CHAPTER II

HISTORICAL

Part I : Phytochemical Study

The Chemistry of Genus *Derris*

Chemical constituents isolated from the genus *Derris* were reported as flavonoids and coumarins. List of the compounds found in various parts of *Derris* spp. is shown in Table 1.

Table 1 Chemical Constituents of *Derris* spp.

Plant and chemical compound	Category	Plant Part	Reference
Derris amazonica			
Flavan, iso : 7-hydroxy-2',4'-dimethoxy : (3-S) :	Flavonoid	Aerial parts	Braz Filho *et al.*, 1975
Lupenone	Triterpene	Aerial parts	Braz Filho *et al.*, 1975
Pterocarpin, homo : demethyl : (6-A-S-11-A-S) :	Flavonoid	Aerial parts	Braz Filho *et al.*, 1975
Rotenone	Flavonoid	Root	Moretti and Grenand, 1982
Sitosterol, β :	Steroid	Aerial parts	Braz Filho *et al.*, 1975
Vestitol, 2'-O-methyl : (3-S) :	Flavonoid	Aerial parts	Braz Filho *et al.*, 1975

Plant and chemical compound	Category	Plant Part	Reference
Derris araripensis			
Chromeno-(7-8-2"-2")-flavone, 3, 6-dimethoxy-6"-6"-dimethyl :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Chromeno-(7-8-2"-3")-flavone, 3, 6-dimethoxy-6", 6"-dimethyl-3, 4-methylenedioxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Furano-(3'-4'-2"-3")-dihydrochalcone, 5'-hydroxy-2', 3'-dimethoxy-3,4-methylenedioxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Furano-(7-8-2"-3")-flavanone, 5-hydroxy-6-methoxy-3'-4'-methylenedioxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Furano-(7-8-2"-3")-flavanonol, 3-5-6-trimethoxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Furano-(7-8-2"-3")-flavone, 3-5-6-trimethoxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Furano-(7-8-2"-3")-flavone, 3-methoxy-3'-4'-methylenedioxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Furano-(7-8-2"-3")-flavone, 5,6-dimethoxy-3'-4'-methylenedioxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Furano -(7-8-2"-3")- flavanone, 5-6-dimethoxy-3'-4'-methylenedioxy :	Flavonoid	Rootbark	Naseimento and Mors, 1981
Furano-(7-8-2'-3')-flavan, 3-4-5-6-tetramethoxy :	Flavonoid	Rootbark	Nascimento and Mors, 1981
Derris brevipes			
Damnacanthal	Quinoid	Stem	Desai *et al.*, 1977
Rotenone	Flavonoid	Stem	Desai *et al.*, 1977
Sitosterol, β :	Steroid	Stem	Desai *et al.*, 1977

Plant and chemical compound	Category	Plant Part	Reference
Derris elliptica			
Deguelin	Flavonoid	Root Suspension culture	Ahmed *et al.*, 1989 Kodama, Yamakawa, and Minoda, 1989
Elliptinol	Oxygen heterocycle	Root	Ahmed *et al.*, 1989
Maackiain, (+) :	Flavonoid	Root	Obara and Matsubara, 1981
Maackiain, (-) :	Flavonoid	Root	Obara, and Matsubara, 1981
Piperidine, 2-(*S*)-carboxy-4-(*R*)-5-(*S*)-dihydroxy :	Proteid	Leaf	Marlier, Dardenne and Casimir, 1976
Piperidine, 2-(*S*)-carboxy-4-(*S*)-5-(*S*)-dihydroxy :	Proteid	Leaf	Marlier, Dardenne and Casimir, 1976
Pyrrolidine, 3-4-dihydroxy-2-5-dihydroxy-methyl :	Alkaloid	Leaf	Marlier, Dardenne and Casimir, 1976
Rotenone	Flavonoid	Callus tissue Suspension culture	Minoda *et al.*, 1977 Kodama, Yamakawa and Minoda, 1980
		Root	Yoxopeus, 1952
		Root	Jone and Graham, 1938
		Entire plant	Cromble, Green and Whiting, 1968
		Bark	Chen and Tsai, 1955
		Root	Petard, 1951
		Stem	Chen and Tsai, 1955
		Root	Chen and Tsai, 1955

Plant and chemical compound	Category	Plant Part	Reference
Rotenone	Flavonoid	Stemwood	Chen and Tsai, 1955
		Leaf	Chen and Tsai, 1955
		Petiole	Chen and Tsai, 1955
		Root	Gaudin and Vacherat, 1938
Tephrosin	Flavonoid	Root	Ahmed et al., 1989
Tubaic acid	Oxygen heterocycle	Root	Obara, Matsubara and Munakata, 1976
Tubaic acid, β :	Oxygen heterocycle	Root	Obara, Matsubara and Munakata, 1976
Derris floribunda			
Cordoin, iso :	Flavonoid	Root	Braz Filho et al., 1975
Derricidin	Flavonoid	Root	Braz Filho et al., 1975
Flavanone, 5-7-dihydroxy-6-prenyl :	Flavonoid	Root	Braz Filho et al., 1975
Lonchocarpin	Flavonoid	Root	Braz Filho et al., 1975
Lonchocarpin, 4-hydroxy :	Flavonoid	Root	Braz Filho et al., 1975
		Root	Braz Filho et al., 1975
Stilbene, 3-4-'-5-trimethoxy-prenyl :	Benzenoid	Root	Braz Filho et al., 1975
Stilbene, 3-4-'-5-trimethoxy-4-prenyl :	Benzenoid	Root	Braz Filho et al., 1975
Stilbene,3-5-dimethoxy-4-prenyl :	Benzenoid	Root	Braz Filho et al., 1975

Plant and chemical compound	Category	Plant Part	Reference
Derris glabrescens			
Derrusnin	Coumarin	Seed	Delle Monache *et al.*, 1977
Glabrescin	Coumarin	Seed	Delle Monache *et al.*, 1977
Glabrescione A	Flavonoid	Seed	Delle Monache *et al.*, 1977
Glabrescione B	Flavonoid	Seed	Delle Monache *et al.*, 1977
Derris laxiflora			
Amyrin, β :	Triterpene	Root	Lin, Chen and Kuo, 1991
Flemichapparin B	Flavonoid	Root	Lin, Chen and Kuo, 1991
Laxifolin	Flavonoid	Root	Lin, Chen and Kuo, 1991
Laxifolin, iso :	Flavonoid	Root	Lin, Chen and Kuo, 1991
Lupeol	Triterpene	Root	Lin, Chen and Kuo, 1991
Lupinifolin	Flavonoid	Root	Lin, Chen and Kuo, 1991
Lupinifolin, 3'-methoxy :	Flavonoid	Root	Lin, Chen and Kuo, 1991
Prunetin	Flavonoid	Root	Lin, Chen and Kuo, 1991
Derris malaccensis			
Rotenone	Flavonoid	Root	Yoxopeus, 1952
Derris negrensis			
Rotenone	Flavonoid	Entire plant	Vasconcelos and Maia, 1976
Rotenone, 6(A)-12(A)-dehydro :	Flavonoid	Entire plant	Vasconcelos and Maia, 1976

Plant and chemical compound	Category	Plant Part	Reference
Derris oblonga			
Elliptone , 12-α-acetoxy-12-deoxo :	Flavonoid	Root	Lin, Chen and Kuo, 1993
Oblongin	Flavonoid	Root	Lin and Kuo, 1993
Oblonginol	Flavonoid	Root	Lin and Kuo, 1993
Derris obtusa			
Aurone, 3',4'-methylenedioxy-furano-(2"-3"-6-7) :	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Aurone, 4-hydroxy-furano-(2"-3-6-7) :	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Aurone, 4-methoxy-furano-(2"-3-6-7) :	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Aurone, Furano-(2"-3"-6-7) :	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Chalcone, 5'-hydroxy-2'-methoxy-3,4-methylenedioxy-(furano-2"-3'-3"-H') :	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Chromeno-(2"-3"-7-8)-flavone, 5-hydroxy-6",6"-dimethyl :	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Chromeno-(2"-3"-7-8)-flavone, 3,6-dimethoxy-6",6"-dimethyl :	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Derriobtusone A	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Derriobtusone B	Flavonoid	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976

Plant and chemical compound	Category	Plant Part	Reference
Heptacosan-1-ol	Alkane	Rootbark	Do Nascimento, De Vasconcellos Dias and Mors, 1976
Derris rariflora			
Flavanone, 5,7-dihydroxy-6-prenyl :	Flavonoid	Wood	Braz Filho, Gottlieb and Mourao, 1975
Flavanone, 5-hydroxy-7-methoxy-6-prenyl :	Flavonoid	Wood	Braz Filho, Gottlieb and Mourao, 1975
Rotenone	Flavonoid	Root	Braz Filho, Gottlieb and Mourao, 1975
		Aerial parts	Braz Filho, Gottlieb and Mourao, 1975
Sitosterol, β :	Steroid	Wood	Braz Filho, Gottlieb and Mourao, 1975
Stilbene,3,5-dimethoxy-4-prenyl :	Benzenoid	Wood	Braz Filho, Gottlieb and Mourao, 1975
Derris robusta			
Alpinumisoflavone dimethyl ether	Flavonoid	Seed	Chibber and Sharma, 1980
Daucosterol	Steroid	Seed	Chibber and Sharma, 1980
Derrone-4'-O-methyl ether	Flavonoid	Seed	Chibber, Sharma and Dutt, 1981
Derrugenin	Flavonoid	Seedcoat	Tsukayama *et al.*, 1980
		Seed hulls	Chibber and Sharma, 1979 a
Derrusnin	Coumarin	Seed	Chibber and Sharma, 1980
Flavone, iso : 5-hydroxy-7-methoxy :	Flavonoid	Seed	Chibber and Sharma, 1979 b
Robustigenin	Flavonoid	Seed hulls	Chibber and Sharma, 1979 c

Plant and chemical compound	Category	Plant Part	Reference
Robustin methyl ether	Coumarin	Seed	Chibber and Sharma, 1980
Robustone	Coumarin	Seed	Chibber and Sharma, 1980
Robustone methyl ether	Coumarin	Seed	Chibber and Sharma, 1980
Sitosterol, β :	Steroid	Seed	Chibber and Sharma, 1980
Derris sp.			
Alpinumisoflavone	Flavonoid	Root	Rocha and Zoghbi, 1982
Alpinumisoflavone, 4'-O-γ-γ-dimethyl-allyl :	Flavonoid	Root	Rocha and Zoghbi, 1982
Alpinumisoflavone-4'-methyl ether	Flavonoid	Root	Rocha and Zoghbi, 1982
Derris spruceana			
Chromene (5",6",3',4')-stilbene, 2,4-dimethoxy-2",2"-dimethyl :	Flavonoid	Root	Garcia *et al.*, 1986
Chromeno (5",6",7,8)-coumarin, 3-methylenedioxy-(3',4')-phenyl-4-5-dimethoxy-2"-2"-dimethyl :	Coumarin	Root	Garcia *et al.*, 1986
Chromeno (5",6",7,8)-coumarin, 3-methylenedioxy-(3',4')-phenyl-4-hydroxy-5-methoxy-2"-2"-dimethyl :	Coumarin	Root	Garcia *et al.*, 1986
Chromeno (5",6",7,8)-coumarin, 3-parahydroxy-phenyl-4-hydroxy-5-methoxy-6-prenyl-2",2"-dimethyl :	Coumarin	Root	Garcia *et al.*, 1986
Chromeno (5",6",7,8)-Iso-flavone,3',4'-methylenedioxy-5-hydroxy-2",2"-dimethyl :	Flavonoid	Root	Garcia *et al.*, 1986

Plant and chemical compound	Category	Plant Part	Reference
Deguelin	Oxygen heterocycle	Root	Menichini, Delle Monache and Marini-Bettolo, 1982
Rotenone	Flavonoid	Root	Menichini, Delle Monache and Marini-Bettolo, 1982
Rotenone, 12-(A)-hydroxy :	Flavonoid	Root	Menichini, Delle Monache and Marini-Bettolo, 1982
Sitosterol, β :	Steroid	Root	Garcia et al., 1986
Tephrosin	Flavonoid	Root	Menichini, Delle Monache and Marini-Bettolo, 1982
Derris trifoliata			
Amyrin, α :	Triterpene	Leaf	Ghosh et al., 1985
Amyrin, β :	Triterpene	Leaf	Ghosh et al., 1985
Campesterol	Steroid	Leaf	Ghosh et al., 1985
Ceryl alcohol	Alkane	Part not specified	Sudachan, 1967
Cholesterol	Steroid	Leaf	Ghosh et al., 1985
Lupeol	Triterpene	Part not specified	Sudachan, 1967
		Leaf	Ghosh et al., 1985
Quercetin-3-O-β-neohesperidoside	Flavonoid	Leaf	Nair and Seetharaman, 1986
Rhannetin-3-O-neohesperidoside	Flavonoid	Leaf	Nair and Seetharaman, 1986
Sitosterol, β :	Steroid	Part not specified	Sudachan, 1967
		Leaf	Ghosh et al., 1985

Plant and chemical compound	Category	Plant Part	Reference
Stigmast-7-en-3-β-ol	Steroid	Leaf	Ghosh *et al.*, 1985
Stigmasterol	Steroid	Part not specified	Sudachan, 1967
		Leaf	Chosh *et al.*, 1985
Derris uliginosa			
Lupeol	Triterpene	Root	Rose, Kirtaniya and Adityachoudhury, 1976
Rotenone	Flavonoid	Root	1. Milsum, 1938
		Root	2. Georgi, 1937
		Root	3. Petard, 1951
		Root	4. Gaudin and Vacherat, 1938
Rotenone, 6(A)-12(A)-dehydro :	Flavonoid	Root	Rose, Kirtaniya and Adityachoudhury, 1976

Introduction to Flavonoids

Flavonoids represent a very widespread group of water-soluble phenylpropane derivatives, many of which are brightly coloured, being red, crimson, purple or yellow. Flavonoids are glycosides and the structure of their aglycones are based on the flavan structure (1) which consists of two aromatic rings jointed in a chroman structure (2) by a three carbon unit (C_6-C_3-C_6). Flavonoids are probably derived in plants from the coupling of a phenylpropane unit produced by the shikimic acid pathway and three C_2 acetate units (Goodwin and Mercer, 1983 ; Harborne, 1973).

Flavan nucleus (1)   Chroman nucleus (2)

The variation in the state of oxidation of the connecting C_3 moiety determining the properties and class of each such compound, the classes are shown below (Ikan, 1991).

Flavone

Flavonol

Flavanone

Flavanonol

Isoflavone

Chalcone

Dihydrochalcone

Aurone

Anthocyanidin

Flavan-3-ol
(Catechin)

Flavan-3,4-diol
(Leucoanthocyanidin)

Flavonoid compounds and the related coumarins usually occur in plants as glycosides in which one or more of phenolic hydroxyl groups are combined with sugar residues. The hydroxyl groups are nearly always found in position 5 and 7 in ring A, while ring B commonly carries hydroxyl or alkoxyl groups at the 4'-positions, or at both 3'- and 4'-positions. Glycosides of flavonoid compounds may bear the sugar on any of the available hydroxyl groups (Ikan, 1991).

Prenylated Flavonoids in Leguminosae

1.   Isoflavonoids

The isoflavonoids represent an important and very distinctive subclass of the flavonoids. These compound are based on a 3-phenylchroman skeleton that is biogenetically derived by an aryl migration mechanism from the 2-phenylchroman skeleton of the flavonoids. In marked contrast to the flavonoids, the isoflavonoids have a very limited distribution in the plant kingdom, and are almost entirely restricted to the subfamily Papilionoideae of the Leguminosae. Even in the subfamilies Caesalpinioideae and Minosoidae of the Leguminosae, only one or two plants have been reported to contain isoflavonoids (Dewick, 1988).

1.1   Isoflavones

Isoflavones (3) constitue the largest group of natural isoflavonoid derivatives. With so many known natural isoflavones, covering a wide range of different oxygenation and substitution patterns, those structures that invite comment are the ones that seem unusual from a biogenetic aspect.

Structural complexity increase enormously as isoprenyl substituents become incorporated into the isoflavonoid system. Alkylation of phenolic groups giving dimethylallyl ethers is not unknown in the isoflavones, but is rare enough that the report of several new ones is of interest. New examples have been isolated from *Calopogonium mucunoides*, *Derris spp.* (Da Rocha and Zoghbi, 1982), *Millettia auriculata* (Gupta *et al.*, 1983) and *Tephrosia maxima* (Murthy and Rao, 1985). It is more usual to find isoprenyl substituents alkylating the aromatic ring systems at nucleophilic sites generated by neighbouring oxygen functions. 3,3-Dimethylallyl substituents are common, wherease 1,1-dimethylallyl substituents are quite rare. The presence of two 3,3-dimethylallyl and three oxygen substituents on the B-ring of an isoflavone (4) from *Piscidia erythrina* (Delle Monache, Ferrari and Menichini, 1984) makes this the first example of an isoflavone with a fully substituted B-ring.

(3)

(4)

Examples of prenylated isoflavones are shown in Table 2.

Table 2   Isoflavones

Isoflavone Compound	Source (s)	Reference
Licoricone	*Glycyrrhiza uralensis*	Kaneda *et al.*, 1973

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
3'-Dimethylallylgenistein : R=H **Licoisoflavone A** : R=OH (phaseoluteone)	*Cajanus cajan* *Lupinus albus*	Dahiya *et al.*, 1984 Tahara *et al.*, 1984 a
Lupinisoflavone C : R=H **Lupinisoflavone D** : R=OH	*Lupinus albus* *L. albus*	Tahara *et al.*, 1984 a Tahara *et al.*, 1984 a
Licoisoflavone B	*Glycyrrhiza* sp. *Lupinus albus*	Hiraga *et al.*, 1984 1. Ingham, Tahara and Harbone, 1983 quoted in Harborne, 1988 2. Tahara *et al.*, 1984

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
2'-Deoxypiscerythrone : R=H Piscerythrone ; R=OH 	*Piscidia erythrina* *P. erythrina*	Delle Monache, Ferrari and Menichini, 1984 Delle Monache, Ferrari and Menichini, 1984
Piscidone ; R_1=OMe, R_2=OH or R_1=OH, R_2=OMe 	*Piscidia erythrina*	1. Radaelli and Santaniello, 1984 2. Delle Monache, Ferrari and Menichini, 1984
Lupiwighteone 	*Lupinus luteus* *Glycyrrhiza uralensis*	Hashidoko, Tahara and Mizutani, 1986 Fukai, Wang and Nomura, 1989

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
Calopogonium isoflavone B : R_1=H. R_2=R_3=OCH$_2$O	*Tephrosia maxima*	Murthy and Rao. 1985
Barbigerone : R_1=R_2=R_3=OMe	*T. barbigera*	Vilain, 1983 quoted in Harborine, 1982
Jamaicin : R_1=OMe. R_2=R_3=OCH$_2$O	*Piscidia erythrina*	1. Pietta and Zio, 1983 2. Delle Monache, Ferrari and Menichini, 1984
Wighteone ; R_1=R_2=R_3=H (erythrinin B)	*Lupinus albus* *L. spp.*	Ingham, Tahara and Harborne, 1983 quoted in harborne. 1988
Luteone ; R_1=OH, R_2=R_3=H	*L. albus* *L. spp.*	Tahara *et al.*, 1984 Harborne *et al.*, 1976
Lupisoflavone ; R_1=R_3=H. R_2=OMe	*L. albus*	Ingham, Tahara and Harborne. 1983 quoted in Harborne. 1988
	L. luteus	Ingham, Tahara and Harborne, 1983 quoted in Harborne. 1988

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
Viridiflorin ; $R_1=R_3=OMe$, $R_2=H$	*Tephrosia viridiflora*	Gomez *et al.*, 1985
Alpinumisoflavone ; $R_1=R_2=OH$	*Derris* sp.	Da Rocha and Zoghbi, 1982 quoted in harborne, 1988
	Lupinus albus	Ingham, Tahara and Harborne, 1983 quoted in Harborne, 1988
	Millettia thonningii	1. Khalid and Waterman 1983 2. Olivares *et al.*, 1982
4'-O-Methylalpinumisoflavone ; $R_1=OH$, $R_2=OMe$	*Derris* sp.	Da Rocha and Zoghbi, 1982 quoted in Harborne, 1988
	Millettia thonningii	1. Khalid and Waterman 1983 2. Olivares *et al.*, 1982
4'-O-Dimethyl - allylalpinumisoflavone ; $R_1=OH$, $R_2=OCH_2CH=CMe_2$	*Derris* sp.	Da Rocha and Zoghbi, 1982 quoted in Harborne, 1988

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
Di-O-Methyl-alpinumisoflavone : $R_1=R_2=OMe$ 	*Millettia* *thonningii*	Khalid and Waterman, 1983
4'-Methylalpinumisoflavone ; $R_1=OH$, $R_2=OMe$ **Robustone** ; $R_1=R_2=OCH_2O$ 	*Millettia* *thonningii* M. thonningii *Tephrosia* *viridiflora* *Derris robusta*	Olivares *et al.*, 1982 Khalid and Waterman, 1983 Gomez *et al.*, 1985 Chibber and Sharma, 1979
Dihydroalpinumisoflavone 	*Crotalaria* *madurensis*	Bhakuni and Chaturvedi, 1984

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
Elongatin	*Tephrosia viridiflora*	Gomez *et al.*, 1985
Parvisoflavone B	*Lupinus albus*	Tahara *et al.*, 1984
Lupinisoflavone A	*Cajanus cajan* *	Dahiya *et al.*, 1984
	Lupinus albus	Tahara *et al.*, 1984

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
Erythrinin C : R=H	*Lupinus albus*	Tahara, Ingham and Mizutani, 1985 quoted in Harborne, 1988
Lupinisoflavone B ; R=OH	*L. albus*	Tahara *et al.*, 1984
2,3-Dehydrokievitone	*Phaseolus vulgaris*	Woodward, 1979
4'-O-Methylderrone	*Derris robusta* *Millettia pachycarpa*	Chibber, Sharma and Dutt, 1981 Singhal *et al.*, 1983

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
Lupalbigenin : R=H	*Lupinus albus*	Ingham and Tahara, 1985 quoted in Harborne, 1988
2'-Hydroxylupalbigenin ; R=OH	*Millettia pulchra*	Baruah, Barua and Sharma, 1984
	L. albus	Tahara *et al.*, 1984
2'-Methoxylupalbigenin ; R=OMe	*M. pulchra*	Baruah, Burua and Sharma, 1984
Lupinisoflavone E ; R=H	*Lupinus albus*	Tahara *et al.*, 1984
Lupinisoflavone F ; R=OH	*L. albus*	Tahara *et al.*, 1984
Flemiphyllin	*Flemingia macrophylla*	Rao and Srimannarayana, 1984

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
6,8-Di-(dimethylallyl) genistein : R_1=H.R_2=OH	*Millettia pachycarpa*	Singhal *et al.*, 1980
6,8-Di-(dimethylallyl) orobol : R_1=R_2=OH	*M. pachycarpa*	Singhal *et al.*, 1980
6,8-Di-(dimethylallyl) pratensein : R_1=OH, R_2=OMe	*M. pachycarpa*	Singhal *et al.*, 1983
Warangalone ; R_1=R_2=H (scandenone)	*Erythrina senegalensis*	Fomum, Ayafor and Wandji, 1985
Auriculatin ; R_1=OH, R_2=H	*Millettia auriculata*	Raju and Srimannarayana, 1978
Auriculasin ; R_1=H, R_2=OH	*M. auriculata*	Minhaj *et al.*, 1976

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
(A) R₁=OMe. R₂=H **(B)** R₁= H. R₂= OMe 	*Millettia pachycarpa* *M. pachycarpa*	Singhal *et al.*. 1981 Singhal *et al.*. 1981
Osajin ; R=H **Pomiferin** ; R=OH 	*Euchresta japonica* *Millettia pachycarpa*	Shirataki *et al.*, 1982 Singhal *et al.*, 1983
(A) : R₁=OH. R₂=H, R₃=OMe 	*Millettia pachycarpa*	Singhal *et al.*, 1981

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
(B) : R₁=H. R₂=OMe. R₃=OH or R₁=H. R₂=OH. R₃=OMe 	*M. pachycarpa*	Singhal *et al.*. 1981
Isoauriculasin ; R₁=H, R₂=OH **Isoauriculatin** ; R₁=OH, R₂=H 	*Millettia auriculata* *M. auriculata*	Minhaj *et al.*, 1976 Minhaj *et al.*, 1976
Cajaisoflavone 	*Cajanus cajan*	Bhanumati, Chhabra and Gupta, 1979

Table 2 (continued)

Isoflavone Compound	Source (s)	Reference
Neobavaisoflavone	*Psoralea corylifolia*	Bajwa. Khanna and Seshadri. 1974
6,3'-Di (dimethylallyl) genistein	*Millettia pachycarpa*	Singhal *et al.*, 1980

* Plant was subjected to physiological stress

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1.2 Isoflavanones

Isoflavanones (5) are much rather than isoflavones. Examples of prenylated isoflavanones are shown in Table 3.

(5)

Table 3 Isoflavanone

Isoflavanone Compound	Source (s)	Reference
5-Deoxykievitone	*Phaseolus vulgaris* *P. aureus* * *P. mungo* *	Woodward, 1979 O'Neill, Adesanya and Roberts, 1983 quoted in Harborne, 1988 Adesanya, O'Neill and Roberts, 1984 quoted in Harborne, 1988
5-Deoxykievitone hydrate	*Phaseolus mungo* *	Adesanya, O'Neill and Roberts, 1984 quoted in Harborne, 1988

Table 3 (continued)

Isoflavanone Compound	Source (s)	Reference
Diphysolone : R₁=R₂=OH	*Desmodium gangeticum* *	Ingham and Dewick. 1984 quoted in Harborne, 1988
	D. robiniodes *	Ingham and Tahara. 1983 quoted in Harborne, 1988
Diphysolidone : R₁=OMe, R₂=OH or R₁=OH, R₂=OMe	*D. robinioides* *	Ingham and Tahara, 1983 quoted in Harborne, 1988
Kievitone ; R=OH	*Phaseolus coccineus* *Lablab niger*	Adesanya, O'Neill and Roberts, 1985 Ingham, 1977
4'-O-Methyl-kievitone ; R=OMe	*Phaseolus mungo* *	Adesanya, O'Neill and Roberts, 1984 quoted in Harborne, 1988

Table 3 (continued)

Isoflavanone Compound	Source (s)	Reference
Kievitone hydrate	*Phaseolus mungo* *	Adesanya. O'Neill and Roberts. 1984 quoted in Harborne. 1988
Cyclokievitone	*Phaseolus aureus* * *P. coccineus* * *P. mungo* *	O'Neill, Adesanya and Robert, 1983 Adesanya, O'Neill and Roberts, 1985 Adesanya, O'Neill and Roberts, 1984 quoted in Harborne, 1988
Cyclokievitone hydrate	*Phaseolus mungo* *	Adesanya, O'Neill and Roberts, 1984 quoted in Harborne. 1988

Table 3 (continued)

Isoflavanone Compound	Source (s)	Reference
Isosophoranone	*Sophora tomentosa*	Shirataki *et al..* 1983
Sophoraisoflavanone A	*Sophora tomentosa*	Komatsu, Yokoe and Shiratoaki, 1978
Sophoraisoflavone B	*Sophora franchetiana*	Komatsu, Yokoe and Shirataki, 1981

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Table 3 (continued)

Isoflavanone Compound	Source (s)	Reference
Cajanone : R=OH 2'-Methylcajanone : R = OMe	*Cajanus cajan* *C. cajan*	Preston. 1977 a Bhanumati. Chhabra and Gupta. 1979 a

* Plant was subjected to physiological stress.

1.3   Rotenoids

Rotenoids are a class of isoflavonoid characterized by the presence of an extra carbon atom in an additional heterocyclic ring (6). This system is derived in nature by oxidative cyclization of a 2'-methoxyisoflavone. Systematic nomenclature for the rotenoids has never been generally adopted, and trivial names are used throughout, though the numbering system of (6) is used. For convenience, these compounds may by subdivided into three major groups according to oxidation levels in the rotenoid ring system, and rotenoids (7). 12a-hydroxyrotenoids (8) and dehydrorotenoids (9) from the usual basis for classification. Almost all of the known natural rotenoids contain isoprenoid-derived substituents. (Dewick. 1988)

(6)

(7)

(8)

(9)

Roots of *Millettia pachycarpa* contain 12a-hydroxyrot-2'-enonic acid as well as rot-2'-enonic acid (Singhal *et al.*, 1982). Again, the uncyclized isoprenyl substituent is of biogenetic interest. Volubinol from branches of *Dalbergia volubilis* (Chawla *et al.*, 1984) contains the rare 2-methoxy-3 hydroxy substitution pattern, also seen in the rotenoid 3-O-demethylamorphigenin (Somleva and Ognyanov, 1985).

Examples of rotenoids contain isoprenoid-derived substituents are shown in Table 4.

Table 4 Rotenoids

Rotenoid Compound	Source (s)	Reference
Rot-2'-enonic acid 	*Millettia pachycarpa*	Singhal *et al.*, 1982a
Deguelin : $R_1=R_2=$OMe 	*Derris elliptica* *Lonchocarpus longifolius* *L. salvadorensis* *L. spruceanus* *Piscidia erythrina* *P. mollis* *Tephrosia strigosa* *T.* sp.	Komada, Yamakawa and Minoda, 1980 Braz Filho *et al.*, 1980 Birch, N., Crombie, L. and Crombie, W.M., 1985 Menichini, Delle Monache, Marini Bettolo, 1982 Delle Monache, Ferrari and Menichini, 1984 Menichini, Delle Monache, Marini Bettolo, 1982 Kamal and Jain, 1980 Menichini, Delle Monache, Marini Bettolo, 1982

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
Millettone : $R_1=R_2=OCH_2O$	*Piscidia erythrina*	Delle Monache, Ferrari and Menichini, 1984
Elliptone	*Piscidia erythrina*	Delle Monache, Ferrari and Menichini, 1984
	Lonchocarpus salvadorensis	Birch, N., Crombie, L and Crombie, W.M., 1985
	Tephrosia strigosa	Kamal and Jain, 1980
Rotenone ; $R_1=R_2=OMe$	*Amorpha fruticosa*	Hohmann *et al.*, 1982
	Derris elliptica	Komada, Yamakawa and Minoda, 1980
	Lonchocarpus longifolius	Braz Filho *et al.*, 1980
	L. salvadorensis	Birch, N., Crombie, L. and Crombie, W.M., 1985
	L. spruceanus	Menichini, Delle Monache and Marini Bottolo, 1982

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
Rotenone : R₁=R₂=OMe	*Millettia pachycarpa*	Singhal *et al.*, 1982
	Piscidia erythrina	Delle Monache, Ferrari and Menichini, 1984
	P. mollis	Menichini, Delle Monache and Marini Bettolo, 1982
	Tephrosia strigosa	Kamal and Jain, 1980
	T. villosa	Chandrasekharan *et al.*, 1983
	T. sp.	Suarez *et al.*, 1980
Isomillettone ; R₁, R₂=OCH₂O	*Piscidia erythrina*	Delle Monache, Ferrari and Menichini, 1984
3-O-Demethylamorphigenin	*Amorpha fruticosa*	Somleva and Ognyanov, 1985

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
Amorphigenin ; R=OH	*Amorpha fruticosa*	1. Somleva and Ognyanov, 1985 2. Hohmann *et al.*, 1982
	Dalbergia monetaria	Abe *et al.*, 1985
Amorphigenin-O-glucoside ; R=OGlc	*Amorpha fruticosa*	Somleva and Ognyanov, 1985
	Dalbergia monetaria	Abe *et al.*, 1985
Amorphigenin O-vicianoside (amorphin) ; R=O-vicianose.	*Amorpha fruticosa*	1. Somleva and Ognyanov, 1985 2. Hohmann *et al*, 1982
Toxicarol	*Lonchocarpus salvadorensis*	Birch, N., Crombie, L. and Crombie, W.M., 1985

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
12a-Hydroxyrot-2'-enonic acid 	*Millettia pachycarpa*	Singhal *et al.*, 1982
Tephrosin (12a-hydroxydeguelin) 	*Amorpha fruticosa* *Lonchocarpus longifolius* *L. spruceanus* *Piscidia mollis* *Tephrosia elata* *T.* sp.	Somleva and Ognyanov, 1985 Braz Filho *et al.*, 1980 Menichini, Delle Monache and Marini Bottolo, 1982 Menichini, Delle Monache and Marini Bottolo, 1982 Lwande, Greene and Bentley, 1985 1. Menichini, Delle Monache and Marini Bottolo, 1982 2. Suarez *et al.*, 1980
12a-Hydroxyrotenone : R=H	*Amorpha fruticosa* *Lonchocarpus longifolius*	Hohmann *et al.*, 1982 Braz Filho *et al.*, 1980

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
	L. spruceanus	Menichini, Delle Monache and Marini Bottolo, 1982
	Millettia pachycarpa	Singha *et al.*, 1982
	Piscidia mollis	Menichini, Delle Monache and Marini Bottolo, 1982
	Tephrosia spp.	Suarez *et al.*, 1980
Dalbinol ; R=OH (12a-hydroxyamorphigenin)	*Amorpha fruticosa*	Hohmann *et al.*, 1982
	Dalbergia monetaria	Abe *et al.*, 1985
Dalbin ; R=OGlc (Dalbinol-O-glucoside)	*Dalbergia monetaria*	Abe *et al.*, 1985
	D. nitidula	Van Herrden, Brandt and Roux, 1980
Hydroxyamorphin ; R=O-vicianose	*Amorpha fruticosa*	Somleva and Ognyanov, 1985

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
Volubinol	*Dalbergia volubilis*	Chawla, Mittal and Rastogi, 1984
11-Hydroxytephrosin	*Amorpha fruticosa* *Tephrosia viridiflora*	Somleva and Ognyanov, 1985 Gomez *et al.*, 1985
Villosinol (12a-hydroxysumatrol)	*Tephrosia viridiflora*	Gomez *et al.*, 1985

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
12-Dihydrodalbinol : R=OH	*Dalbergia monetaria*	Abe *et al.*, 1985
12-Dihydrodalbin ; R=OGlc (12-Dihydrodalbinol-O-glucoside)	*D. monetaria*	Abe *et al.*, 1985
Dehydromillettone	*Piscidia erythrina*	Delle Monache, Ferrari and Menichini, 1984
Dehydrorotenone : R=H	*Lonchocarpus longifolius* *Tephrosia villosa*	Braz Filho *et al.*, 1980 Chandrasekharan *et al.*, 1983

Table 4 (continued)

Rotenoid Compound	Source (s)	Reference
Dehydroamorphigenin : R=OH	*Amorpha fruticosa*	Hohmann *et al.,* 1982
6-Hydroxydehydrotoxicarol	*Amorpha fruticosa*	Somleva and Ognyanov, 1985

1.4 Pterocarpans

Pterocarpan (10) contain a tetracyclic ring system derived from the basic isoflavonoid skeleton by an ether linkage between the 4 and 2' positions. The systematic numbering of (10) rather than that for simple isoflavonoids is used. however. The majority of natural pterocarpans isolated have arisen from phytoalexin studies. using fungal or abiotically stressed plant tissues. and the number of examples continues to grow. making this the second largest group of isoflavonoids after the isoflavones (Dewick. 1988).

(10)

Several phytoalexins from the genus *Glycine* are 6a-hydroxypterocarpans and a range of different isoprenylated structures has been identified. Canescacarpin, isolated from bacteria-infected leaves of *Glycine canescens* (Lyne, Mulheirn and Keen, 1981), contains an isopropenyldihydrofuran grouping with opposite configuration (R) to that observed in the isomeric glyecollin III. Glyceofuran and 9-O-methylglyceofuran from *Glycine max* represent a further variant on this structure, containing a furan rather than dihydrofuran group (Ingham *et al.*, 1981), and an uncommon isopropenylfuran is observed in clandestacarpin from *Glycine clandestina* (Lyne, Mulheirn and Keen, 1981).

In the Phaseolae and related tribes, more complex prenylated isoflavonoid derivatives, e.g. phaseollin and phaseollidin, are produced (Ingham and Markham, 1980).

Example of prenylated pterocarpans are showns in Table 5.

Table 5 Pterocarpans

Pterocarpan Compound	Source (s)	Reference
Homoedudiol	*Neorautanenia edulis*	Brink, Rall and Engelbrecht, 1974

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Sophorapterocarpan A (homoedudiol[+]) 	*Calopogonium mucunoides* *Sophora franchetiana*	Ingham and Tahara, 1985 quoted in Harborne, 1988 Komatsu, Yokoe and Shirataki, 1981
Nitiducol 	*Dalbergia nitidula*	Van Heerden, Brandt and Roux, 1978
Nitiducarpin 	*Dalbergia nitida*	Van Heerden, Brandt and Roux, 1978

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Edudiol ; R=OH **Edulenol** ; R=OMe 	*Neorautanenia edulis* *N. amboensis*	Brink, Rall and Breytenbach, 1977 Brink, Rall and Breytenbach, 1977
Neoraucarpanol ; R=OH **Neoraucarpan** ; R=OMe 	*Neorautanenia amboensis* *Neorautanenia edulis*	Brink, Rall and Breytenbach, 1977 Brink, Rall and Breytenbach, 1977
Isoneorautenol 	*Calopogonium mucunoides* *	Ingham and Tahara, 1985 quoted in Harborne, 1988

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Phaseollidin ; R=OH 	*Dolichos biflorus* *	Keen and Ingham, 1980
	Erythrina abyssinica	Kamat *et al.*, 1981
	E. corallodendron	Ingham, 1980 quoted in Harborne, and Mabry, 1982
	E. crista-galli	Ingham and Markham, 1980
	E. lysistemon	Ingham, 1980 quoted in Harborne and Mabry, 1982
	E. sandwicensis	Ingham, 1980 quoted in Harborne and Mabry, 1982
	Lablab niger	Ingham, 1977 quoted in Harborne and Mabry, 1982
	Psaseolus aureus *	O'Neill, Adesanya and Roberts, 1983 quoted in Harborne, 1988
	P. coccineus *	Adesanya, O'Neill and Roberts, 1985
	Psophocarpus tetragonolobus	1. Preston, 1977 2. Ingham 1978

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Sandwicensin ; R=OMe	*Erythrina sandwicensis*	Ingham, 1980 quoted in Harborne and Mabry, 1982
Lespedezin	*Lespedeza homoloba*	Ueno *et al.*, 1973
1-Methoxyphaseollidin	*Psophocarpus tetragonolobus*	Preston, 1977

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Gangetinin	*Desmodium gangeticum*	Purushothaman *et al.*, 1975
Phaseollin	*Erythrina abyssinica* *Phaseolus coccineus* * *P. vulgaris* *	Kamat *et al.*, 1981 Adesanya, O'Neill and Roberts, 1985 Bailey and Berthier, 1981
Calopacarpin ^	*Alysicarpus spp.* * *Calpogonium mucunoides* * *Pueraria phaseoloides* *	Ingham and Tahara, 1985 quoted in Harborne, 1988 Ingham and Tahara, 1985 quoted in Harborne, 1988 Ingham and Tahara, 1985 quoted in Harborne, 1988

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Dolichin A (6a*R*, 11a*R*, 2'*R*) **Dolichin B** (6a*R*, 11a*R*, 2'*S*)	*Dolichos biflorus* * *D. biflorus* *	Ingham *et al.*, 1981a Ingham *et al.*, 1981a
Neodunol	*Calopogonium mucumoides* *	Ingham and Tahara, 1985 quoted in Harborne, 1988
Apiocarpin	*Apios tuberosa* *	Ingham and Mulheirn, 1982

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Cristacarpin ; R=OMe (erythrabyssin I) **Sandwicarpin** ; R=OH 	*Erythrina abyssinica* *E. sandwicensis*	Kamat *et al.*, 1981 Ingham and Markham, 1980
Erythrabyssin II 	*Erythrina abyssinica*	Kamat *et al.*, 1981
Glyceollidin I 	*Glycine max* *	Zahringer, Schaller and Grisebach, 1981

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Glyceocarpin (glyceollidin II)	*Glycine max* *	Ingham *et al.*, 1981b
Tuberosin	*Calopogonium mucunoides* *	Ingham and Tahara, 1985
Lespein	*Lespedeza homoloba*	Ueno *et al.*, 1973

Table 5 (continued)

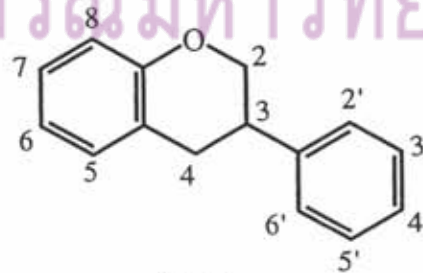| Pterocarpan Compound	Source (s)	Reference
Glyceollin I	*Glycine max*	1. Burden and Bailey, 1975 2. Lyne, Mulheirn and Loworthy, 1976
Glyceollin II	*Glycine canescens* * *G. max* *	Lyne, Mulheirn and Keen, 1981 1. Ingham *et al.*, 1981 b 2. Osman and Fett, 1983 3. Komives, 1983 4. Banks and Dewick, 1983 5. Osswald, 1985
Canescacarpin (6a*S*, 11a*S*, 5'*R*) **Glyceollin III** (6a*S*, 11a*S*, 5'*S*)	*Glycine canescens* * *G. max* *	Lyne, Mulheirn and Keen, 1981 1. Ingham *et al.*, 1981 b 2. Osman and Fett, 1983 3. Komives, 1983 4. Osswald, 1985

Table 5 (continued)

Pterocarpan Compound	Source (s)	Reference
Glyceollin IV	*Glycine max*	Lyne and Mulheirn, 1978
Glyceofuran ; R=OH **9-O-methylglyceofuran ; R=OMe**	*Glycine max* *	Ingham *et al.*, 1981b Ingham *et al.*, 1981b

* Plant was subjected to physiological stress,+ Revised structure for homoedudiol

^ Structure previously assigned to homoedudiol

1.5 Isoflavans

All plant-derived isoflavans (11) contain a 2'-oxygen substituent, a feature which appears to be a consequence of the close relationship for the biosynthetic pathways to isoflavans and pterocarpans (Dewick, 1982).

(11)

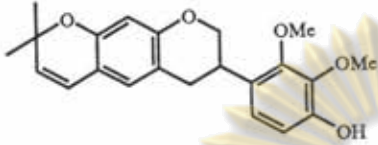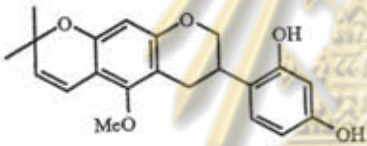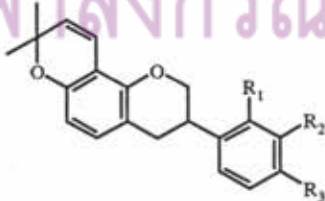Examples of prenylated isoflavans are shown in Table 6.

Table 6 Isoflavans

Isoflavan Compound	Source (s)	Reference
Spherosinin	*Sphaerophysa salsula*	Kattaev, Nikonov and Rashkes, 1975
Neorauflavan	*Neorautanenia edulis*	Brink, Rall and Engelbrecht, 1974b
Glabridin ; R$_1$=R$_3$=OH, R$_2$=H	*Glycyrrhiza glabra*	Saitoh, Kinoshita and Shibata, 1976
4'-Methylglabridin ; R$_1$=OH, R$_2$=H, R$_3$=OMe	*G. glabra*	Mitscher *et al.*, 1980
3'-Methoxyglabridin ; R$_1$=R$_3$=OH, R$_2$=OMe	*G. glabra*	Mistscher *et al.*, 1980

Table 6 (continued)

Isoflavan Compound	Source (s)	Reference
Leiocin	*Dalbergia nitidula*	Van Heerden, Brandt and Roux, 1978
Leiocinol	*Dalbergia nitidula*	Van Heerden, Brandt and Roux, 1978
2'-Methylphaseollidinisoflavan	*Vigna unguiculata*	Preston, 1975

Table 6 (continued)

Isoflavan Compound	Source (s)	Reference
Licoricidin 	*Glycyrrhiza uralensis*	Chang *et al.*, 1983
Heminitidulan ; R_1=OH, R_2=R_4=H, R_3=OMe	*Dalbergia nitidula*	Van Heerden, Brandt and Roux, 1978
Nitidulin ; R_1=R_2=OH, R_3=OMe, R_4=H	*D. nitidula*	Van Heerden, Brandt and Roux, 1978
Nitidulan ; R_1=OH, R_2=H, R_3=R_4=OCH$_2$O 	*D. nitidula*	Van Heerden, Brandt and Roux, 1978
Phaseollinisoflavan ; R=OH	*Glycyrrhiza glabra* * *Phaseolus coccineus* *	Mitscher *et al.*, 1980 Adesanya, O'Neill and Roberts, 1985

Table 6 (continued)

Isoflavan Compound	Source (s)	Reference
Hispaglabridin B	*Glycyrrhiza glabra*	Mitscher *et al.*, 1980
Crotmarine	*Crotalaria madurensis*	Bhakuni and Chaturvedi, 1984

* Plant was subjected to physiological stress.

1.6   3-Arylcoumarins

Examples of 3-arylcoumarins (12) carry prenyl substituents are shown in Table 7.

(12)

Table 7 3-Arylcoumarins

3-Arylcoumarin Compound	Source (s)	Reference
Glycycoumarin ; R=OH	*Glycyrrhiza uralensis*	Zhu *et al.*, 1984
Glycyrin ; R=OMe	*Glycyrrhiza* sp.	Zhu *et al.*, 1984

1.7   3-Aryl-4-Hydroxycoumarins

Examples of 3-aryl-4-hydroxycoumarins (13) have been isolated from seed of *Millettia thonningii* by two independent groups (Olivares *et al.*, 1982). Robustic acid is a known compound, but thonningine-A and thonningine-B are new structures in this small group. All the known examples have a 5-methoxy substituent (Dewick, 1988).

(13)

Examples of prenylated 3-aryl-4-hydroxycoumarins are shown in Table 8.

Table 8 3-Aryl-4-Hydroxycoumarins

3-Aryl-4-Hydroxycoumarin Compound	Source (s)	Reference
Glabrescin	*Derris glabrescens*	Delle Monache *et al.,* 1977
Robustic acid ; R=H **Thonningine B** ; R=OMe	*Derris robusta* *Millettia thonningii*	Subba Rao, 1965 Olivares *et al.,* 1982
Robustin ; R=H **Thonningine A** ; R=OMe	*Derris spruceana* *D. robusta* *Millettia thonningil*	Subba Rao, 1965 Subba Rao, 1965 Olivares *et al.,* 1982

1.8   Coumestans

Coumestans (14) are widely distributed and are easily recognized in solution or on chromatograms from their intense bright-blue or violet fluorescence under UV light (Dewick, 1982).

(14)

Examples of prenylated coumestans are shown in Table 9.

Table 9 Coumestans

Coumestan Compound	Source (s)	Reference
Psoralidin	*Phaseolus lunatus*	Rich, Keen and Thomason, 1977

Table 9 (continued)

Coumestan Compound	Source (s)	Reference
Psoralidin oxide 	*Psoralea corylifolia*	Gupta, *et al.*, 1980
Corylidin 	*Psoralea corylifolia*	Gupta, Dhar and Atal, 1977
Sophoracoumestan A ; R=OH **Tuberostan ; R=OMe** 	*Sophora franchetiana* *Pueraria tuberosa*	Komatsu, Yokoe and Shirataki, 1981 Prasad, Kapil and Popli, 1985

Table 9 (continued)

Coumestan Compound	Source (s)	Reference
Isosojagol	*Phaseolus coccineus* *	O'Neill, Adesanya and Roberts, 1984
Phaseol	*Phaseolus aureus* *	O'Neill, 1983
Glycyrol	*Glycyrrhiza uralensis* G. spp.	Zhu *et al.*, 1984 Hiraga *et al.*, 1984

* Plant was subjected to physiological stress

1.9 Coumaronochromones

For many years, only a single example of the coumaronochromone (15) class of isoflavonoid has been regcognized. This is lisetin, isolated from *Piscidia erythrina* (Pietta and Zio, 1983). Millettin was isolated from seeds of *Millettia auriculata* (Raju *et al.*, 1981). Lupinalbins B-E all contain isoprenyl substituents were isolated from *Lupinus albus* (Tahara, Ingham and Mizutani, 1985 quoted in Harborne, 1988).

(15)

Example of coumaronochromones all contain isoprenyl substituents are shown in Table 10.

Table 10 Coumaronochromones

Coumaronochromone Compound	Source (s)	Reference
Millettin	*Millettia auriculata*	Raju *et al.*, 1981
Lupinalbin B	*Lupinus albus*	Tahara, Ingham and Mizutani, 1985 quoted in Harborne, 1988

Table 10   (continued)

Coumaronochromone Compound	Source (s)	Reference
Lupinalbin C	*Lupinus albus*	Tahara, Ingham and Mizutani, 1985 quoted in Harborne, 1988
Lupinalbin D ; R=H **Lisetin ; R=OMe**	*Lupinus albus* *Piscidia erythrina*	Tahara, Ingham and Mizutani, 1985 quoted in Harborne, 1988 1. Pietta and Zio, 1983 2. Delle Monache, Ferrari and Menichini, 1984
Lupinabin E	*Lupinus albus*	Tahara, Ingham and Mizutani, 1985 quoted in Harborne, 1988

1.10   2-Arylbenzofurans

A wide variety of 2-arylbenzofuran structure (16) is encountered in nature, and several different biosynthetic origins are undoubtedly involved. Some structures are of lignan/neolignan origin and derived from phenylpropane dimers, and others are probably produced by cyclization of stilbenes. Compound in this group are derived from leguminous plants, and almost always occur with structurally related isoflavonoids (Dewick, 1982).

(16)

Ambofuranol from bulbs of *Neorautanenia amboensis* was the first example of a 2-arylbenzofuran with an oxygen substituent on the heterocyclic ring (Breytenbach and Rall, 1980).

Example of 2-arylbenzofurans all contain isoprenyl substituents are shown in Table 11.

Table 11 2-Arylbenzofurans

2-Arylbenzofuran Compound	Source (s)	Reference
Neoraufurane	*Neorautanenia edulis*	Brink, Rall and Engelbrecht, 1974 b

Table 11 (continued)

2-Arylbenzofuran Compound	Source (s)	Reference
Ambofuranol	*Neorautanenia amboensis*	Breytenbach and Rall, 1980
Licobenzofuran (liconeolignan)	*Glycyrrhiza uralensis* G. sp.	Chang *et al.*, 1983 Chang *et al.*, 1981

2.   **The Minor Flavonoids**

2.1   **Chalcones**

Chalcone, and dihydrochalcones, are C_6-C_3-C_6 compounds that lack a central heterocyclic ring. Position on these compounds, unfortunately, are identified using a numbering system unique to these groups. Chalcones were apparently recognized as being structurally related to acetophenones whose ring carbons were identified by primed numbers. Hence, chalcone (and dihydrochalcone) A-ring carbons are also identified with primed numbers ; the B-ring carbons are identified with unprimed numbers. This system is illustrated below (17) (Bohm, 1988).

(17)

2.1.1 Chalcones Lacking B-Ring Oxygenation

Members of the legume genus *Flemingia* exhibit a rich and varied flavonoid chemistry as reference to the earlier reviews will attest. Five chalcones belonging to this subgroup from *F. stricta*, of which two are new. The known natural products include flemistrictin-A which clearly serves as the starting material for formation of the others. The other two known compounds are flemistrictin-B and flemistrictin-C. In both of these compounds ring closure has occurred involving the 4'-hydroxyl, in one case yielding a furan derivative, and in the other a pyran derivative. The new compounds are isomeric to this pair involving cyclization with the 2'-hydroxyl to yield compounds flemistrictin-E and flemistrictin-F respectively. Further examples of the biosynthetic versatility of *Flemingia* were revealed in a study of *F. wallichii* (Sivarambabu *et al.*, 1985).

A new prenylated chalcone from *Flemingia fruticulosa*, called "flemiculosin" (Khattri *et al.*, 1984).

2.1.2 Chalcones Having One B-Ring Oxygenation

A series of mono-and di-prenylflavanones and the related 3,5-di-C-prenylchalcone were isolated from *Erythrina abyssinica*. The chalcone is "abyssinone-V" (Kamat *et al.*, 1981).

2.1.3 Chalcones Having Two B-Ring Oxygenations

C-Prenylation of flavonoid is common with cyclization to form a variety of derivatives frequently seen. Most of these derivatives have six- or five- membered rings (Bohm, 1988).

Pyrano derivatives would be formed by reaction between C-3" of a 3-methylbuten-2-yl side chain and a phenolic hydroxyl as shown in the conversion of

model compound (18) to (19). A five-membered heterocycle would be formed by the reaction at C-2" of the side chain (Bohm, 1988).

(18)   (19)

2.1.4   Chalcones Having Three B-Ring Oxygenations

A trioxygenated B-ring compound ; "pongachalcone-I" was isolated from *Pongamia glabra* (Subramanyam, K., Rao, J.M. and Rao, K.V.J., 1977).

Examples of prenylated chalcones are shown in Table 12.

Table 12 Chalcones

Chalcone Compound	Source (s)	Reference
Derricidin ; R_1=H, R_2= (cordoin)	*Derris floribunda*	Braz Filho *et al*, 1975
	Lonchocarpus sp.	Delle Monache, Cuca Suraez and Marini-Bettolo, 1978
Flemistrictin-A ; R_1= , R_2=H	*Flemingia stricta*	Rao *et al.*, 1976
	Derris floribunda	Braz Filho *et al*, 1975b

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
ψ-Isocordoin ; R₁=, R₂=H	*Lonchocarpus* sp.	Delle Monache *et al.*, 1974 quoted in Harborne and Mabry, 1982
Flemistrictin-B	*Flemingia stricta*	Subrahmanyam *et al.*, 1982
Flemistrictin-C	*Flemingia stricta*	Subrahmanyam *et al.*, 1982

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
Flemistrictin-E	*Flemingia stricta*	Subrahmanyam *et al.*, 1982
Flemistrictin-F	*Flemingia stricta*	Subrahmanyam *et al.*, 1982
Flemiwallichin-A ; R₁=OH, R₂=H	*Flemingia wallichii*	1. Subrahmanyam *et al.*, 1982 2. Sivarambabu *et al.*, 1985
Flemiwallichin-B ; R₁=H, R₂=OH	*Flemingia wallichii*	1. Subrahmanyam *et al.*, 1982 2. Sivarambabu *et al.*, 1985

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
Flemiwallichin-D	*Flemingia wallichii*	1. Subrahmanyam *et al.*, 1982 2. Sivarambabu *et al.*, 1985
Flemiwallichin-E	*Flemingia wallichii*	1. Subrahmanyam *et al.*, 1982 2. Sivarambabu *et al.*, 1985
Flemiwallichin-F	*Flemingia wallichii*	1. Subrahmanyam *et al.*, 1982 2. Sivarambabu *et al.*, 1985

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
	Ammothamnus lehmanni	Sattikulov *et al.*, 1983 quoted in Harborne, 1988
Ovalichalkone	*Millettia ovalifolia*	Gupta and Krishnamurti, 1979
Ovalichalkone-A	*Millettia ovalifolia*	Gupta and Krishnamurti, 1979

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
Pongachalcone-I	*Pongamia glabra*	Subramanyam, K., Rao, J.M. and Rao, K.V.J., 1977
Praecanosone-A ; R=H **Praecanosone-B** ; R=CH₃	*Tephrosia praecans* *T. praecans*	Camele *et al.*, 1980 Camele *et al.*, 1980
4-Hydroxyisocordoin ; R₁= , R₂=H **4-Hydroxyderricin** ; R₁= , R₂=CH₃	*Lonchocarpus* sp. *L.* sp.	Delle Monache *et al.*, 1974 quoted in Harborne and Mabry, 1982 Delle Monache *et al.*, 1974 quoted in Harborne and Mabry, 1982

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
Xanthoangelol ; $R_1=$ ⟶⟶⟶ , $R_2=H$	*Lespedeza cyrtobotrya*	Miyase *et al.*, 1980
Lonchocarpin ; R=H	*Derris floribunda*	Braz Filho *et al.*, 1975
4-Hydroxylonchocarpin	*Derris floribunda*	Braz Filho *et al.*, 1975

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
Glabrachalcone	*Pongamia glabra*	Pathak, Saini and Khanna, 1983
Bavachromanol	*Psoralea corylifolia*	Suri *et al.*, 1980
Lespeol	*Lespedeza cyrtobotrya*	Miyase *et al.*, 1980

Table 12 (continued)

Chalcone Compound	Source (s)	Reference
Licochalcone-A	*Glycyrrhiza glabra*	Saitoh and Shibata, 1975
3,4-Dihydroxylonchocarpin ; $R_1=R_2=R_3=H$	*Derris floribunda*	Braz Filho *et al.*, 1975
Pongachalkone II ; $R_1=R_2=H$, $R_3=CH_3$	*Pongamia glabra*	Subramanyam, K., Rao, J.M. and Rao, K.V.J. 1977
Glabrachromene-II ; $R_1=H$, $R_2 = R_3 = -O-CH_2-O-$	*P. glabra*	Subramanyam, K., Rao, J.M. and Rao, K.V.J., 1977
Glabrachromene ; $R_1=OMe$, $R_2 = R_3 = -O-CH_2-O-$	*P. glabra*	Subramanyam, K., Rao, J.M. and Rao, K.V.J. , 1977

Table 12 (continued)

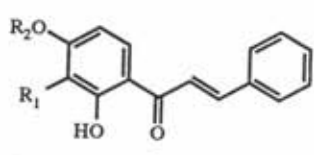| Chalcone Compound	Source (s)	Reference
Flemiculosin	*Flemingia fruticulosa*	Khattri *et al.*, 1984
Crotmadine	*Crotalaria madurensis*	Bhakuni and Chaturved, 1984
Abyssinone-V	*Erythrina abyssinica*	Kamat *et al.*, 1981

2.2   Dihydrochalcones

Identification of positions in the dihydrochalcones follows the pattern described for chalcones. This section also treats dihydrochalcones that have substitutions on the α- and β- carbons of the bridge (Bohm, 1988).

C-Prenylation is frequently seen in flavonoids isolated from members of the genus *Flemingia*. "flemistrictin-D" was isolated from *F. stricta* along with a series of chalcones and flavanones most of which vary principally in the manner of ring closure of the prenyl group (Subrahamanyam *et al.*, 1982).

Examples of prenylated dihydrochalcones are shown in Table 13.

Table 13 Dihydrochalcones

Dihydrochalcone Compound	Source (s)	Reference
Dihydrocordoin 	*Lonchocarpus* sp.	Delle Monache *et al.*, 1974
Flemistrictin-D 	*Flemingia stricta*	Subrahmanyam *et al.*, 1982

2.3 Flavanones

Flavanones are colorless substances which are simple reduction products of flavones (Harborne, 1973). The numbering system for flavanone (19) uses primed number for the A ring and unprimed numbers for the B ring (Bohm, 1982). Since C-2 of the flavanone molecule is a centre of asymmetry ; the phenyl substituent at that position can be either in the (2S) configuration with that group indicated as being above the plane of the page. The former configuration is considered to be the natural one. Many, if not most , reports on flavanones do not comment on the stereochemistry of the compounds. This is usually because of the very small amounts of material available ; in some cases identifications are assumed on the basis of chromatographic comparisons (Bohm, 1988).

Flavanone nucleus (20)

2.3.1 Flavanones Lacking B-Ring Oxygenation

The simplest known flavanone is (21), 7-hydroxyflavanone. It was originally discovered in members of the Leguminosae. C-prenyl derivative of this compound are fairly well known. The parent C-prenyl derivative (22), known from the genus *Tephrosia*, has now been isolated from another member, namely *T. falciformis*, where it occurs with the new natural product "falciformin" (Khan, Chandrgsekharan and Ghanim, 1986).

(21)

(22)

Prenylated pinocembrin derivatives were first obtained from members of two plant families, Leguminosae and Compositae. These two families continue to yield new and interesting members of this group of flavonoids (Bohm, 1988).

Greater levels of complexity are seen in the prenyl ethers isolated from seed of *Lonchocarpus costaricensis* (Waterman and Mahmound, 1985).

Tephrowatsin-C was isolated from *Tephrosia watsoniana*. Hydration of the prenyl function also occurs to yield this compound (Gomez *et al.*, 1985).

2.3.2   Flavanones Having One B-Ring Oxygenation

No group of flavonoids seems immune from prenylation in the Legumes. Several examples are found in this subgroup. 6-Prenyl-, 8-prenyl, 6,8-diprenyl and 8,3',5'-triprenyl derivatives (Bohm, 1988).

A series of flavanones having two or three prenyl groups has been isolated from *Amorpha fruticosa*. Despite five of the seven having 3',4'-dioxygenation it is convenient to present them all at this time. All seven share the 6,8-diprenyl substitution. The simplest compound identified was "amoridin" which is 6,8-diprenyl-7-O-methylnarigenin. "Amorilin" is 6,8,3'-triprenylnarigenin (Rozsa *et al.*, 1982 b).

The triprenyl compound found in *Sophora*, from which its common name "sophoranone". It has rcently been described from another legume, *Millettia pulcha* (Baruah *et al.*, 1984).

Multi C-prenylatiun is commonly met feature of flavanones in the Leguminosae. Some of the most highly C-alkylated flavanones known have been found in members of the Leguminosae (Bohm, 1988).

2.3.3   Flavanones Having Two B-Ring Oxygenations

Three C-prenylated flavanones were isolated from *Euchresta japonica*. One of these is a derivative of narigenin. The other two compounds were "euchrestaflavanone-B" and "euchrestaflavanone-C".

Examples of prenylated flavanones are shown in Table 14.

Table 14 Flavanones

Flavanone Compound	Source	Reference
6- Prenylpinocembrin ; R=H	*Derris rariflora*	Braz Filho, Gottieb and Mourao, 1975
6-Prenyl-7-methyl ether pinocembrin ; R=CH$_3$	*D. rariflora*	Braz Filho, Gottieb and Mourao, 1975
Sophoraflavanone B	*Sophora tomentosa*	Komatsu, Yokoe and Shiritaki, 1978
7-Methylglabranin	*Tephrosia villosa*	Jayaraman, Ghanim and Khan, 1980

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Candidone 	*Tephrosia elata*	Lwande, 1985
(A) R_1=H, R_2= **(B)** R_1=R_2= 	*Millettia ovalifolia* *M. ovalifolia*	Gupta and Krishnamurti, 1976a Gupta and Krishnamurti, 1976a
Sigmoidin A 	*Erythrina sigmoidea*	Fomum *et al.*, 1986

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Sigmoidin B	*E. sigmoidea*	Fomum *et al.*, 1986
Sigmoidin C	*E. sigmoidea*	Fomum *et al.*, 1986
Amoridin ; R_1=Me, R_2=R_3=H	*Amorpha fruticosa*	Rozsa *et al.*, 1982 b
Amoradicin ; R_1=Me, R_2=H, R_3=OH	A. fruticosa	Rozsa *et al.*, 1982 b
Amoradinin ; R_1=Me, R_2=H, R_3=OMe	A. fruticosa	Rozsa *et al.*, 1982 b
Amoritin ; R_1=H, R_2= , R_3=OMe	A. fruticosa	Rozsa *et al.*, 1982 b
Amorisin ; R_1=H, R_2= , R_3=OH	A. fruticosa	Rozsa *et al.*, 1982 b
Amorilin ; R_1=R_3=H, R_2=	A. fruticosa	Rozsa *et al.*, 1982 b

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Amorinin	*Amorpha fruticosa*	Rozsa *et al.*, 1982 a
Tephrowatsin-C	*Tephrosia watsoniana*	Gomez *et al.*, 1985
Falciformin	*T. falciformis*	Khan, Chandrasekharan and Ghanim, 1986

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Ovalichromene : R=OMe **(-)-Isolonchocarpin** ; R=H 	*Milletia ovalifolia* *Tephrosia* *purpurea*	Gupta and Krishnamurti, 1976 Gupta, Krishnamurti and Parthasarathi, 1980
Obovatin methylether : $R_1=CH_3$, $R_2=H$ **Fulvinervin** ;R1=H, R2= 	*Tephrosia* *bracteolata* *T. fulvinervis*	Khalid and Waterman, 1981 Rao, Venkataratnam and Vilain, 1985
Cajaflavanone 	*Cajanus cajan*	Bhanumati *et al.*, 1978

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Erythrisenegalone	*Erythrina senegalensis*	Fornum, Ayafor and Wandji, 1985
Lupinifolin ; R$_1$=H, R$_2$=OH	*Tephrosia lupinifolia*	Smalberger, Vleggaar and Weber, 1974
Minimiflorin ; R$_1$=OH, R$_2$=H	*Lonchocarpus minimiflorus*	Mahmound and Waterman, 1985
Ovalichromene-A ; R=OMe	*Millettia ovalifolia*	Islam, Gupta and Krishnamurti, 1980
Ovalichromene-B ; R=H	*M. ovalifolia*	Islam, Gupta and Krishnamurti, 1980

Table 14 (continued)

Flavanone Compound	Source	Reference
Ovaliflavanone-C ; R=H	*Milletia ovalifolia*	Islam Gupta and Krishnamurti, 1980
Ovaliflavanone-D ; R=	*M. ovalifolia*	Islam Gupta and Krishnamurti, 1980
Flemichin-A	*Flemingia wallachii*	1. Roa *et al.*, 1975 2. Sivarambabu, S., Rao, J.M. and Rao, K.V.J., 1979
Flemichin-E	*F. wallichii*	1. Roa *et al.*, 1975 2. Sivarambabu, S., Rao, J.M. and Rao, K.V.J., 1979

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Flemichin-D	*Flemingia stricta*	Sivarambabu, S., Rao, J.M. and Rao. K.V.J., 1979 a
Flemiflavanone-C **(enantiomer of flemichin-D)**	*Flemingia stricta*	Sivarambabu, S. Rao, J.M. and Rao, K.V.J., 1979 a
Flemiflavanone-A	*Flemingia stricta*	Sivarambabu, S., Rao, J.M. and Rao, K.V.J., 1979 a
Flemiflavanone-B	*Flemingia stricta*	Sivarambabu, S., Rao, J.M. and Rao, K.V.J., 1979 a

Table 14 (continued)

Flavanone Compound	Source	Reference
Sophoranol	*Sophora tomentosa*	Komatsu, Yokoe and Shiritaki, 1978
Abyssinone I ; R=H	*Erythrina abyssinica*	Kamat *et al.*, 1981
Abyssinone III ; R=	*E. abyssinica*	Kamat *et al.*, 1981
Abyssinone II ; $R_1=R_3=H$; $R_2=$	*Erythrina abyssinica*	Kamat *et al.*, 1981
Abyssinone IV ; $R_1=H$, $R_2=R_3=$	*E. abyssinica*	Kamat *et al.*, 1981
Abyssinone V ; $R_1=H$, $R_2=R_3=$	*E. abyssinica*	Kamat *et al.*, 1981
Sophoranone ; $R_1=R_2=R_3=$	*Millettia pulchra*	Baruah *et al.*, 1984

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Euchresta flavanone-A ; $R_1=R_3=$ ▵, $R_2=R_4=R_5=H$ **Flemiflavanone-D** ; $R_1=$ ▵, $R_2=R_4=R_5=H$, $R_3=$ $CH_2CH \overset{CH_3}{-} CCH_3$ (epoxide) **Hydroxysophoranone** ; $R_1=R_5=H$; $R_2=R_3=R_4=$ ▵ 	*Euchresta japonica* *Flemingia stricta* *Millettia pulchra*	Shirataki *et al.*, 1981 Mitscher *et al.*, 1985 Baruah *et al.*, 1984
Fleminone 	*Flemingia macrophylla*	Rao and Srimannaryana, 1983
Euchrestaflavanone-B 	*Euchresta japonica*	Shirataki *et al.*, 1982

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Euchrestaflavanone-C 	*Euchresta japonica*	Shirataki *et al.*, 1982
Exiguaflavanone A ; R=H **Exiguaflavanone B ; R=Me** 	*Sophora exigua* *S. exigua*	Nijsiri Ruangrungsi *et al.*, 1992 Nijsiri Ruangrungsi *et al.*, 1992
Exiguaflavanone C 	*Sophora exigua*	Iinuma *et al.*, 1993

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Exiguaflavanone D	*Sophora exigua*	Iinuma *et al.*, 1993
Exiguaflavanone E	*Sophora exigua*	Iinuma *et al.*, 1993
Exiguaflavanone F	*Sophora exigua*	Iinuma *et al.*, 1993

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Exiguaflavanone G	*Sophora exigua*	Iinuma *et al.*, 1994
Exiguaflavanone H	*Sophora exigua*	Iinuma *et al.*, 1994
Exiguaflavanone I	*Sophora exigua*	Iinuma *et al.*, 1994

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Exiguaflavanone J	*Sophora exigua*	Iinuma *et al.*, 1994
Exiguaflavanone K	*Sophora exigua*	Iinuma *et al.*, 1994
Exiguaflavanone L	*Sophora exigua*	Iinuma *et al.*, 1994

Table 14 (continued)

Flavanone Compound	Source (s)	Reference
Exiguaflavanone M 	*Sophora exigua*	Iinuma *et al.*, 1994

* Stereochemistry unknown

2.4 Dihydroflavonols

Dihydroflavonols, or 3-hydroxyflavanones, are numbered in the same fashion as flavanones. Carbons -2 and -3 are asymmetric in dihydroflavonols, so four isomers are possible for each compound. The majority of naturally occurring dihydroflavonols exist in the (2R : 3R) configuration, but a few compounds are known with (2S : 3S) stereochemistry (Bohm, 1982).

C-Prenylated dihydroflavonols are a somewhat better known group of natural products. Compound (23), which exhibits two C-prenyl groups one of which has been further modified through cyclization with the 7-hydroxyl function and it has (2R : 3R) stereochemistry. This compound was isolated from *Tephrosia lupinifolia* (Smalberger, Vleggaar and Weber, 1974).

(23)

Prenylated dihydroflavonols are shown in Table 15.

Table 15 Dihydroflavonols

Dihydroflavonol Compound	Source (s)	Reference
	Millettia pachycarpa	Singhal *et al.*, 1980
Tirumalin	*Rhynchosia cyanosperma*	Adinarayana *et al.*, 1980

Table 15 (continued)

Dihydroflavonol Compound	Source (s)	Reference
Sophoronol	*Sophora tomentosa*	Delle Monache *et al.*, 1976
Glepidotin-B	*Glycyrrhiza lepidota*	Mitschor *et al.*, 1983
Lupinifolinol	*Tephrosia lupinifolia*	Smalberger, Vleggaar and Weber, 1974

* Stereochemistry unknown

3. Flavones and Flavonols

A series of flavonoids, C-prenylated flavones and pyranoflavones, these were from *Tephrosia*. *Trans*-tephrostachin and *trans*-anhydrotephrostachin are remarkable for the unusual *trans* configuration in the side chain (Khalid and Watermann, 1981).

Prenylated flavones and flavonols are shown in Table 16.

Table 16 Flavones and Flavonols

Flavone and Flavonol Compound	Source (s)	Reference
cis-Tephrostachin ; R=CH=CHC (OH) Me$_2$	*Tephrosia bracteolata*	Kalid and Waterman, 1981
trans-Tephrostachin ; R=CH=CHC (OH) Me$_2$	*T. bracteolata*	Kalid and Waterman, 1981
trans-Anhydrotephrostachin	*T. bracteolata*	Kalid and Waterman, 1981
Isopongachromene	*Pongamia glabra*	Pathak, Saini and Khanna, 1983 a

4. Flavans and Leucoanthocyanidins

Prenylated flavan, namely nitenin has been isolated from the leaf of *Tephrosia nitens* (Gomez *et al.*, 1984) and *T. watsoniana* (Gomez *et al.*, 1985).

Tephrowatsonin A is leucoanthocyanidins (natural flavan-4-ols) isolated from the leaf of *T. watsoniana* contains prenyl substituents (Gomez *et al.*, 1985).

Examples of prenylated flavans and luecoanthocyanidins are shown in Table 17

Table 17 Flavans and Leucoanthocyanidins

Flavan and Leucoanthocyanidin Compound	Source (s)	Reference
Nitenin	*Tephrosia nitens* *T. watsoniana*	Gomez *et al.*, 1984 Gomez *et al.*, 1985
Tephrowatsonin A	*Tephrosia watsoniana*	Gomez *et al.*, 1985

5. C-glycosylflavonoids

C-glucosylisoflavanone, namely dalpanin has been isolated from *Dalbergia paniculata*, with a phloroglucinol derived A ring and a 2',4'-di-O-substitution (Adinarayana and Rao, 1975).

Example of prenylated C-glycosylflavonoids is shown in Table 18.

Table 18 C-glycosylflavonoids

C-glycosylflavonoid Compound	Source (s)	Reference
Dalpanin	*Dalbergia paniculata*	Adinarayana and Rajasekhara Rao, 1975

Biosynthesis

1. Biosynthesis of Flavonoids

In the past few years, considerable progress has been made in elucidating the biosynthesis of flavonoids. In particular, the knowledge of the enzymology has developed rapidly. With the exception of the anthocyanins, where a few reactions still remain unknown, the essential steps of the biosynthetic pathway of the main flavonoid classes are now clear. There are two main reasons for this rapid development. The improvement in chalcone synthase preparation and handling on the one hand and the realization that flowers are rich sources of enzymes for further reactions in flavonoid biosynthesis on the other, have provided the means for producing ^{14}C-labelled substrates, particularly (*S*)-naringenin and (2*R*, 3*R*)-dihydrokaempferol, in enantiomeric pure form and with high specific activity. Furthermore, studies in cell cultures are being supplemented of replaced by studies in flowers from intact plants. The extensive genetic information already available about flavonoid biosynthesis in

flowering plants has proved to be of great advantage. The detection of enzymes in cell-free extracts has also been improved considerably and high-performance liquid chromatography has been more and more applied to the separation, identification and quantification of substrates and products. The general overview of the origins of flavonoid precursors and the interlocking of the individual reactions leading to the various flavonoid classes. Since all flavonoids derive their carbon skeletons from two basic compounds, malonyl-CoA and the CoA ester of a hydroxycinnamic acid, the biosynthesis of these compounds are discussed as followed (Heller and Forkmann, 1988).

The origins of the direct flavonoid precursors 4-coumaroyl-CoA and malonyl-CoA and the biosynthetic interrelations of the various flavonoid classes are demonstrated in Fig. 1, with particular reference to substances with a single hydroxyl group in the B-ring. The enzymes involved in their biosynthetic pathway are summarized in Table 19.

Both flavonoid precursors are derived from carbohydrates. Malonyl-CoA is synthesized from the glycolysis intermediate acetyl-CoA and carbon dioxide, the reaction being catalysed by acetyl-CoA carboxylase. The supply of 4-coumaroyl-CoA is more complex. It involves the shikimate/arogenate pathway, the main route to the aromatic amino acids, phenylalanine and tyrosine in higher plants. Subsequent transformation of phenylalanine to *trans*-cinnamate is catalysed by phenylalanine ammonia-lyase which provides the link between primary metabolism and the phenylpropanoid pathway. Aromatic hydroxylation of cinnamate by cinnamate 4-hydroxylase leads to 4-coumaroyl-CoA by action of 4-coumarate : CoA ligase.

The central step in flavonoid biosynthesis is the condensation of three molecules of malonyl-CoA with a suitable hydroxycinnamic acid CoA ester, ordinarily 4-coumaroyl-CoA, to the C_{15} chalcone intermediate (4,2',4',6'-tetrahydroxychalcone, see Fig. 1). The reaction is catalysed by chalcone synthase. Flavonoids, aurones and other diphenylpropanoids are derived from the chalcone intermediate. Transformation by stereospecific action of chalcone isomerase provides the first flavonoid, a (2S)-flavanone (naringenin).

Oxidative rearrangement of the flavanone, involving a 2,3-aryl shift, yields an isoflavone (genistein). The reaction is catalysed by 'isoflavone synthase'. Introduction of a double bond between C-2 and C-3 of flavanone leads to the abundant class of flavones (apigenin). Two different enzymes are known to catalyse this reactin : a

dioxygenase and a mixed-function mono-oxygenase. Dihydroflavonols (dihydrokaempferol) are formed by direct hydroxylation of flavanones in the 3 position. This reaction is catalysed by the dioxygenase, flavanone 3-hydroxylase.

Dihydroflavonols are biosynthetic intermediates in the formation of flavonols, catechins, proanthocyanidins and anthocyanidins. The large class of flavonols (e.g. kaempferol) is formed by introduction of a double bond between C-2 and C-3 of dihydroflavonols. Flavonol synthase, the enzyme catalysing this reaction, is also a dioxygenase. Reduction of the carbonyl group of dihydroflavonols in the 4 position gives rise to flavan 2,3-*trans*-3,4-*cis*-diols (leucopelargonidin). These compounds, also named leucoanthocyanidins, are the immediate precursors for the synthesis of catechins and proanthocyanidins. Catechins (afzelechin) are synthesized from leucoanthocyanidins by action of flavan 3,4-*cis*-diol reductase. Proanthocyanidins (propelargonidin B-3) are formed by a condensation of catechins and leucoanthocyanidins.

The reaction steps from leucoanthocyanidins to anthocyanidins (pelargonidin) are still unknown. An obligatory reaction in the sequence is a glycosylation, usually a glucosylation, in the 3 position of the anthocyanidin or of a suitable intermediate. This reaction leads to the first stable anthocyanin (e.g. pelargonidin 3-glucoside).

Modification by hydroxylation of the A- and, in particular, the B-ring, methylation of hydroxyl groups as well as glycosylation and acylation reactions result in the immense diversity of flavonoids found in nature. Numerous enzymes catalysing these modifications have been described. Some enzymes can act on both intermediates (flavanone or dihydroflavonol) and end products (flavone, isoflavone, flavonol or anthocyanidin 3-glycoside), others exclusively on the end products.

As a result of extensive genetic studies in a wide variety of plants, mutants are available for each step in flavonoid biosynthesis from chalcone formation up to complex modifications of the anthocyanin molecule. In recent years, this immense amount of genetic information has increasingly been used in the elucidation of flavonoid biosynthesis. Flowers of genetically defined plants have proved to be very valuable for supplementation experiments with potential precursors, as well as for correlating single genes with particular enzymes. Such correlations clearly establish that an enzyme capable of catalysing a particular step *in vitro* has the same function *in vivo*.

Figure 1 Scheme illustrating the pathways to phenylalanine and acetyl-CoA, and the following reaction steps leading to the various flavonoids classes. The enzymes marked with numbers are listed in Table 2

Table 19   List of Enzymes Leading to Various Flavonoid Classes.

Enzymes of the preflavonoid pathways are marked with the roman numerals, enzymes of the flavonoid pathway with arabic numerals.

Enzymes
Non-flavonoid precursors
I   Acetyl-CoA carboxylase
II   Phenylalanine ammonia-lyase
III   Cinnamate 4-hydroxylase
IV   4-Coumarate : CoA ligase
Flavonoid classes
1   Chalcone synthase
2   Chalcone isomerase
3   2-Hydroxyisoflavanone synthase
4   Flavone synthase
5   (2*S*)-Flavanone 3-hydroxylase
6   Flavonol synthase
7   Dihydroflavonol 4-reductase
8   Flavan-3, 4-*cis*-diol 4-reductase
9   Anthocyanidin/flavonol 3-*O*-glucosyltransferase

2.   Biosynthesis of Isoflavonoids

The isoflavonoids share a common biosynthetic pathway with the flavonoids as far as chalcone-flavanone intermediates, but then a 1,2-aryl migration occurs to produce the rearranged 3-phenylchroman skeleton that differentiates isoflavonoids from other flavonoids. The last decade or so has seen significant progress towards our understanding of isoflavonoids biosynthesis, and the main reason for this must lie in exploitation of the fact that some isoflavonoids are produced in plant tissues as stress metabolites or phytoalexins. Their production can be induced by treatment of plants, or plant parts, with fungi, abiotic inducers or fungal elicitors. This leads to rapid synthesis over a short period of time, during which labelled precursors can be applied,

resulting in high incorporations and allowing the use of modern stable isotope methodologies. Similarly, enzymes catalysing parts of the pathway can be found at much higher levels of activity during the stress period. Research efforts into isoflavonoid biosynthesis have been expended in two main areas, how the various isoflavonoid classes are interrelated, and secondly the mechanism of the unusual aryl migration (Dewick, 1988).

4.2.1 Biogenetic Relationships Among the Isoflavonoids

As a result of many studies in several plant systems, a scheme (Figure 2) interrelating ten of the known classes of naturally occurring isoflavonoids can be present (Dewick, 1982).

Figure 2 General scheme of isoflavonoid biosynthetic relationships.

Part II : Pharmacognostic Specification

Leaf Measurements

1. Palisade Ratio

1.1 Definition of Terms Used

Palisade Cells are a type of photosynthetic cells of the mesophyll of a leaf occurring mostly just beneath the upper epidermal surface layer (Esau, 1972). The cells are elongated and more or less cylindrical and arranged in one or more rather regular, relatively compact layers near the ventral, or upper side of the leaf with the long axis of the cells perpendicular to the leaf surface (Eames and MacDaniels, 1974).

Palisade Ratio is the average number of palisade cells beneath each upper epidermal cell (Evans, 1989). It is obtained by counting the total number of palisade cells beneath four contiguous upper epidermal cells and dividing the number by four (Youngken, 1950).

1.2 History

The determination of palisade ratio was introduced by Zorning and Weiss in 1925 in their studies of the Compositae (Trease and Evans, 1978). They stated that the average number of palisade cells beneath an upper epidermal cell was of diagnostic value. Although the number of palisade cells per unit area increased successively from the base of the leaf to the apex, but since there was a corresponding diminution in the area of the epidermal cells, the ratio remained almost constant (Zorning and Weiss, 1925 quoted in Youngken, 1950).

In 1973 Wallis and Dewar used the measurement to distinguish some different varieties of buchu. They found it possible to distinguish *Barosma pulchella*, *B. venusta*, *B. ovata* and *B. peglerae* from *B. betulina*. They introduced the term "palisade ratio" as a figure obtained by counting the total number of palisade cell beneath four upper epidermal cells and dividing the number by four (Evans, 1989).

Wallis and Forsdike, in their investigation of the palisade ratio of *Atropa belladonna* Linn., *Scopolia corniolica* Jacquin and *Solanum nigrum* Linn., found that the palisade ratio did not change with the age of the leaf, the habitat of the plant, or

from year to year within either of these species (Wallis and Forsdike, 1938 quoted in Youngken, 1950).

Figures for senna have been published by George in 1943 and 1946 (Evans, 1989). He determined the palisade ratio ranges for the upper and lower epidermises of Alexandrian and Tinnevelly Senna leaflets. He showed that the lower epidermis of *Cassia acutifolia* Delile. and the upper epidermis of *C. angustifolia* Vahl. both have palisade ratios very near 7.5, that the upper epidermis of *C. acutifolia* Delile. has a palisade ratio of 9.5 and the lower epidermis of *C. angustifolia* Vahl. has a palisade ratio of 5.0, and that the identity of a powder of either species of Senna can be established from the mean of 20 to 30 palisade ratio determinations on epidermal fragments, a value above 7.5 indicating *C. acutifolia* Delile and a value below, *C. angustifolia* Vahl. (George, 1943 quoted in Youngken, 1950).

The development of the palisade tissue depends largely upon light intensity. There may, therefore, be great variation in the proportion and arrangement of the palisade parenchyma in the same species growing under different conditions (Eames, 1974). However, it is evidently found that the palisade cells of the mesophyll bear a definite relation to the epidermal cells and the palisade ratio is sufficiently constant to serve as a diagnostic character of species (Wallis, 1960).

The palisade ratio can be used for the determination of quite fine powders (Evans, 1989).

2. Stomatal Number and Stomatal Index

2.1 Definition of Terms Used

Stomata are the openings in the epidermis through which gaseous interchange takes place between the intercellular spaces of the subepidermal cells and the atmosphere. These openings are spaces between two specialized cells known as guard cells. The term "stoma" is also applied to the opening in the epidermis plus the surrounding guard and subsidiary cells (Eames and MacDaniels, 1974). In the latter case the term "stomatal apparatus" is preferred.

Stomatal Number is the average number of stomata per square millimetre of epidermis. In recording results the range as well as the average value should be recorded for each surface of the leaf and the ratio between the two surfaces (Evans, 1989).

Stomatal Index is the percentage proportion of the ultimate divisions of the epidermis of a leaf which have been converted into stomata (Evans, 1989).

$$\text{Stomatal Index (I)} = \frac{S}{E + S} \times 100$$

Where   S = number of stomata per unit area

and   E = number of ordinary epidermal cells in the same unit area.

2.2   History

The stomata are most common on green aerial parts of plants particularly the leaves. In green leaves they occur either on both surfaces or on one only, either the upper or more commonly the lower (Esau, 1972). The number of stomata per square millimetre of epidermis is different in different plants. It is apparently related to the humidity of the environment; variations in amount of light seem to have no effect (Salisbury, 1927 quoted in Eames, 1974). The density of stomata has been established as 100 to 300 per square millimetre for leaves of many species (Stalfelt, 1956 quoted in Esau, 1972).

The investigations of Timmerman indicated that stomatal number varies considerably with the age of the leaf, thus the actual number of stomata per square millimetre is variable for the same plant if records are made for different years (Timmermann, 1927 quotedin Wallis, 1960). It is also indicated that stomatal numbers are usually useless for distinguishing between closely allied species, but that in certain cases the ratio between the number of stomata on the two surface may be of diagnostic importance. It is possible, for example, to distinguish *Datura innoxia* from other species of *Datura* as follows (Evans, 1989).

Species	Upper Surface		Lower Surface		Ratio
	Range	Mean	Rang	Mean	Lower / Upper
D. stramonium	59-140	87	145-254	200	2.3
D. tatula	93-175	126	155-331	208	1.65
D. laevis	108-115	111	188-215	201	1.80
D. innoxia	82-172	41	105-223	165	1.17

In 1925, Salisbury indicated that the number of stomata increases toward the apex and margin of the leaf where the cells decrease in size, the proportion of stomata to epidermal cells remaining the same (Salisbury, 1927 quoted in Eames and MacDaniels, 1974). He also showed that a high correlation coefficient exists between the number of stomata and the number of epidermal cells per unit area of leaf surface of a given species. He proposed the following formula for the calculation of the stomatal index :

$$\text{Stomatal Index (I)} = \frac{S}{E + S} \times 100$$

where S is the number of stomata per unit area and E is the number of ordinary epidermal cells in the same unit area. The stomatal index expresses the percentage proportions of the ultimate divisions of the epidermis of a leaf which have been converted into stomata (Youngken, 1950).

Whilst stomatal number varies considerably with the age of the leaf, stomatal index is highly constant for a given species and may be determined on either entire or powdered samples (Evans, 1989).

Rowson showed that stomatal index values may be used to distinguish between leaves of co-generic species (Rowson, 1943 quoted in Wallis, 1960 ; Evans, 1989).

Species	Stomatal index	
	Upper surface	Lower surface
Atropa acuminata	1.7 to **4.8** to 12.2	16.2 to **17.5** to 18.3
Atropa belladonna	2.3 to **3.9** to 10.5	20.2 to **21.7** to 23.0
Cassia senna	11.4 to **12.4** to 13.3	10.8 to **11.8** to 12.6
Cassia angustifolia	17.1 to **19.0** to 20.7	17.0 to **18.3** to 19.3
Datura inermis	18.1 to **18.3** to 18.7	24.5 to **24.9** to 25.3
Datura metel	12.7 to **17.4** to 19.4	21.2 to **22.3** to 23.9
Datura stramonium	16.4 to **18.1** to 20.4	24.1 to **24.9** to 26.3
Datura tatula	15.6 to **20.2** to 22.3	28.3 to **29.8** to 31.0

Digitalis lanata	13.9 to **14.4** to 14.7	14.9 to **16.1** to 17.6
Digitalis lutea	2.5 to **5.5** to 8.4	21.6 to **22.9** to 25.2
Digitalis purpurea	1.6 to **2.7** to 4.0	17.9 to **19.2** to 19.5
Digitalis thapsi	5.9 to **7.0** to 7.8	11.9 to **12.4** to 13.5
Erythroxylum coca	Nil	12.2 to **13.2** to 14.0
Erythroxylum truxillense	Nil	8.9 to **10.1** to 10.7
Phytolacca acinosa	Nil	**15.0**
Phytolacca americana	2.9 to **4.2** to 5.7	13.0 to **13.2** to 13.4

Forsdike showed that stomatal index values were used to distinguish those of Alexandrian senna (Forsdike, 1949 quoted in Wallis, 1960 ; Trease and Evans 1978).

Stomatal index is employed in the European Pharmacopoeia, 1969, to distinguish leaflets of Indian and Alexandrian sennas (Evans, 1989).

3   Vein-Islet Number

3.1   Definition of Terms Used

Vein-Islet   is the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands of a leaf (Evans, 1989).

Vein-Islet Number   is the number of vein-islets per square millimetre of leaf surface calculated from four contiguous square millimetres in the central part of the lamina, midway between the midrib and the margin. The result should be given to the nearest 0.5 (Trease and Evans, 1978).

3.2   History

The number of vein-islets per unit area of leaf surface is constant for any given species of plant and can be used as a character for the identification of species (Wallis, 1960).

The term "vein-islet" was first introduced in 1915 by Benedict, who defined it as the size of aggregation of photosynthetically active cells surrouning by the veinlets. He suggested that the average vein-islet area is of physiological significance (Benedict, 1915 quoted in Wallis, 1960).

The vein-islets increase in size as the leaf matures, their growth being a part of the general growth throughout the leaf. In full growth leaf of any one species, the number of vein-islets per unit area of leaf surface is apparently fairly constant, regardless of the size of the leaf or the age of the individual plant (Ensign, 1919 quoted in Pisetpakasit 1976 ; Levin, 1927 quoted in Wallis, 1960).

Levin determined the vein-islet numbers of a number of species of senna, coca, digitalis and buchu leaves. Dewar has subsequently determined the value for various species of *Digitalis*. The vein-islet number frequently serve to distinguish closely related plants. In the case of the *Barosma* species it will be noted that *B. serratifolia* and *B. bathii*, which cannot be distinguished from *B. betulina* by their palisade ratios, are distinguished from the official leaves by their vein-islet numbers. The values of vein-islet number that determined by Levin are follows (Levin, 1927 quoted in Trease and Evans, 1978).

		Range of veinislet numbers
(a) Senna	*Cassia senna*	15 to **26** to 29.5
	Cassia angustifolia	19.5 to **21** to 22.5
(b) Coca	*Erythroxylum coca*	8 to **11** to 12
	Erythroxylum truxillense	15 to **20** to 26
(c) Digitalis	*Digitalis purpurea*	2 to **3.5** to 5.5
	Digitalis lanata	2 to **2.7** to 3.5
		3 to **4.4** to 8
	Digitalis lutea	1 to **1.2** to 1.5
	Digitalis thapsi	8.5 - 16
(d) Buchu	*Barosma bathii*	15 to **16.8** to 20
	Barosma serratifolia	9 to **16.6** to 24
	Barosma crenulata	10 to **13.0** to 16.5
	Barosma betulina	10 to **12.7** to 15
	Barosma ovata	15 - 20
	Barosma pulchella	6 to **7.2** to 8.5
	Barosma venusta	5 to **6.0** to 7

The shape of the vein-islets is also frequently characteristic and will often enable one to sort out a mixture of leaves which have been broken into small fragments (Wallis, 1960). Forsdike showed that the appearance of leaf venation under a hand lens, when viewed by transmitted, and by reflected light, may be used to distinguish medicinal leaves from their common adulterants (Forsdike, 1949 quoted in Wallis, 1960).

4. Veinlet Termination Number

4.1 Definition of Term Used

A Veinlet Termination is a small vein-tip running out from the surrounding veinlets into the centre of each vein-islet (Wallis, 1960). It is the ultimate free termination of a veinlet or branch of a veinlet (Evans, 1989).

Veinlet Termination Number is the number of veinlet terminations per square millimetre of leaf surface (Evans, 1989).

4.2 History

The number of veinlet terminations per unit area of leaf surface is of diagnostic value in certain cases. Hall and Melville who defined the term "veinlet termination" as the ultimate free terminatin of a veinlet or branch of a veinlet, suggest that the number of veinlet terminations per square millimetres of leaf surface may be used to differentiate coarse powders of certain leaves belonging to co-generic species. They have found veinlet termination number useful in distinguishing between Peruvian and Bolivian coca leaves and between Alexandrian and Tinnevelly senna leaflets (Hall and Melville, 1951 quoted in Trease and Evans, 1978). They also indicated that the veinlet termination number is not significantly dependent on the position at which it is determined (Hall and Melville, 1954 quoted in Pisetpakasit, 1976). The values of veinlet termination number that they distinguished are follows (Evans, 1989).

Erythroxylum truxillense	23.1 - 32.3	*Atropa belladonna*	6.3 - 10.3
Erythroxylum coca	16.8 - 21.0	*Atropa acuminata*	1.4 - 3.5
Cassia senna	32.7 - 40.2	*Digitalis purpurea*	2.5 - 4.2
Cassia angustifolia	25.9 - 32.8	*Hyoscyamus niger*	12.4 - 19.0
		Datura stramonium	12.6 - 20.1

Thin-Layer Chromatography (TLC)

1.   **Introduction to TLC** (Sherma, 1991)

TLC, which together with paper chromatography comprise "planar" or "flat-bed" chromatography, is the simplest of all of the widely used chromatographic methods to perform. A suitable closed vessel containing solvent and a coated plate are all that are required to carry out separations and qualitative and semiquantitative analysis. With optimization of techniques and materials, highly efficient separations and accurate and precise quantification can be achieved. TLC can be used also for preparative-scale separations by employing specialized apparatus and techniques.

Basic TLC is carried out as follows. An initial zone of mixture is placed near one end of the stationary phase, a thin layer ; the sample is dried ; and the end of the stationary phase with the initial zone is placed into a mobile phase, usually a mixture of pure solvents, inside a closed chamber. The components of the mixture migrate at different rates during movement of the mobile phase through the stationary phase, which is termed the development of the chromatogram. When the mobile phase has moved an appropriate distance, the stationary phase is removed, the mobile phase is rapidly dried, and the zones are detected by application of a suitable visualization reagent.

Differential migration is the result of varying degrees of affinity of the mixture components for the stationary and mobile phases. Different separation mechanisms are involved, the predominant forces depending on the exact nature of the two phases and the solutes. The interactions involved in determining chromatographic retention and selectivity include hydrogen bonding, eletron-pair donor/electron-pair acceptor (charge transfer), ion-ion, ion-dipole, and van der Waals interactions. Among the latter are dipole-dipole, dipole-induced dipole, and instantaneous dipole-induced dipole interactions.

Sample collection, preservation, and purification are problems common to TLC and all other chromatographic methods. For complex samples, the TLC development will usually not completely resolve the analyte (the substance to be determined) from interferences unless a prior purification is carried out. This is most often done by selective extraction and column chromatography. In some cases,

substances are converted, prior to TLC, to a derivative that is more suitable for separation, detection, and/or quantification than the parent compound.

Detection is most simple when the compounds of interest are naturally colored or fluorescent or absorb ultraviolet (UV) light. However, application of a location or visualization reagent by spraying or dipping is usually required to produce color or fluorescence for most compounds. Absorption of UV light is common for many compounds, e.g., aromatics and those with conjugated double bonds. This leads to a simple, rather universal detection method on layers impregnated with a fluorescence indicator (fluorescence quench detection).

Compound identification in TLC is based initially on Rf values compared to authentic standards. Rf values are generally not exactly reproducible from laboratory to laboratory or even in different runs in the same laboratory, so they should be considered mainly as guides to relative migration distances and sequences. Factors causing Rf values to vary include : dimensions and type of the chamber, nature and size of the layer, direction of mobile-phase flow, the volume and composition of the mobile-phase, equilibration conditions, humidity, and sample preparation methods preceding chromatography. Further characterization of separatd substances can be obtained by scraping the layer and elution of the analyte followed by infrared (IR), nuclear magnetic resonance (NMR), or mass spectrometry (MS).

2.   History of TLC

The history of liquid chromatography, which dates back to the first description of chromatography by Michael Tswett in the early 1900s, was reviewed by Sherma (Sherma, 1977 quoted in Sherma and Fried, 1991) Stahl (Stahl, 1969 quoted in Sherma and Fried, 1991 ; Stahl, 1979 quoted in Sherma and Fried, 1991), Kirchner (Kirchner, 1978 quoted in Sherma and Fried, 1991 ; Kirchner, 1980 quoted in Sherma and Fried, 1991), and Pelick et al. (Pelick et al., 1966 quoted in Sherma and Fried, 1991) have reviewed the history of TLC. TLC is a relatively new discipline, and chromatography historians usually date the advent of modern TLC from 1958. The review by Pelick et al. tabulates significant early developments in TLC and provides translations of classical TLC studies by Izmailov and Schraiber and by Stahl. In 1938, Izmailov and Schraiber separated certain medicinal compounds on unbound alumina spread on glass plates. Since they applied drops of solvent to the plate containing the sample and sorbent layer, their procedure was called "drop chromatography." Meinhard and Hall in 1949 used a binder to adhere alumina to microscope slides, and

these layers were used in the separation of certain inorganic ions using drop chromatography. In the early 1950s, Kirchner and colleagues at the U.S. Department of Agriculture developed TLC as we know it today. They used sorbents held on glass plates with the aid of a binder, and plates were developed with conventional ascending procedures used in paper chromatography. Kirchner coined the term "chromatostrips" for his layers. Stahl introduced the term "thin-layer chromatography" in the late 1950s. His major contributions were the standardization of materials, procedures, and nomenclature and the description of selective sovent systems for resolution of important compound classes. His first laboratory manual popularized TLC, and he obtained the aid of chemical manufacturers in offering standard materials for TLC (Stahl, 1962 quoted in Sherma and Fried, 1991). Other early books that had great influence on the development of TLC include those by Kirchner (Kirchner, 1967 quoted in Sherma and Fried, 1991), Bobbitt (Bobbitt, 1963 quoted in Sherma and Fried, 1991), Randerath (Randerath, 1963 quoted in Sherma and Fried, 1991), and Truter (Truter, 1963 quoted in Sherma and Fried, 1991).

Quantitative TLC was introduced by Kirchner *et al.* in 1954 (Kirchner, Miller and Rice, 1954 quoted in Sherma and Fried, 1991) when they described an elution method for determination of biphenyl in citrus fruits and products. Densitometry was first used for direct measurement of bands separated by means of electrophoresis and was later used on paper chromatograms. Densitometry in TLC was initially reported in the mid-1960s by Dallas *et al.* (Dallas, Barret and Padley, 1964 quoted in Sherma and Fried, 1991) using the Joyce Loebl Chromascan and by Genest (Genest, 1965 quoted in Sherma and Fried, 1991) and Thomas *et al.* (Thomas, Scharoun and Ralston, 1965 quoted in Sherma and Fried, 1991) using the Photovolt densitometer. A symposium on quantitative TLC held in 1968 in Great Britain led to the first book published on this topic (Shellard, 1968 quoted in Sherma and Fried, 1991).

High-performance TLC plates (Halpaap and Ripphahn, 1977 quoted in Sherma and Fried, 1991) were produced commercially in the mid-1970s and provided impetus for the improvements in practice and instrumentation that occurred in the late 1970s and 1980s and led to the methods termed "high-performance TLC (HPTLC)" (Zlatkis and Kaiser, 1977 quoted in Sherma and Fried, 1991) and "instrumental HPTLC" (Bertsch *et al.*, 1980 quoted in Sherma and Fried, 1991). Centrifugally accelerated preparative-layer chromatography (Harrison, 1979 quoted in Sherma and Fried, 1991) and forced-flow techniques in TLC (overpressured layer chromatography,

OPLC) (Tyihak, Mincsovics and Kalasz, 1979 quoted in Sherma and Fried, 1991) were introduced in the late 1970s.

These and other high-performance and quantitative methods have caused a renaissance in the field of TLC. There is no doubt that TLC will continue to evolve and grow in the 1990s and beyond as a highly selective, sensitive, quantitative, rapid, and automated technique for analysis of all types of samples and analytes, and for preparative separations. To keep abreast of this inevitable progress in TLC, the biennial review of advances in theory, practice, and applications by Sherma (Sherma, 1988 quoted in Sherma and Fried, 1991) is indispensible.

3. Two-Dimensional Thin-Layer Chromatography

Two-dimensional development is particulary valuable for mixtures of many components (Stahl, 1969). If the components of a mixture are not completely separated by development in a single direction, it may be possible to resolve them by this method (Randerath, 1968).

It must be stressed that the factors that determine the reproducibility of results in the one dimensional method have an even greater effect in the two-dimensional method (Randerath, 1968). The major point of variation in this technique is what is done to the solvent system or to the layer between the two developments (Robbit, 1963). In order to obtain reproducible results, the layer must always be treated in exactly the same way before development in the second direction. Thus, for instance, the conditions of the intermediate drying must never be altered (Randerath, 1968).

If very small quantities of substance have to be detected, it should be noted that the lower limits of detection are higher for two-dimensional than for one-dimensional chromatograms, since diffusion effects cause greater dilution of the substance in the longer development time of the two-dimensional method. Nevertheless, the detection sensitivity is still considerably greater than on a two-dimensional paper chromatogram. A further substantial advantage is that two different separation principles (e.g. adsorption and partition chromatography or partition chromatography and electrophoresis) can be combined in the two-dimensional technique (Randerath, 1968).