CHAPTER 2

HISTORICAL

A. Chemical Constituents of the *Sophora* Linn.

Studied on the chemical constituents from the genus *Sophora* began 50 years ago. There are 2 main groups of chemicals isolated from the *Sophora.*. The first group of compounds was flavonoids , in addition *Sophora* was one of the genus which enriched of various kinds of flavanone. The second group of compounds was quinolizidine alkaloids.

The distribution of alkaloids in the genus *Sophora* is shown in Table 1.

Table 1 Distribution of *Sophora* alkaloids

Plant	Alkaloids	Reference
Sophora *alopecuroides* Linn. (entire plant)	aloperine, allylaloperine	Zhao (1980)
	13,14-dehydrosophoridine	Kuchkarov *et al* (1978)
	13,14-dehydrosophoridine N-oxide	Kuchkarov *et al* (1977)
	N-methylaloperine,	Zhao (1980)
	N-methylcytisine,	
	matrine,	
	sophocarpine,	
	oxysophocarpine	Qi et al (1989)
	sophocarpine N-oxide	Zhao (1980)
	sophoramine	Bain,and Toda (1988)
S. alopecuroides	baptifoline	Monakhova *et al* (1973)
	3 -hydroxysophoridine	Monakhova *et al.*(1976)
	matrine N-oxide	Plekhanova,and Smirnova

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora alopecuroides Linn. (aerial parts)	N-(2-hydroxyethyl) Cytisine	Monakhova *et al* (1973)
	N-hydroxysophoridine	Kuchkarov *et al* (1974)
	neosophoramine	Monakhova *et al* (1974)
	pachycarpine	Phekhanova, and Smirnova (1965)
	sophoridine	Zhao (1980)
	sophoridine N-oxide	Kuchkarov, and Kushmuradov (1979)
	sophorine	Southon, and Buckingham (1989)
	tricrotonyltetramine	Monkhova *et al* (1974b)
S.alopecuroides Linn (roots)	matrine, oxymatrine, oxysophocarpine, sophocarpine, sophoridin	Cui, and Zhang (1986)
S.alopecuroides Linn (leaves)	aloperine, matrine, sophocarpine, sophoramine sophoridine	Orekhov *et al* (1938)
S.alopecuroides Linn (seeds)	aloperine	Orekhov *et al.*(1935)
	cytisine	Qi *et al.*(1989)
	matrine	Orekhov *et al* (1935)
	matrine N-oxide	Plekhanova, and Smirnova (1965)
	oxymatrine, oxysophocarpine	Qi *et al.* (1989)
	pachycarpine	plekhanova, and smirnova (1965)
	sophocarpine, sophoridine	Orekhov *et al.* (1935)
S. alopecuroides Linn. (stems)	aloperine, matrine, sophocarpine, sophoridine	Zhao *et al.*(1984)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora arizonica S. Wats (seeds)	anagyrine, cytisine, N-methyl cytisine	Murakoshi *et al.*(1993)
S. chrysophylla Seem. (leaves,stems)	ammodendrine, (-)-anagyrine, kuraramine, (-)-lupanine, 5,6-dehydrotupanine, (+)-matrine, (+)-matrine N-oxide, (-)-pohakuline	Murakoshi *et al.*(1984)
S.chrysophylla Seem. (seeds)	anagyrine, cytisine, matrine, matrine N-oxide, N-methyl cytisine (-)-rhombifoline sophocrysine	Hoeneisen *et al* (1993) Murakoshi *et al* (1984) Briggs,and Russell(1942)
S.chrysophylla Seem. (stems)	trilampholobine, lampholobine, (+)-mamarine, (+)-matrine N-oxide, (-)-N-formylcytisine, (-)-N-methylcytisine	Murakashi *et al.*(1984)
S.chrysophylla Seem. (barks)	(+)-matrine, (-)-pohakuline	Murakoshi *et al* (1984)
S.chrysophylla Seem. (leaves)	(-)-mamanine N-oxide, 17-oxolupanine	Murakoshi *et al* (1984)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora denudata Bory (seeds)	anagyrine,cytisine heptifoline, (-)-matrine N-methylcytisine,oxymatrine	Favgeras *et al* (1973)
S.exigua Craib (roots)	(-)-anagyrine, (-)-12-autamide cytisine, (-)-baptifoline, (-)-cytisine, (+)-5,6-dehydroluparine, (-)-12-hydroxycytisine, luparine,N-formylcytisine, N-methylcytisine	Takamatsu *et al* (1991)
S.flavescens Ait (aerial parts)	anagyrine, baptifoline, (-)-7,8-dehydrosophoramine (-)-13,14-dehydrosophoridine, (+)-9-hydroxymatrine, (-)-9-hydroxysophoramine, (+)-lehmannine matrine, matrine N-oxide, (-)-N-methylcytisine oxymatrine (-)-sophocarpine, (-)-sophoramine, sophoranol (-)-sophoridine	Ueno *et al.*(1978b) Murakoshi *et al* (1982a) Ueno *et al.*(1978b) Wei *et al.*(1988) Ueno *et al.*(1978b) Murakoshi *et al.*(1982a)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora flavescens Ait. (roots)	(+)-allomatrine, (+)-matrine, (+)-matrine N-oxide oxymatrine oxysophocarpine (+)-sophocarpine N-oxide, (-)-sophoramine, (+)-sophoranol sophoridine	Murakoshi *et al.*(1982a) Bai *et al.* (1982) Cui,and Zhang(1986) Murakoshi *et al.* (1982a) Bai *et al* (1982)
S.flavescens Ait (flowers)	(-)-anagyrine, (-)-baptifoline, (-)-7,11-dehydromatrine, (+)-5,9-dihydroxymatrine, (-)-9-hydroxysopramine, iskuraramine (+)-kuraramine lupanine, (+)-mamanine, (+)-matrine, (+)-matrine N-oxide, (-)-rhombifoline, (+)-sophocarpine N-oxide, (-)-sophoramine, (+)-sophoranol, (+)-sophoranol N-oxide	Murakoshi *et al.*(1982a) Murakoshi *et al.*(1981) Murakoshi *et al.*(1982a)
S.flavescens Ait. (seeds)	(-)-anagyrine, (-)-baptifoline, (+)-5,2-dihydroxymatrine (+)-9-hydroxymatrine, (+)-matrine, (-)-N-methylcytisine, (+)-sophocarpine N-oxide	Murakoshi *et al.*(1982a)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora flavescens var. *angustifolia* Sieb et Zucc. (seeds)	anagyrine, baptifoline (+)-5,2-dihydroxy matrine, (+)-9-hydroxy matrine (-)-5-hydroxy-sophocarpine leontabinine, (+)-leontalbinine N-oxide, matrine,matrine N-oxide N-methylcytisine, (-)-sophocarpine, (+)-sophocarpine N-oxide, (+)-sophoranol N-oxide,	Cai *et al.*(1990) Sekine *et al* (1993) Saito *et al.*(1990) Sekine *et al.*(1993) Cai *et al.*(1990)
S.flavescens var. *angustifolia* Sieb et Zucc.(callus tisuve)	anagyrine, 5,6-dehydrolupanine, matrine, (+)-sophoranol N-oxide	Saito *et al.*(1989)
S.franchetiana Dunn (leaves,seeds,stems, aerial parts)	(-)-anagyrine, (-)-N-formyl cytisine, (-)-rhombifoline, (-)-tsukushinamine A	shiniya *et al* (1981)
S.franchetiana Dunn (aerial parts)	(+)-ammodendrine, matrine,(-)-baptifoline, cytisine, (-)-tsukushinamine B, tsukushinamine C	Ohimiya *et al* (1981)
S.japonica Linn. (seeds)	anagyrine,cytisine,matrine, matrine N-oxide,	Abdusalamov *et al*(1972)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora japonica Linn. (seeds)	N-methyl cytisine, sophocarpine stizolamine	Yostrida,and Hasegawa (1977)
S.japonica Linn. (leaves)	lupanine	Winte *et al* (1983)
S.leachiana M.E.Peck (seeds)	anagyrine, cytisine, N-methyl cytisine	Iinuma *et al* (1991)
S.mollis R.Grah (leaves,stems)	ammodendrine, (-)-anagyrine,(-)-baptifoline, (-)-cytisine, 5,6-dehydrolupanine, (-)-N-formylcytisine, (-)-N-melhylcytisine, rhombifoline,(+)-sparteine	Murakoshi *et al* (1982b)
S.moorcroftiana Benth ex Baker (seeds)	anagyrine, cytisine, N-methylcytisine	Mimaki *et al*.(1991)
S.moorcroftiana Benth ex Baker (aerial parts,flowers,leaves)	(-)-matrine,oxymatrine, matrine N-oxide, sophocarpine	Cui,and Zhang(1986)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora pachycarpa C.A.May(entire plants)	anabasine, cytisine, goebeline matrine, matrine N-oxide, N-metlyl cytisine pachycarpine sophocarpine, sophocarpine N-oxide, sophoramine	Markman,and Glvshenkova (1963) Zainutdinov *et al.* (1968) Aslanov *et al.*(1966) Zainutdinov *et al.*
S.pachycarpa C.A.May (seeds)	ammothamnine,cytisine pachycarpidine matrine,matrine N-oxide sophocarpidine, sophocarpine sophoramine	Aslanov *et al* (1966) Zainutdinov *et al.* (1968) Southon,and Buckingham (1989) Zuinutdinov *et al* (1968)
S.pachycarpa C.A.May (roots)	pachycarpine sophocarpidine, sophocarpine	Orekhov *et al.*(1933) Orekhov *et al.*(1934)
S.prodanii E.Anders (roots)	allantotin cytisine sophoridine sophocarpine, sparteine	Constantinescu *et al* (1969) Paslarasu and Padautatocam(1973) Pislarasu, and Dragut (1978)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora secundiflora . Lag.ex DC.(seeds)	anagyrine, cadaverine cytisine, 5-dehydrolupanine, epilupanine, N-methylcytisine pachycarpine sparteine thermopsine	Keller,and Hatfield (1979) Keller,and Hatfield (1979) Abdel Baky (1989) Keller,and Hatfield (1979) Izaddoost *et al.*(1976)
S.secundiflora Lag ex DC.(leaves)	(-)-anagyrine, (-)cytisine,(-)-N-acetylcytisine, (-)-N-formylcytisine, N-methylcytisine, (-)-N-methylcytisine sparteine	Murakushi *et al.*(1986) Abdel-Baky,and Makboul (1985)
S.secundiflora Lag. ex DC.(stems)	anagyrine,cytisine 5,6-dehydrolupanine, lupanine, N-acethylcytisine, N-formylcytisine N-methylcytisine, oxosparteine, sparteine	Chavez,and Sullivan (1984)
S.subprostrata hChun et TC.chen (roots)	anagyrine matrine matrine N-oxide methytisine oxymatrine sophocarpine	Komatsu *et al.*(1970b) Qui,and Zhang.(1986) Takeshita *et al.*(1991) Komatsv *et al.*(1970b) Qui,and Zhang (1986) Chuang *et al.*(1983)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora tetraptera J.Mill (fruits)	ammodendrine,anagyrine, N-formylcytisine, rhombifoline	Kinghorn *et al.*(1982)
S.tetraptera J.Mill (leaves)	baptifoline,cytisine, matrine N-methylcytisine	Reyes *et al.*(1988)
S.tetraptera J.Mill (seeds)	cytisine,matrine N-methylcytisine sophochrysine	Urza,and Cassels (1970) Briggs,and Taylor (1938) Briggs,and Mangan (1948)
S.tomentosa Linn. (leaves,fruits,seeds, stems)	(+)-ammodendrine, (-)-baptifoline,(-)-cytisine, (-)-epilamprolobine, (+)-epilamprolobine N- oxide, (+)-matrine,(+)-matrine-N-oxide 5-(3-methoxycarbonylbutyroyl) aminomethyl-trans- quinolizidine, (-)-N-acethylcytisine,(-) -N-formylcytisine, (-)-N-methylcytisine,(+)- sophocarpine N-oxide	Murakoshi *et al.*(1981)
S.tomentosa Linn. (seeds)	stizolamine	yoshida,and Hasegawa (1977)
S.tonkinensis Gagnep. (roots)	(-)-cytisine,(+)-matrine, (+)-matrine N-oxide,(+)-sopho- carpine N-oxide,(+)- sophoramine,(+)-sophoranol	Dou *et al.*(1989)

Table 1 (continue)

Plant	Alkaloids	Reference
Sophora velutina Lindl. var *zinbabweensis* Gilleti& Brummitt (leaves)	cytisine, (+)-9-hydroxylamprolobine, (+)-lampropine	Asres *et al.*(1986)
S.viciifolia Hance (flowers,leaves)	oxidized sophocarpine, sophocarpine	Li,and Chang.(1981)

The distribution of flavonoids in the genus *Sophora* is shown in Table 2

Table 2 Distribution of flavonoids in the genus *Sophora* .

Plant	Flavonoids	Reference
Sophora alopecuroides Linn. (roots)	ammothamnidin, glabrol isobavachin leachianone A trifolirhizin vexibidin,rexibinol	Batirov *et al.*(1985) Yusupova *et al* (1984) Yusupova *et al.*(1984) Batirov *et al.*(1985)
S.chrysophylla Seem. (roots)	4,7-dihydroxyflavone, isoliquiritigenin, maackian, sophoraisoflavanone C, sophoraisoflavanone D, (-)-tuberusin	Shirataki *et al.*(1990)

Table 2 (continue)

Plant	Flavonoids	Reference
Sophora exigua Craib. (roots)	exiguaflavanone A, exiguaflavanone B exiguaflavanone C, exiguaflavanone D, exiguaflavanone exiguaflavanone F,maackian sophoraflavanone G 2',5,7-trihyodroxy-8-lavandulylflavanone	Ruangrungsi *et al* (1992) Ruangrungsi *et al* (1993) Ruangrungsi *et al* (1992) Ruangrungsi *et al* (1993)
S.flavescens Ait . (roots)	formononetin formononetin-7-0-B-0-Glucoside isokurarinone kuroridin, kuraridnol,kurarinol kushenin,kushenol A, kushenol B kushenol C,kushenol D kushenol E,kushenol F, kushenol G, kushenol H,kushenol I kushenol I,kushenol K, kushenol L kushenol M kushenol N,kushenol O maackian,(-)-maackian 5-0-methylkushenol C noranhydroicaritin	Kyogoku *et al* (1973a) Ji, and Daze (1991) Kyogoku *et al* (1973a) Ji , and Daze (1991) Kyogoku *et al* (1973 a) Mu *et al* (1985 a) Wu *et al* (1985 b) Wu *et al* (1985c) Wu *et al* (1986) Yamaki *et al.*(1990) Yagi *et al* (1989) Komatsu *et al* (1970d)

Table 2 (continue)

Plant	Flavonoids	Reference
Sophora flavescens Ait. (roots)	pterocarpin sophoraflavanone G trifolirhizin vexibinol xanthohumol	Furuya,and Ikuta (1968) Yamaki *et al* (1990) Yagi *et al* (1989) Yamahara *et al.*(1990) Komatsu *et al.*(1970d)
S.flavescens Ait . (leaves)	luteolin-7-o-β-Glucoside	Yamaki *et al* (1990)
S.flavescens var. *angustifolia* Sieb et Zucc.(leaves+stem)	apigenin-7-o-β-o-glucoside,cynaroside	Yamamoto *et al* (1992)
S.flavescens var. *angustifolia* Sieb et Zucc. (roots)	methylanhydroicaritin, kushenol I, sophoraflavanoneG,trifolirhizin	Yamamoto *et al.*(991)
S.franchetiana Dunn. (roots)	3-hydroxy-4-methoxy-8,9-methylenedioxy, pterocarrpan,(-)-maackian (+)-sophoraisoflavanone B, (-)-sophorapterocarpam A	Komatsu *et al* (1981b) Komatsu *et al* (1981a)
S.japonica Linn. (roots)	biochanin A,5,7-dihydroxy-3,4-methylenedioxy isoflavone,flemmichapparin B, irisolidone,(+)-maackian sophojaponicin	Komatsu *et al* (1976a) Shibata, and Nishikawa (1963)
S.japonica Linn. (wood)	biochanin A,biochanin A-7-β-D-gentiobioside,biochanin A-7-β-D-xyloxylglucoside,	Takeda *et al* (1977)

Table 2 (continue)

Plant	Flavonoids	Reference
Sophora japonica Linn. (wood)	irisolidone,irisolidone-7 -0-glucoside,maackian, pratensein,rutin,sissotrin	Takeda *et al.*(1977)
S.japonica Linn. (fruits)	genistein, kaempferol, kaempferol-3,7-diglucoside, sophorabioside,sophoraflavano- loside,sophoricoside	Szabo *et al.*(1967) Akhmedkhodzhaeva *et al* (1986)
S.japonica Linn. (flowers)	genistein, isorhamnetin-3-rutiroside kaempferol-3-rutinoside Quercetin rutin	Lui,and changling (1990) Kimura, and Yamada (1984) Ishida *et al.*(1987) Balbaa *et al.*(1974)
S.japonica Linn. (entire plants)	5,7,4-trihydroxyflavone -3-rhamno diglucoside, 5,4-dinydroxyisoflavone -7- diglucorhamnoside, genistien,genistein-7-β- D-cellobioside,kaempferol sophoraflavanoloside, sophoricoside	Ho *et al.* (1984)
S.leachiana M.E.Peck. (roots)	8-methylkaempferol-7-0-β- D-Glucoside leachianone B,leachianone C leachianone D, leachianoneE,maackian poriol,poriolin sophoraflavanone I	Iinuma,and Ohyama (1991) Iinuma *et al.* (1992) Iinuma *et al.* (1991) Iinuma,andOhyama(1991) Iinuma *et al.*(1992)

Table 2 (continue)

Plant	Flavonoids	Reference
Sophora mollis R.Grah. (heartwood)	Biochanin A	Jain, and Koul (1972)
S.moorcroftiana Benth ex.Baker. (seeds)	calycosin, euchrestaflavanone A, licoisoflavone A, licoisoflavanone B,(-)-maackiain,phaseolidin, sophoraflavanone B, sophoraflavanone G, sophoraflavanone H, sophoraflavanone I, sophoraflavanone I, sophoraflavanone A	Shirataki *et al.*(1991)
S.moorcroftiana Benth ex Baker. (roots)	licoisoflavanone B,(-)-maackiain, sophoraflavanoneB, sophoraflavanone G, sophoraisoflavanone A	Shirataki *et al.*(1988)
S.pachycarpa C.A.May. (leaves)	genistein-7-0-xyloglucoside	Sattikulav *et al.*(1983)
S.prostrata J.Buch. (leaves)	apigenin-7-0-rhamnosylglucoside, 7,4-dihydroxyflavone,	Markham (1973)

Table 2 (continue)

Plant	Flavonoids	Reference
*Sophora prostrata*J.Buch (leaves)	luteolin-7-0-rhamnosyl-glucoside, luteolin-7-0-trihydroxyflavone, 7,3,4--trihydroxyflavone-7-0-rhamnosylglucoside,vicenin-2	
S.secundiflora Lag. ex DC. (leaves)	apigenin,cosmosiin	Makboul, and Abadel Baley (1984)
	fisetin,5-deoxykaempferol kaempferol,kaempferol-3-0-rhamoglucoside, kaempforol-3-0-rhamnoside 3-methoxyquercetin quercetin,rutin	Hasen *et al.* (1987) Makboul, and Abdel-Baky (1984) Hasan *et al.*(1987) Makboul,and Abdel-Baky (1984)
S.secundiflora Lag.ex DC.(entrire plants)	(-)unanisoflavan, calycosin,liquiritigenin secondifloran	Minhaz *et al.*(1976) Minhaz *et al.*(1977)
S.subprostrata Chun et T.C. Chen. (roots)	2-[(7-hxdroxy-2,2-dimethyl -2H-benzopyran) -6-yl]-7-hydroxy-8-(3-methyl-2-butenyl) chroman-4-one	Kyogoku *et al.*(1973c)
	2-[{3-hydroxy-2,2-dimethyl-8 -13-methyl-2-butenyl)}chroman -6-yl]-7-hydroxy-8-(3-methyl -2-butenyl)-8-(3-methyl chroman-4-one,	Kyogoku *et al.*(1973b)

Table 2 (continue)

Plant	Flavonoids	Reference
Sophora subprostrata Chun et T.C. Chen. (roots)	2-[{2-(1-hydroxy-1-methyl ethyl)-7-(3-methyl-2-butenyl)-2 ,3-dihydrobenzofuran}-5 -yl]-7-hydroxy-8-(3-methyl -2-butenyl)chroman-4-one	Kyogoku *et al.*(1973b)
	l-maackiain	Komatsu *et al.*(1970a)
	l-pterocarpin	Shibata,and Nisshikawa (1963)
	sophoradin	Komatsu *et al.*(1970b)
	sophoradochromene	Komatsu *et al.*(1970c)
	sophoraflavone A, sophoraflavone B	Shirataki *et al.*(1986)
	sophoranochromene	Komatsu *et al.*(1970c)
	sophoranone	Komatsu *et al.*(1970c)
	trifolirhizin	Shibata, and Nishikawa (1963)
	(-)-trifolirhizin 6-monoacetate	Komatsu *et al.*(1976b)
	2,4,7-trihydroxy-6,8-bis (3-methyl-2-butenyl) flavanone	Kyogoku *et al.*(1973b)
S. tetraptera J.Mill. (leaves)	apigenin-7-0-glucoside, apigenin-7-0- rhamnosylglucoside, apigenin-7-0- rhamnosylglucoside-4-0- glucoside, 7,4-dihydroxyflavone, 7,4-dihydroxyflavone-7-0- glucoside, 7,4-dihydroxyflavone-7-0- rhamnosylglucoside, luteolin-7-0-rhamnosylglucoside	Markham (1973)
	quercetin-3-0-galactoside,rutin	Reyes *et al.*(1988)

Table 2 (continue)

Plant	Flavonoids	Reference
Sophora tetraptera J.Mill (leaves)	7,3,4-trihydroxyflavone, 7,3,4-trihydroxyflavone -7-0-glucoside, 7,3,4-trihydroflavone-7-0- rhamnosylglucoside,vicenin-2	Markham (1973)
S.tetraptera J.Mill. (bark+wood)	Maackiain	Brigqs *et al.*(1975)
S.tomentosa Linn. (aerial parts)	2-(2,4-dihydroxyphenyl)-5 6-methylenedioxy benzofuran, 2-(2-hydroxy-4-methoxyphenyl -5,6-methylendioxybonzofuran, formononetin, isoliquiritigenin	Komatsu *et al.*(1978a)
	isobavachin.(-)-isosophoranone	Komatsu *et al.*(1978b)
	l-maackiain,medicagol	Komatsu *et al.*(1978a)
	sophoracarpan A, sophoracarpan B	Kinoshita *et al.*(1986)
	sophoraflavanone B, sophoraisoflavanone A, sophoronol	Komatsu *et al.*(1978b)
S.tomentosa Linn. (roots)	(-)-isosophoranone A, sophoraflavanone	Shirataki *et al.*(1983)
	sophoronol	DelleMonache *et al.*(1976)
S.tomentosa Linn. (entrie plants)	isosophoronol	Delle Monache *et al.* (1977)
	pterocarpin	Mizuno *et al.*(1989)
	sophorabioside	Farkas *et al.*(1968)
	wighteone	Mizuno *et al.*(1989)
S.viciifolia Hance (entire plants)	diosmin	Plouvier (1966)

B.   Chemistry of Flavonoids

1. Introduction to Flavonoids

Flavonoids represent a very widespread group of water-soluble polyphenolic derivatives which the basic structure is flavan or 2-phenyl benzopyran (1). In plant, flavonoid aglycones (flavonoids without attached sugars) occur in a variety of structural forms. All contain fifteen carbon atoms in their basic nucleus and these are arranges in a C_6-C_3-C_6 configuration, that is, two aromatic rings (C_6 group) linked by a three carbon unit which may or may not form a third ring. For convenience the rings are labelled A, B, and C and the individual carbon atoms are refered to a numbering system which utilizes ordinary numerals for the A-and C-rings and "primed" numerals for the B-ring (1) (Goodwin and Mercer, 1983; K.R. Markham,1982).
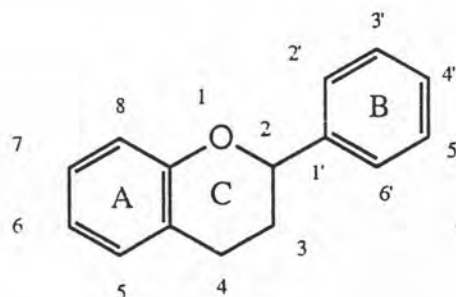
Flavan (2-phenyl benzopyran)

(1)

In the great majority of case, the A ring (the left hand ring) is either monohydroxylated or dihydroxylated. By contrast, the B ring (the right hand benzene ring) is either monohydroxylated, dihydroxylated or trihydroxylated. This difference is due to the fact that the two rings have different biosynthetic origins: ring A is formed by the condensation of three moleules of acetic acid, while the B ring is derived from sugars by the shikimic route. In addition,the substituents may be -OCH_3, -OCH_2O,O-glucosides or C-glycosides (Kibereau-Gayon, 1972).

2. Flavonoids Glycosides

Flavonoids frequently occur as glycosides. The various aglycone having one or more of their hydroxyl groups attached to a sugar by a glycosidic linkage is O-glycoside. The glycosidic linkage is usually β. The α-configuration has only been reported in the case of L-arabinoside and L-rhamnosides.

The unusual flavonoid derivative is the C-glycoside, in which a sugar residue is combined, through its carbon atom, with an aglycone, through one of the latter's carbon atoms forming a C-C bond. The best known C-glycosides are vitexin (2), 8-C-glycosylapigenin, and orientin (3), 8-C-glucosylluteolin. The C-glycosides are highly resistant to hydrolysis by acids and by enzymes
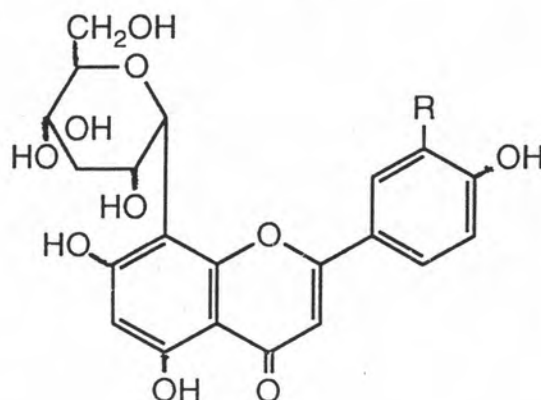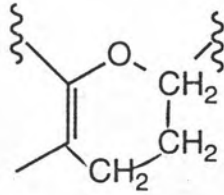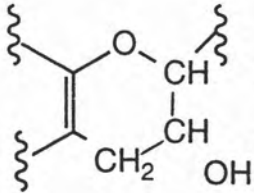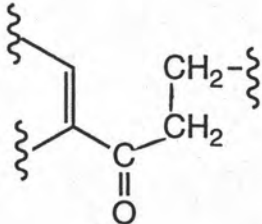

vitexin, R=H (2)
orientin, R=OH (3)

The sugars involved in the structure of flavonoid glycosides are almost exclusively aldoses. The presence of D-fructose, a ketone, is quite exceptional. D-glucose is by far the most common sugar found in glucosidic combination, but L-rhamnose, D-galactose, L-arabinose, D-xylose and a derivative of glucose, D-glucuronic acid, may be found. Besides the monosaccharides, disaccharides and even trisaccharides may be involved in the glycosidic structure which termed monosides, diosides and triosides, respectively (Goodwin and Mercer, 1983, Ribereau-Gayon, 1972, Robinson, 1980).

3. Classification of Flavonoids

Flavonoids are classified according to the state of oxidation of the central C unit (C-2,3,4) in the molecule going from most reduced to most oxidized, the structure and their classes are denoted in Table 3 (only the key portion of the molecule is shown).

Table 3 The different flavonoid classes listed in increasing oxidation level of the three central carbon atoms of the flavonoids.

Oxidation level	Structure / Class	Principal substance	
		Name	Hydroxylation (*)
1	Flavans	koaburanin	5(7-0-glucose)
2	Catechins	Catechin	5,7,3',4'
	Dihydrochalcones	phloretin	3,2',4',6'
		hydroxyphlo -retin	3,4,2',4',6'
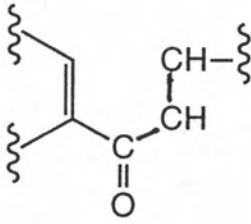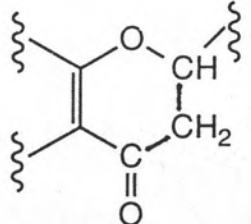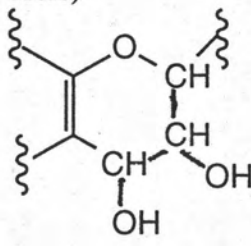
Oxidation level	Structure / Class	Principal substance	
		Name	Hydroxylation (*)
3	chalcones	butein	3,4,2',4'
	Flavanones	naringenin	5,7,4'
		butin	7,3',4'
		eriodictyol	5,7,3;',4'
	leucoanthocy-anidins (Flavan-3,4-diols)	leucocyani-din	5,7,3',4 '
		leucodelphi-nidin	5,7,3',4',5'

Table 3 (continue)
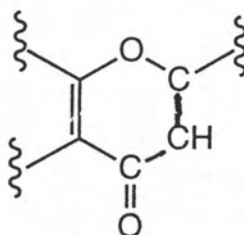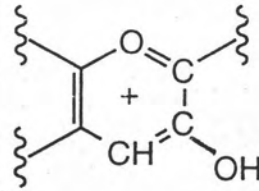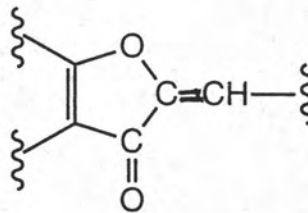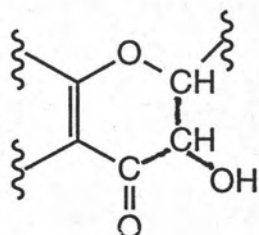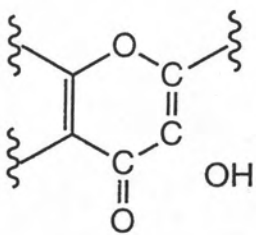
Oxidation level	Structure / Class	Principal substance	
		Name	Hydroxylation (*)
4	Flavones	apigenin	5,7,4'
		luteolin	5,7,3',4
	Anthocyanidins	pelargonidin	5,7,4'
		cyanidin	5,7,3',4'
		delphinidin	5,7,3',4',5'
	Aurones	sulphuretin	6,3',4'
		aureusidin	4,6,3',4'
	Flavanonols (dihydroflavonols)	fustin	7,3',4'
		taxifolin	5,7,3',4'

Table 3 (continue)

Oxidation level	Structure / Class	Principal substance	
		Name	Hydroxylation (*)
5	Flavonols	kaempferol	5,7,4'
		quercetin	5,7,3',4'
		myricetin	5,7,3',4',5'

* Numbering system : (1) most flavonoids
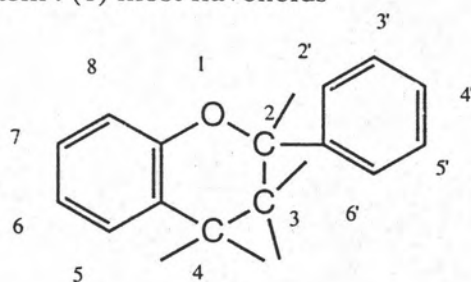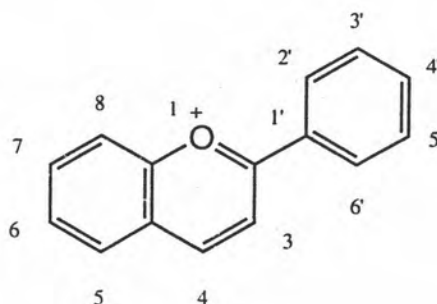
(2) chalcones, dihydrochalcones
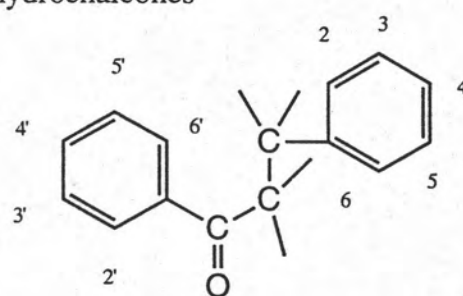
(3) aurones
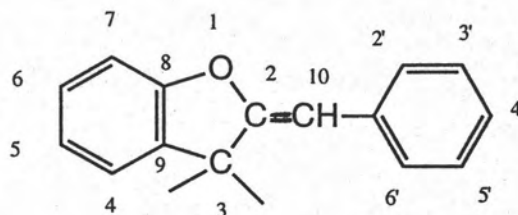
According to Harborne and Mabry (1982), flavonoid aglycones are divided into groups as follow:

3.1 <u>Anthocyanidins</u>

The structure common to all anthocyanidins is the flavalium, or 2-phenyl benzopyrylium ion (4).

flavylium (2-phenyl benzopyrylium) ion

(4)

The anthocyanidins is one of the main classes of flavonoids which contribute color to plant. The color properties depend on the number of hydroxyl groups in ring B. Both the intensity and shade of color also with changes in pH because of their ionic character. The decolorization of an anthocyanin solution in the pH 4-5 is though to occur via the anhydrobase (2.2) which is blue by hydration. There is an acid-base equilibrium between flavalium salt (2.1) and the anhydrobase (2.2), and that the formation of the colorless carbinol base (2.3) is due solely to the hydroxylation of the flavylium salt (2.1).
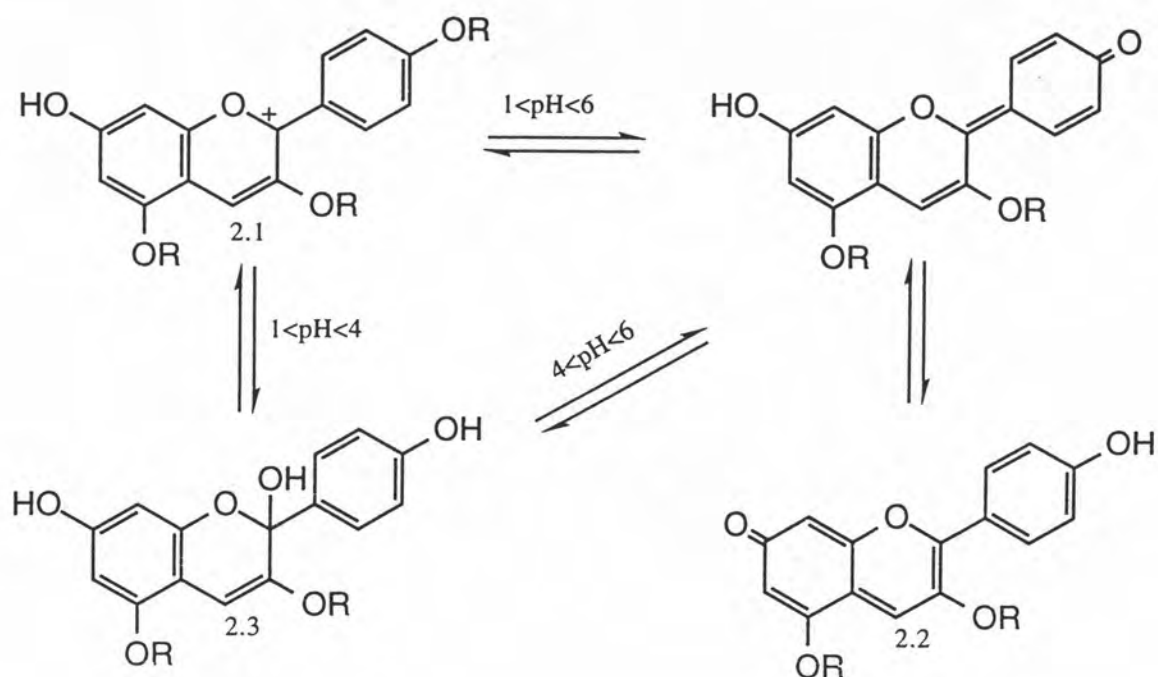
Figure 2 Structural transformation reaction of anthocyanin

The transformation mechanism can be suggested: in aqueous acidic media there is an acid-base equilibrium between the flavylium salt (2.1) and the quinoidal anhydrobase (2.2) , with increasing stability of the flavylium salt under pH 4-4.5 as the pH decreases. The stability of the anhydrobase increases with increasing pH upto 6-6.5. Nucleophilic attack of water on the flavylium salt (2.1) produces the carbinol base (2.3), which can also be produced by hydration of the anhydrobase (2.2) (Brouillard and El Hage Chanine,1980).

3.2 Flavones and Flavonols

The flavones and flavonols contribute much less to color in plants than do the anthocyanins, but many yellows in nature are due to them and they do modify color when the occur with the anthocyanins (Harborne,1967).

The flavonols differ from the flavones only in having an-OH at position 3, but as Harborne (1967) points out, this simple distinction goes hand in hand with spectral and color properties, which chromatographic characters, and with differences in distribution.

3.2.1 <u>Flavones</u>

Kostanecki and Tambor named the compound 2-phenylbenzopyrone (5) as flavone. The numbering shown is that now universally accepted (Gripenberg, 1962).
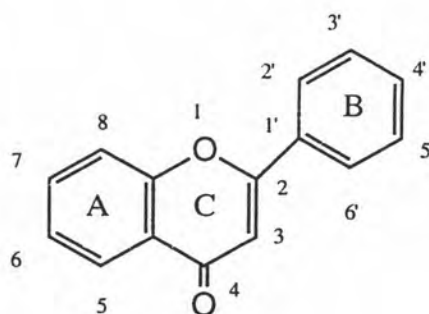
flavone
(2-phenylbenzopyrone)
(5)

Among the floral pigments, structures base on the flavone nucleus are now know that contain from 0 to 8 hydroxyl groups, all intermediate hydroxyl levels being represented. It considers the possible ways in which intermediate numbers of hydroxyls may be attached to the basic skeleton, and the fact that varying number of these groups may be either methylated to sugar residues (Fowden, 1965).

Harborne points out the close correspondence between flavones and anthocyanins. The same types of glucosides occur, often together. Some of the flavone glucosides, however, are C-glycosyl compound, which are rare or unknown among other flavonoids.

Flavone itself, and one or more simple hydroxy-derivatives occur as farina or meal on the surface of *Primula* and a few closely related members of the Primulaceae (Ribereau-Gayon,1972). Flavone carrying isoprenoid substituents are common occurence in *Artocarpus, Morus* and *Tephrosia* species. The isoprenoid substituent mostly is dimethylallyl unit (Venkataraman.1975; Wollenweber.1982).

The common flavones are apigenin (7) and luteolin (8), which widely distributed in the angiosperms. Tricin (9) is common only   grasses.

Flavone	substiuent positions				
	5	7	3'	4'	5'
apigenin (7)	OH	OH	-	OH	-
luteolin (8)	OH	OH	OH	OH	-
tricetin (9)	OH	OH	OH	OH	OH

3.2.2 Flavonols

The Flavonols (10) are 3-hydroxyflavones (6) Harborne (1967) prefers to separate the flavones and flavonols, the distinction between flavones and flavonols is an arbitrary one,since flavonols are simply a class of flavone in which the 3-position is substituted by a hydroxy group. Furthermore, the two groups differ in their spectral and color properties and are usually distinguishable by chromatographic means. Finally, phytochemical survey indicate that the simple difference in structure between flavones and flavonols is one that is of considerable phytogenetic significance. The glycosides of flavonols and of anthocyanidins are often of very similar structure, that they occur together in flowers, and that are biosynthetically related.
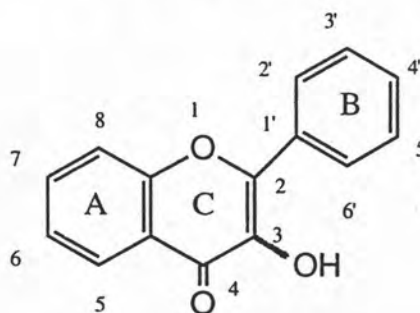
flavonol

(3-hydroxyflavone)

(10)

Just as apigenin and luteolin are the common flavones, so kaempferol (11), quercetin (12) and myricetin (13) are the common flavonols.

While flavones occur characteristically in the more herbaceous plant families such as, Umbelliferae, Labiatae, Compositae, etc., flavonols are most abundant in wood angiosperms. They are very widely distributed in plant, both as co-pigments to anthocyanin in petals and also in leaves of higher plants.

Flavonol	Substituent positions				
	5	7	3'	4'	5'
kaempferol (11)	OH	OH	-	OH	-
myricetin (12)	OH	OH	OH	OH	OH
quercetin (13)	OH	OH	OH	OH	-

3.3 The Minor Flavonoids

According to Bohm (1982), the minor flavonoids consist of chalcones, aurones including auronols), dihydrochalcones (including β-oxygenated derivatives), flavanones and dihydroflavonols. Each of these classes is limited natural distribution. Occurrence is either plant group (Harborne 1984).

3.3.1 Chalcones

The Chalcones (sometimes chalkones) are open chain flavonoids, characterized by their possession of two aromatic ring (A and B) are linked by an aliphatic three-carbon chain which dose not participate in forming a hetero ring as is usually found in other types of flavonoid compounds. The chalcone numbering is different from the system used for flavonoid types that possess heterocylic rings. In chalcones the A ring, always written to the left, is given the primed numbers, while the B–ring carbon are given the unprimed numbers. The position are shown numbered in structure (14). Bridge carbons are marked relative to the carbonyl function (Bohm, 1975 a, 1982; Shimokoriyama, 1962).
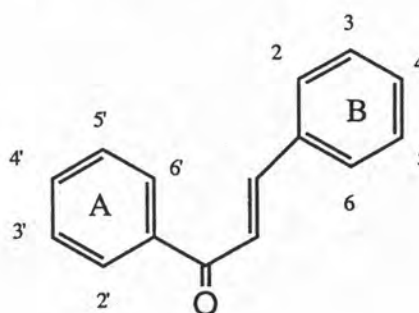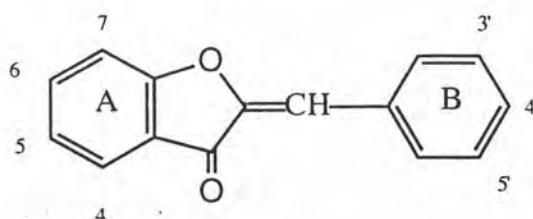

chalcone

(14)

Chalcones are generally considered to be the immediate precursors of the commoner flavonoids so they are classified in group of flavonoids. Furthermore on acid treatment they isomerize to flavanones and the reverse reaction occur in base.

Chalcones occur in widely separated plant genera, in a fern (Pityrogramma), in a monocotyledon (Xanthorrhoea), in a primitive angiosperm genus such as *Paeonia* and in a highly advanced one such as *Coreopsis*.. This distribution fit in with the biogenetic position of chalcones as primitive pigments (Harborne,1973).

3.3.2 Aurones

Aurones are hydroxylated 2-benzylidenecoumaranones (15) , which the ring system is numbered in the following way (Bohm, 1982; Robinson, 1980).


aurone (benzylidencoumaranone)

(15)

The chalcones and the aurones are often grouped as anthochlor pigments Harborne (1966) believes that chalcones are more primitive than the aurones. These name aurones derives from the fact that they are often golden yellow. They may arise in laboratory, by oxidation of chalcones. Both aurones and chalcones are yellow pigments which the colors change to red-orange when fumed with ammonia or the alkaline vapor of lighted cigarette. They occur together characteristically in the petal of the flowers in the Compositae.(Bohm 1975 a;Harborne,1973,1984)

The most widely distributed aurones is aureusidin (16) which occurs naturally as glycosides.Sulfuretin (17) is also one of the most common aurones (Bohm, 1975a,1982).

Aurone	substituent positions			
	4	6	3'	4'
aurensidin (16)	OH	OH	OH	OH
sulfuretin (17)	-	OH	OH	OH

3.3.3 Dihydrochalcones

Dihydrochalcones (19) are derived from chalcones by reduction of the chalcones, β–double bond. As with chalcones the A-ring is derived from acetate and thus has the phloroglucinol hydroxylation pattern. Similarily, the B-ring, derived from a phenylpropanoid precursor, usually shows the 4-mono or 3,4-dihydroxylation pattern. The numbering system is the same as that for chalcones (Bohm,1975a).
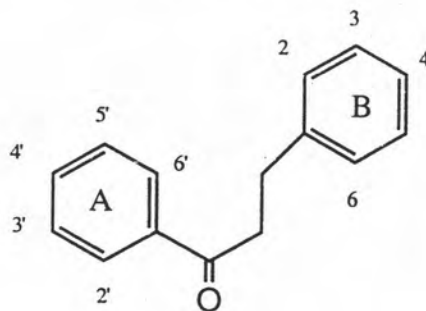

Dihydrochalcone

(18)

Dihydrochalcones being mainly confined to the Rosaceae and Ericaceae (Harborne, 1984). In this class only a limited number of substances are known. Davidogenin (19), 4-0-methyldavidogenin (20) and uvangoletin (21) examples of dihydrochalcones (Bohm,1982).

Dihydrochalcone	substituent positions			
	2'	4'	6'	4
Davidogenin (19)	OH	OH	-	OH
4-0-mehyldavidogenin (20)	OH	OCH3	-	OH
uvangoletin (21)	OH	OH	OCH3	-

3.3.4 Flavanones

Flavanones are dihydroflavones or 2-phenylbenzopyran-4-one (23). The numbering system for flavanones uses unprimed numbers for the A- and C-ring and primed for the B-ring.(Bohm,1975b)
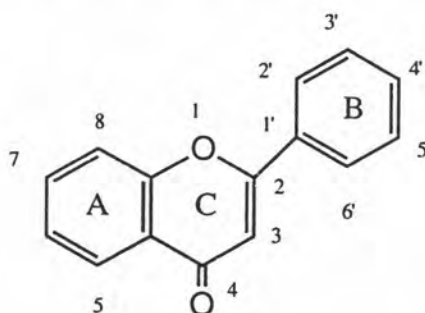

Flavanone (2-phenylbenzopyran 4-one)

(22)

Since carbon-2 of the flavanone molecular is a center of asymmetry, two isomoric forms of each structure are possible. Evidence leading to the estabishment of the (2S) configuration for the naturally occuring flavanones was presented in the earlier review (Bohm,1975).

The parent compound, flavanone (22) is not known to be naturally occuring; the simplest plant flavanone has a hydroxy group at position 7. The most widely occuring of flavanones is naringenin (24) which has been report from about 26 genera. Naringenin occurs as free phenol in many species and as a variety of glycosides in several of the others C-Alkylation of the naringenin ring occur in several plants to provide a variety of derivative. The simple 6-C-β–D-glucopyranosyl derivatives is the first flavanone reported from the Liliaceae (Bndzianoeski and Shizypczakowa,1978). The simplest flavanone that bears the phloroglucinol hydroxylation pattern on the A-ring is pinocembrin (25). Several C-methyl derivatives of pinocembrin has also been report from nature, such as strobopinin (26), cryptostrobin (23) (Wollenweber,1979 a; Bohm 1975b)

Flavanone	substituent position				
	5	7	6	8	4'
cryptostrobin (23)	OH	OH	-	CH$_3$	-
naringenin (24)	OH	OH	-	-	OH
pinocembrin (25)	OH	OH	-	-	-
strobopinin (26)	OH	OH	CH$_3$	-	-

3.3.5 Dihydroflavonols

Dihydroflavonols (27), or 3-hydroxyflavanones, are numbered in the same fashion as flavanones. They are often called flavanonols. Carbon-2and-3 are asymmetric in dihydroflavonols, so four isomers are possible for each compound. The majority of naturally occuring dihydroflavonols exist in the (2 R:3 R) configuration.
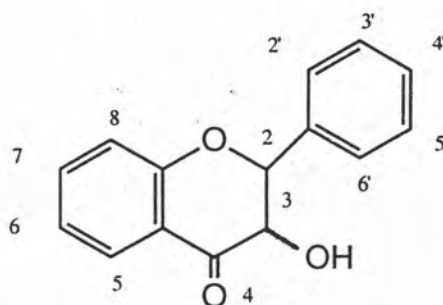
Dihydroflavonol

(27)

The simplest dihydroflavonol exhibiting the phloroglucinol A-ring substitution pattern is pinobanksin (30) which is widely distributed in *Pinus*. Dihydrokaempferol, or aromadendrin (28) is the most widely occuring of dihydroflavonol. The simplest dihydroflavonol which has long been known as a constituent of Anarcardiaceae is fustin (29) (Bohm,1975 b; Harborne, 1973; Young,1979).

| Dihydroflavonol	substituent positions			
	5	7	3'	4'
aromadendrin (28)	OH	OH	-	OH
fustin (29)	-	OH	OH	OH
pinobanksin (30)	OH	OH	-	-

A dihydroflavonol and a flavonol with identical hydroxylation pattern in the A- and B-ring often occur together in the same plant. This suggest that the relationship between dihydroflavonol and flavonol is closer. Therefore, dihydroflavonols and flavonols may be derived from the common precursor through the same initial state, or that one may be converted into the other.

3.4 Proanthocyanidins

All the colorless substances isolated from plants which when heated with acid form anthocyanidins is "proanthocyanidin". This is a chemical term and dose not imply any biogenetic relationship (Freudenberg and Weinges,1960). The terminology of leucoanthocyanidin was reserved (Weinges et al., 1969) for the monomeric proanthocyanidins such as the phenolic flavan-3,4-diols and the name condensed proanthocyanidin for the various flavan-3-ol dimers and higher oligomers.Haslam (1982) suggested that the term "leucoanthocyanin" should no longer be used as it was a generic expression and not specific enough. So the term leucoanthocyanidin as described above, and the name condensed proanthocyanidin, which means for the various flavan-3-ol dimers and higher oligomers.

3.4.1 Natural Leucoanthocyanidins (Flavan-3,4-diols)

Natural leucoanthocyanidins are flavan-3,4-diols. These colorless substances give red solution with acid (Haslam,1975).
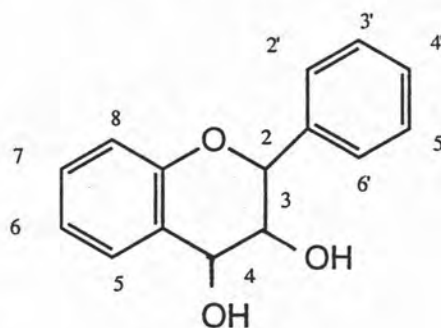

Flavan -3,4-diols

(31)

The majority of natural leucoanthocyanidins have been isolated from the wood or bark of *Acacia* species. They are frequently found in plant tissues, where they are

concerned in the formation of the condensed proanthocyanidins. They are distinguished from other flavonoids in that they rarely exist as glycosides (Haslam 1982; Ikan, 1969; Robinson, 1980).

3.4.2 Condensed Proanthocyanidins

The name proanthocyanidin is used alternatively for condensed tannins because on treatment with hot acid, some of the carbon-carbon linking bonds are broken and anthocyanidin monomers are released. Most condensed proanthocyanidins are procyanidins (32), which means that they yield cyanidin on acid treatment (Harborne,1984,Haslam, 1975, 1982).
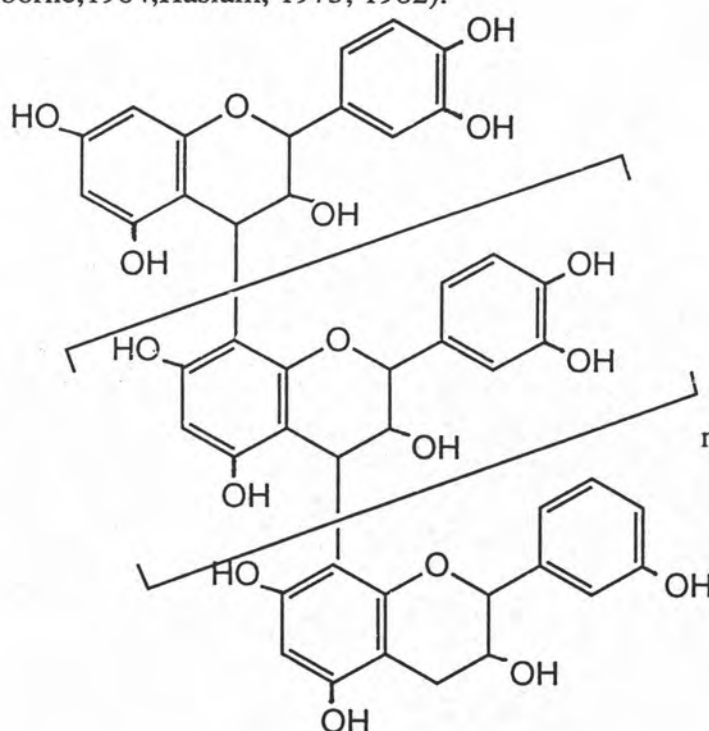
Procyanidin

(32)

3.5 Biflavonoids

Biflavonoids are flavonoid dimers. The dimeric compounds which form by carbon-carbon or carbon-oxygen. The numbering system of these compounds are as follows: the frist numberal refers to the type interflavonyl link, the letter refer to the type of flavonoid moieties present A=biflavones, B=flavanone-flavone, C= biflavanones and D=other; and the last numeral refer to the individual compound in

each group. The name of the compounds are againthose given to them by the authers,since no generally accepted system for naming biflavonoids.

3.6 Isoflavonoids

The isoflavonoids are biogenetically related to the flavonoids but constitute a distinctly separate class in that they contain a rearranged C 15 skeleton and may be regarded as derivatives of 3-phenylchroman. The enzyme(s) responsible for this biochemical rearrangement would appear to be rather specialized, since isoflavonoids have a very limited distribution, being confined essentially to the subfamily Papilionoideae (Lotoideae)of the Leguminosae. There are, however, occasional examples of their occurrence in the subfamily Caesalpinioideae, and in other families (Rosaceae, Moraceae, Amaranthaceae, Podocarpaceae, Chenopodiaceae, Cupressaceae, Iridaceae, Myristaceae, Stemonaceae) together with recent reports of their isolation from microbial sources (Dewick,1982,1988)

Structurally, the isoflavonoids may be subdivided into several classes according to oxidation levels in the skeleton, are the complexity of the skeleton, for instance, formation of further heterocyclic rings.

3.6.1 Isoflavones

Isoflavones (33) continue to be the most abundant of the natural isoflavonoid derivatives. Being 3-phenylchromones, they are isomers of the flavones; the lateral benzene ring is attached to the carbon atom in position 3 instead of position 2 (Dewick, 1982, 1988; Riberoan, 1972, Wong, 1975).
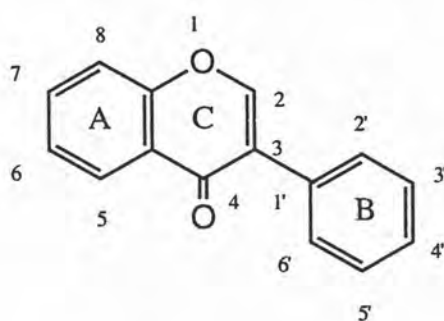
isoflavone (3-phenylchromone)

(33)

The isoflavones are colorless substances, though they are capable of forming colors with metals and were once used as mordant dyes (Harborne,1973). They are much less wide entirely to the subfamily Papilionoideae of the Leguminosae. The four

simple isoflavones daidzein (35), formononetin (36) genistein (37) and biochanin A (34) are extremely common (Dewick, 1982, 1988, Harborne, 1984).

Isoflavone	substituent positions		
	5	7	4'
biochanin A (34)	OH	OH	OCH$_3$
daidzein (35)	-	OH	OH
formononetin (36)	-	OH	OCH$_3$
genistein (37)	OH	OH	OH

3.6.2 Isoflavanones

Isoflavanones (38) are much rarer than isoflavones. They are isomeric with the flavanones; the lateral benzene ring is attached to the carbon atom in position 3 instead of position 2 (Dewick, 1982).
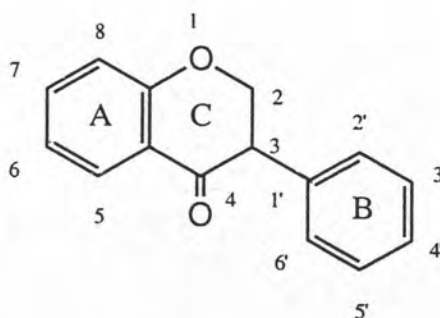

Isoflavanone

(38)

Isoflavanones (38) are much rarer than isoflavones, though the number of known naturally occurring examples continues to grow. Padmakastein (39) from the bark of the Indian plant *Prunus puddum* Roxb.ex Brand (Rosaceae) was the frist natural example of this class.Secondifloran (40) is 3-hydroxyisoflavanones, has been isolated from *Sophora secondiflora* Lag.ex DC. , and although such isoflavonoids derivatives are known chemically, this is the only example reported in the nature.

| Isoflavanone	substituent positions						
	3	5	7	2'	3'	4'	5'
padmakastein (39)	-	OH	OCH$_3$	-	-	OH	-
secondifloran (40)	OH	-	OH	OH	OH	OCH$_3$	-

3.6.3 Rotenoids

Rotenoids is a general name for a class of isoflavonoid compound containing an- extra carbon atom in an addittional heterocyclic ring. They have in common the four-ring chromanochroman system (41) as the basic structure unit. The system is derived in nature by oxidative cyclization of a 2-methoxyisoflavone A considerable in nature are treated in three subdivisions:rotenoids (42), dehydrorotenoids (43) and 12 a-hydroxyrotenoids (44) (Dewick,1982,1988).
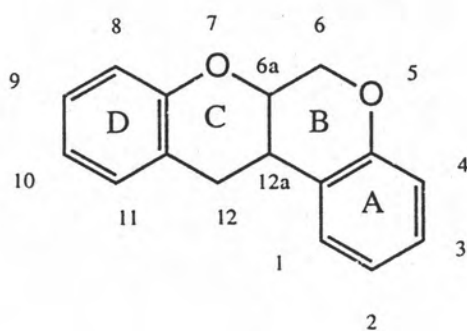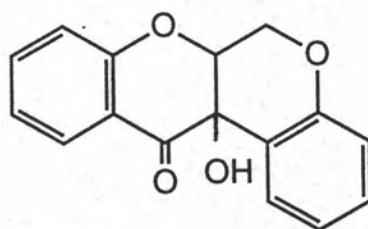
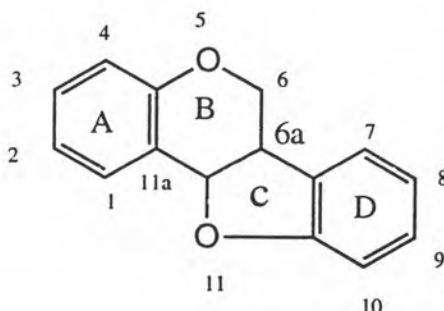chromanochroman

(41)
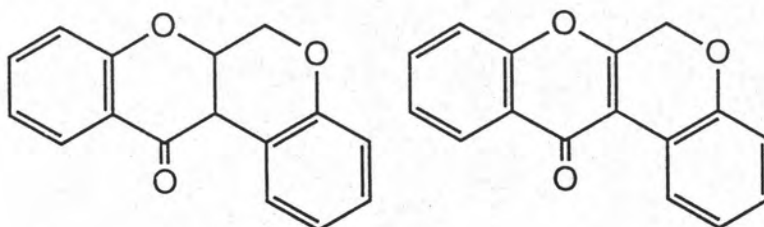
rotenoid

(42)

dehydrorotenoid

(43)

12 a-hydroxyrotenoid

(44)

3.6.4 Pterocarpans

Pterocarpans (45) contain coumaronochroman derived form isoflavonoid skeleton by an ether linkage between the 4 and 2 position. However the systematic numbering of pterocarpan rather than that for simple isoflavonoids is used (Dewick,1988).
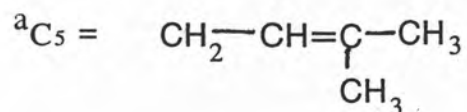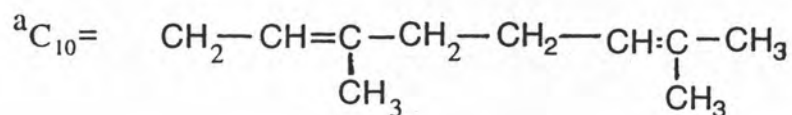

pterocarpan (coumaranochroman)

(45)

The most common of natural pterocarpans is medicarpin (48), it functions as a phytoalexin in tissue of many legumes. A wide variety of substitution patterns is encountered, but 3,9-and 3,8,9-oxygenation patterns predominate, and 1-oxygenated compound (corresponding to position 5 in isoflavones) are relatively uncommon. Unusual geranyl substitutents have been found in lespedezin (46).from *Lespedeza homoloba* Nakai bark, lespein (47) also isolated from this plant is 6a-dimethylallylphaseollidin.

Pterocarpan	substituent positions			
	3	6a	9	10
lespedezin (46)	OH	-	OH	$^aC_{10}$
lespein (47)	OH	$^aC_5$	OH	$^aC_5$
medicarpin (48)	OH	-	OCH_3	-

Explanation of symbols:

$$^aC_5 = \quad CH_2-CH=\underset{CH_3}{\overset{|}{C}}-CH_3$$

$$^a C_{10} = CH_2 - CH = C - CH_2 - CH_2 - CH:C - CH_3$$
$$\quad\quad\quad\quad\quad\quad\quad | \quad\quad\quad\quad\quad\quad\quad |$$
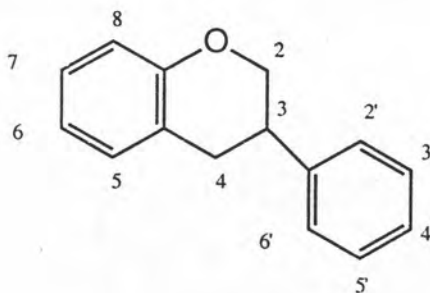$$\quad\quad\quad\quad\quad\quad\quad CH_3 \quad\quad\quad\quad\quad\quad CH_3$$

The pterocarpans are widely distributed in leguminous both as heartwood and bark constituents, and also in young tissue challenged by microorganisms, since many function as phytoalexins (Dewick, 1982, Wong, 1975).

3.6.5 Isoflavans

Isoflavans (49) represent the most reduced of the isoflavonoid modification (Wong,1975).

isoflavan

(49)

The simplest example of the isoflavans is 7,4-dihydroxyisoflavan (equol), an animal metabolite undoubtedly produced by degradation of simple isoflavones such as formononetin (36) and daidzein (35) obtained in the diet (Wong 1975). Every plant-derived isoflavan, however, contain a 2-oxygen substituent, as well as 7,4,-oxygenation. This feather has been associated with posible biosynthetic derivative by reduction of pterocarpans (45) Isoflavans often function as phytoalexins, and many of the new structure has been isolated during antifungal screens (Wong,1975).

The examples of this class are duartin (50), mucronulatol (51) and vestitol (52) which all occur in various Brazilian wood of the *Dalbergia* and *Machaerium* genera (Wong,1975).

3.6.7 2-Arylbenzofurans

2-Arylbenzofuran (57) possible derivation from the isoflavonoid skeleton is via loss of C-6 from a coumestan or by a sequence in which the benzofuran moiety is obtained from the acctate-derived ring, rather than the shikimate-derived ring.
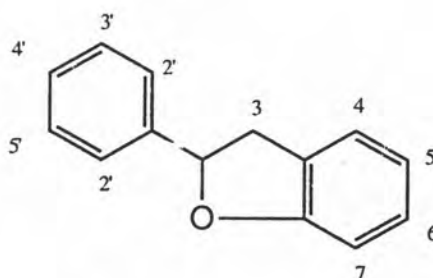

2-arylbenzofuran

(57)

2-Arylbenzofuran (57) mostly co-occur in leguminous plants with isoflavonoids having related substitution patterns. Pterofuran (59) from *Pterocarpus indicus* Willd. was frist to be isolated in 1964. A biosynthetic sequence involving loss of C-6 from a coumestan, by analogy with alkaline degradation of coumestans, is generally postulated (Dewick, 1982,1988, Wong, 1975). Vignafuran (60) is isolated from *Collectotrichum lindemuthianum* Schinz, infected cowpea (*Vigna unguiculata* DC.).Demethylvignafuran and isopterofuran (58) have also been found to function as phytoalexins (Dewick, 1978, 1982, 1988).

2-Arylbenzofuran	substituent positions			
	6	2	3'	4'
isopterofuran (58)	OH	OCH$_3$	OCH$_3$	OH
pterofuran (59)	OH	OCH$_3$	OH	OCH$_3$
vignafuran (60)	OCH$_3$	OCH$_3$	-	OH

3.6.8 Coumaranochromones

Coumaronochromones (61) are known to co-occur with structurally analogous 2-hydroxyisoflavone derivatives, and it is likely that the 2-hydroxyisoflavones could be their biosynthetic precursors. Indeed 2-hydroxyisoflavones may be cyclized to the corresponding coumaranochromones using

a variety of oxidizing agent, and selenium dioxide is a recently reported reagent for this conversion (Dewick, 1982, 1988).
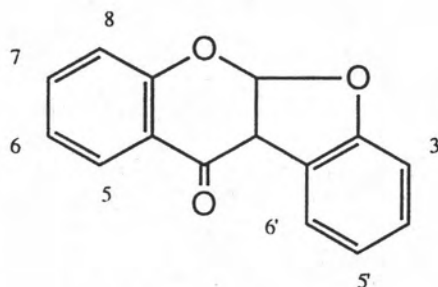

coumaranochromone

(61)

Lisetin (62) has been isolated from only source *Piscidia erythrina* Linn. The coumaronochromones known include lupinalbin A (63) and lupinalbin B (64), they found in roots of white lupin, *Lupinus albus* Linn.(Dewick,1988).

Coumaranochromone	substituent positions					
	5	6	7	3'	4'	5'
lisetin (62)	OH	-	OH	$^{a}C_5$	OH	OCH3
lupinalbin A (63)	OH	-	OH	-	OH	-
upinalbin B (64)	OH	$^{a}C_5$	OH	-	OH	-

Explanation of Symbols

$$^{a}C_5 = \quad CH_2{-}CH{=}\underset{\underset{CH_3}{|}}{C}{-}CH_3$$
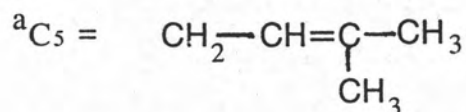
4. Biosynthesis of Flavonoids

4.1 General Aspects

All classes of flavonoids are biosynthetically closely related, with a chalcone being the first common intermediate. Earlier feeding experiments with radioactively labelled precursor have established that the carbon skeleton is derived from acetate and phenylalanine ; ring A is formed by a head to tail condensation of three acetate units and ring B as well as carbon atoms 2,3 and 4 of the heterocyclic ring C arise from phenylalanine (Goodwin and Mecer, 1983; Hahlbrock and Grisebach, 1975)

4.2 Chalcones, Flavanones, Flavones and Flavonols

The biosynthetic relationships of the flavonoids as concluded mainly from labelling experiments in vivo are illustrated in Figure3.
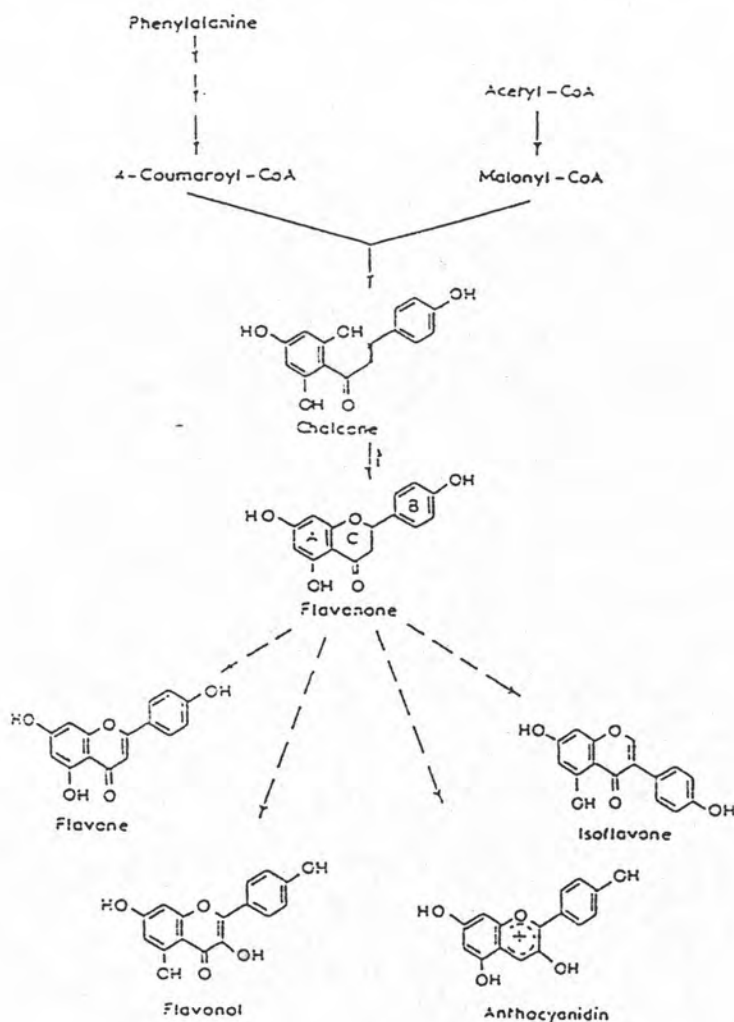

Figure 3 Scheme illustrating the position of the chalcone as the first intermediate in the biosynthesis of all classes of flavonoids.

The chalcone or flavonone isomers are the central intermediates in the synthesis of all flavonoids. The chalcone is the immediate product of the synthase reaction. In a second step, chalcone isomerase stereospecific formation of (-) (2S)-flavanone from the corresponding chalcone (Hahlbrock, 1981). The frequent co-occurence of chalcones, flavanones, flavones and flavonols suggests that their biosynthetic pathways are closely related. The chalcones with a phloroglucinol-type substitution in ring A are exclusively intermediates in the formation of 5,7-dihydroxyflavonoids, while chalcones with a resorcinol type substitution in ring A are selectively converted into 7-hydroxyflavonoids (Halhbrock and Grisebach, 1975).

The formation of a chalcone is the exceptional step in flavonoid biosynthesis which is both specific for this pathway and common to all flavonoids. The enzyme catalysing this step, chalcone synthase, can therefore be regarded as the key enzyme of flavonoid biosynthesis. The best substrates for chalcone synthase are Co A esters of malonic acid and 4-coumaric acid. The sequence of reactions are shown in Figure 4 which was deduced from the occurrence of several side product of the chalcone synthase reaction with the partially purify enzymes from parsley and Haploppus (Dewick 1986, 1988; Halbrock, 1982).

Chalcone isomerase was the first enzyme of the flavonoid metabolism. It catalyses the formation of the 6-membered heterocyclic ring C of flavanones from the corresponding chalcones and has no cofactor requirements(Ebel and Hahlbrock, 1982; Halbrock and Grisebach, 1975).

Figure 4 Scheme illustrating the proposed mechanism of action of chalcone synthase from parsley.

The stereochemistry of the reaction has been studied using a chalcone deuterated in the a position as substrate. The resulting flavanone had the S-configuration at C-2 and the deuterium took up the equatorial position at C-3 (A.Fig.5). On the other hand, when the reaction was carried out in D$_2$O with unlabelled substrate, the deuterium occupied the axial position at C-3 (B.Fig.5). This means that cyclization is formally a cis addition to the a,*b*-double bond (Goodwin, 1983).

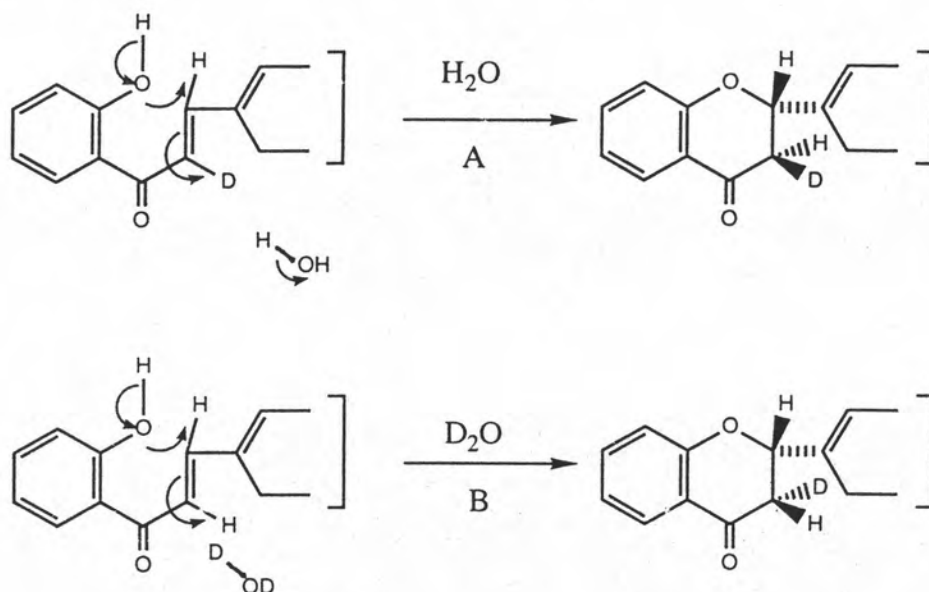
Figure 5 Stereochemistry of the reaction catalysed by chalcone-flavonone isomerase

A= Reaction proceeds in H$_2$O with the chalcone labelled at the a-carbon atom with deuterium

B=Reaction proceeds in D$_2$O with unlabelled chalcone

The flavanone, naringenin, is the putative substrate for flavone and dihydroflavonol formation in flavone and flavonol glycosides biosynthesis (Figure 6).
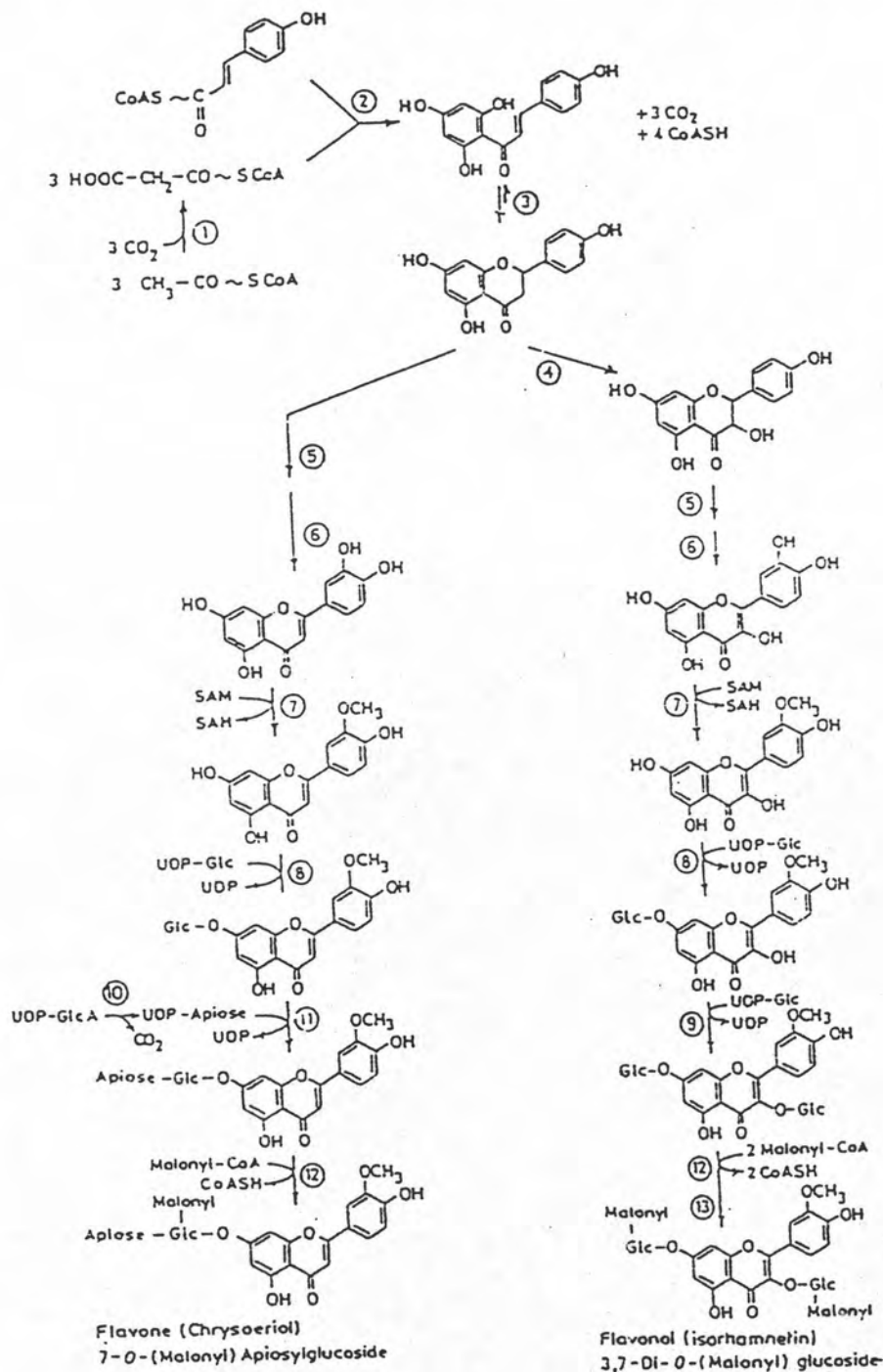

Figure 6 Scheme illustrating the sequence of the reactions of the flavone and flavonol glycoside pathways. The enzyme marked by numbers are listed in Table 4. SAM, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocystein.

Table 4 List of enzymes mentioned in Figure 6

Key to No in Fig.2.5	Enzyme
No.1	Acetyl-Co A carboxylase
No.2	Chalcone synthase
No.3	Chalcone isomerase
No.4	Fiavonoid-3-hydroxylase
No.5	Flavonoid oxidase
No.6	Flavonoid 3-hydroxylase
No.7	S-adenosyl-L-methionine: flavonoid 3-0-methyl transferase
No.8	UDP-glucose: flavonoid 7-0-glucosyltransferase
No.9	UDP-glucose:flavonol 3-0-glucosyltranferase
No.10	UDP-apiose/UDP-xylose synthase
NO.11	UDP-apiose:flavone 7-0-glucoside 2-0-apiosyltranferase
No.12	Malonyl-Co A: flavonoid 7-0-glucoside malonyl transferase
No.13	Malonyl-Co A: flavonol 3-0-glucoside malonyltransferase

4.3 Anthocyanins

Trace studied with 3H and 14C-labelled precursors have confirmed the general rule that anthocyanins, like other flavonoids, are synthesized from acetate and phenylalanine. These experiments also established that chalcones and dihydroflavonols are intermediates in anthocyanin biosynthesis (Grisebach, 1981).

The co-occurence of anthocyanins and flavonols in a large variety of plant suggests that anthocyanin biosynthesis may be closely related to flavonol biosynthesis (Fig 6 and Fig 7). In agree with enzymes chalcone synthase, chalcone isomerase and flavanone-3-hydroxylase have been demonstrated in a number of anthocyanidin-forming plants and plant tissue. Flavanone-3-hydroxylase activity was demonstrated in cell-free extracts from flowers of *Matthiola incana* R.Br. which synthesize large amounts of anthocyanins(Forkmann, 1980).

Figure 7 Conversion by 3-hydroxylation and oxidation of naringenin to various substituted flavones, dihydroflavonols, flavonols and anthocynidins by cell-free extracts from *Petroselinum hortense* Hoffm. cell culture(a), *Haplopappus gracilis* A.Gray cell cultures(b), flowers of *Matthiola incana* R.Br.(c) and flowers of *Antirhium majus* Linn.(d)

4.4 Isoflavonoids

The biosynthesis of isoflavonoids was established that acetate gives rise to ring A and that phenylalanine or cinnamate (or ring substituted cinnamic acids) are incorporated into ring B and carbon atoms 2,3,4 of the heterocyclic ring C (Hahlbrock and Grisebach,1975). The mode of incorporation of phenylpropanoid compounds into isoflavonoids is shown in Figure 8. Since chalcones and flavanones are efficient precursors of isoflavonoids, the migration of ring B from the former b to the former a carbon atom of the phenylpropanoid precursor must take place after formation of the basic C15 skeleton (Ebel, 1979).
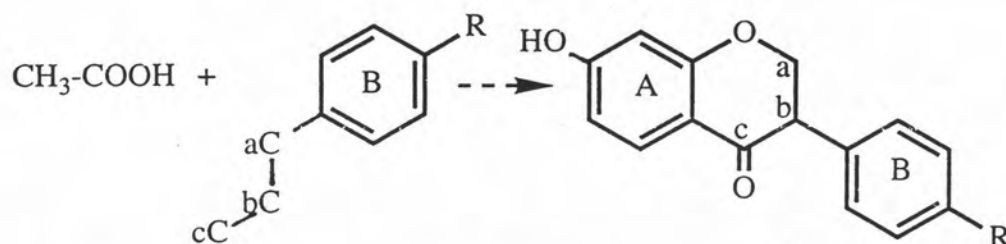
Figure 8 Mode in corporation of phenylpropanoid compounds into isoflavonoids

4.5 Other flavonoids

Minor group of flavonoids are the biflavonoids, proanthocyanidins, aurones, C-glycosylflavonoids. Feeding experiments with radioactively labelled precursor have demonstrated that they are synthesized according to the general scheme for flavonoid formation shown in Figure 3 (Dewick 1988; Goodwin and Mercer, 1983).

C. The Activities

The discovery of new plant flavonoids increased number of pharmacologic effects such as spasmolytic , anti-anginal, anti-ulcer, anti-hepatotoxic, anti-inflammatory, anti-allergic, antimicobial, antiviral, etc. In recent years , a number of flavonoid properties have been recognized which may play a role in these activities.

1.1 Anti-inflammatory Activity

The flavonoids exhibited selective inhibition of the pathways of the biosynthesis of proinflammatory arachidonic acid metabolites. The possible mechanism of action of certain flavonoids might be at the level of arachidonate release and subsequent metabolism. This activity used for treatment of edema and inflammation associated with traumatic injuries.

1.2 Anti-allergic Activity

Flavonoids were inhibitors of allergic mediator release from mast cells and basophils. This was especially true for flavone. In an effort to improve the smooth muscle relaxant properties of flavonoids and therefore employed in case bronchial asthma and other allergic diseases.

1.3 Circulatory Activity

Flavonoids have been shown to reduce the rapid local increase in the permeability of skin cappillary and found to increase tensile strength of cappillary wall in the skins and decrease cappillary fragility. This activity used for treatment of hemorrhoids , circulatory disorders , varicose veins , hypertension , and radiation injuries.

1.4 Antiviral Activity

The basic flavonoids structures whose derivatives have antiviral activity were flavan. Their activity appeared to be mediate by inhibition of viral replication before the initiation of viral RNA synthesis. Translation of virion RNA or cleavage of polyproteins may also be inhibited.

1.5 Inhibit Platelet Aggregation Activity

Flavonoids inhibited platelet aggregation through the mechanism involving cyclic nucleotide phosphodiesterase. So Flavonoids exhibited potential use in the prevention or treatment of thrombosis or atherosclerosis.