelopathic plants requires identification of the enzyme(s) responsible for (-)-catechin biosynthesis. (-)-Catechin has the opposite stereochemistry at C-2 and C-3 to that of most flavonoids, and it is likely that (-)-catechin biosynthesis proceeds through the achiral anthocyanidin in a reaction similar to that catalyzed by ANR.

5.2. Isoflavonoid Phytoalexins

Isoflavonoids are a subclass of flavonoids, restricted primarily to legumes, that play important roles in plant and animal health (Dixon and Steele, 1999). Many of the more complex isoflavonoids such as the antimicrobial pterocarpan phytoalexins synthesized in response to fungal pathogens and other stresses are optically active (Ingham, 1982) (Figure 1.2). Pterocarpans have diastereomeric carbons at 6a and 11a; thus, four stereoisomers are possible, although due to chemical constraints only one pair of naturally occurring stereoisomers is found (i.e., 6a*R*;11a*R* and 6a*S*;11a*S*). In the majority of legumes the (-)-enantiomers predominate; examples include (-)-medicarpin (alfalfa, chickpea, clover), (-)-maackiain (chickpea, clover), and (-)-glycinol (soybean) (Ingham, 1982).

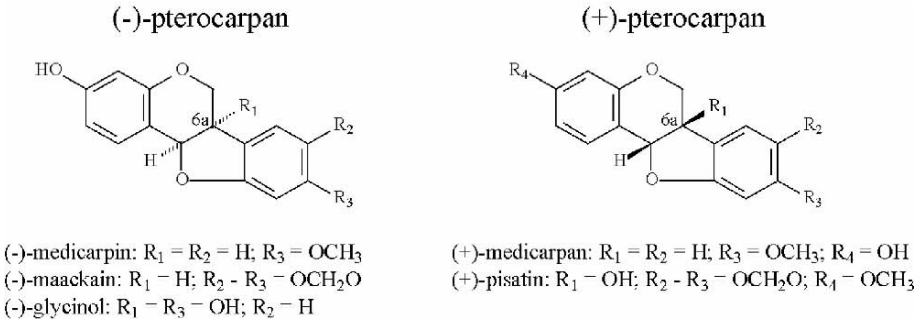


Figure 1.2 *Structure and stereochemistry of isoflavonoid pterocarpan phytoalexins.*

Two well-known examples of pterocarpan phytoalexins with opposite stereochemistry include (+)-medicarpin from peanut and (+)-pisatin from pea. Although molecular models of (+)- and (-)-pterocarpans are nearly superimposable (with the exception of the B ring), the absolute configuration of these phytoalexins can be an important factor in plant–pathogen interactions. Studies on the toxicity of maackiain and pisatin enantiomers to phytopathogenic fungi demonstrated that in general fungi were more sensitive to pterocarpans of the opposite stereochemistry to that found in their host plant. For example, fungal pathogens of (-)-maackiain-producing plants were more sensitive to (+)-maackiain (Delserone et al., 1992). It has been suggested that this differential sensitivity of fungal pathogens to

phytoalexins of the opposite stereochemistry may be exploited for disease control by engineering plants to synthesize enantiomers of the opposite stereochemistry. Further support for this strategy comes from work on the detoxification enzymes of phytopathogenic fungi, which convert phytoalexins to less toxic forms by demethylation, hydroxylation, or reductive cleavage (VanEtten et al., 1989). These enzymes often display a high degree of stereospecificity for their host's phytoalexins. For example, an isolate of *Nectria haematococca* specifically hydroxylated (-)-maackiain and (-)-medicarpin but not their (+)-enantiomers (VanEtten et al., 1983). Similarly, a purified pterocarpan hydroxylase from the chickpea pathogen *Ascochyta rabiei* hydroxylated (-)-maackiain and (-)-medicarpin but not (+)-maackiain (Tenhaken et al., 1991). Pisatin demethylase from *N. haematococca* and *Ascochyta pisi* preferred (+)-pisatin over (-)-pisatin (53–58% of activity), although the demethylase from *Mycosphaerella pinodes* and *Phoma pinodella* had the same activity on both enantiomers (George and VanEtten, 2001). Furthermore, (+)-pisatin but not its (-)-enantiomer induced pisatin demethylase activity in *N. haematococca* (VanEtten et al., 1989).

Although the biosynthetic pathway leading to (-)-medicarpin and related compounds is well characterized, the *in vivo* enzymatic routes to (+)-pterocarpans remain unknown. In (-)-pterocarpan biosynthesis the key enzyme determining the stereochemistry of the 6a (and 11a) positions of the pterocarpan is isoflavone reductase (IFR), which stereospecifically reduces 2'-hydroxyisoflavone to (3*R*)-isoflavanone (Fischer et al., 1990a; Paiva et al., 1991). This (3*R*)-isoflavanone is further reduced to isoflavanol, then dehydrated to pterocarpan with retention of stereochemistry (Dixon, 1999). An initial hypothesis for the biosynthesis of (+)-pisatin suggested that pea IFR would specifically generate (3*S*)-isoflavanone. However, cloning and expression of pea IFR in *Escherichia coli* later showed that this reductase produced (3*R*)-isoflavanone, identical to that of alfalfa IFR (Paiva at al., 1994). The 6a-hydroxymaackiain 3-*O*-methyltransferase catalyzing the final step in the biosynthesis of (+)-pisatin is specific for (+)-6a-hydroxymaackiain (Preisig et al., 1989; Wu et al., 1997), suggesting that the reversal of stereochemistry occurs between reduction of isoflavanone and formation of (+)-6a-hydroxymaackiain. Possible mechanisms for the synthesis of (+)-pterocarpans include formation of an isoflav-3-ene intermediate or epimerase-mediated inversion of configuration. Support for the latter hypothesis comes from an unpublished report that in peanut, which synthesizes (+)-medicarpin, IFR produces (3*R*)-vestitone, but that the following enzyme vestitone reductase accepts only (3*S*)-vestitone (Guo and Paiva, 1995).

6. REFERENCES

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