FLAVONOIDS : CHEMISTRY AND BIOCHEMISTRY†

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The carbon skeletons of the flavonoids are C_6-C_3-C_6 units which called A, C, and B ring, respectively. Flavonoids, in their occurrence, represent a very large number of types with different properties. In this review, we wish to express the identification of this compounds and the flavonoids as taxonomic markers.

1. Preliminary identification
2. Chromatography
3. UV spectra
4. PMR spectra
5. Mass spectra
6. Chemical taxonomy

Recently, the scientists have been interested in the flavonoid compoumds for biochemical activities and as taxonomic markers. The flavonoids occurr in all parts of plants : roots, stems, leaves, flowers, pollen, fruit, seed , wood, and bark, generally as glycosides.

1. Preliminary identification

Many methods are available which aim to subdivide these groups further and specific sections. The simple color test is quite useful for preliminary identification. Generally, the color test of the hydroxy groups is the determination of the hydroxyl position. The typical example is the ferric chloride reaction. The color tests for flavonoid compounds are shown in Table

† Dedicated to Professor Tsunematsu Takemoto on the occasion of his retirement.

1, and 2.

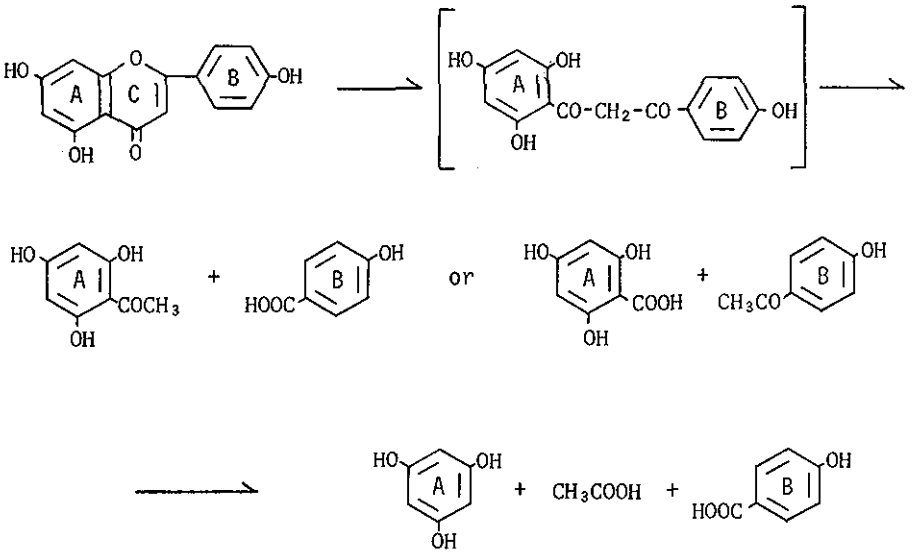
Table 1. The color tests for preliminary identification.

Flavonoid types	Aqueous NaOH	conc. H_2SO_4	Mg - HCl in MeOH	Na/Hg - Acid in MeOH
Flavanones	Yellow to orange ⟶ deep red or purple.	Orange to crimson	Red, mangenta, violet.	Red
Flavones	Yellow	Yellow to orange	Yellow to red	Red
Flavonols	Yellow to orange		Red to mangenta	Yellow to pale red
Isoflavones	Yellow	Yellow	Yellow	Pale red to pink

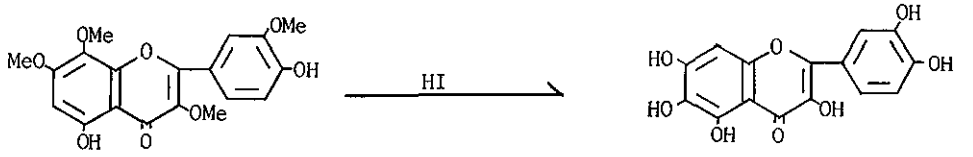
Table 2. The phenolic color tests for flavonoid compounds.

Reactions or tests	Detectable formations	Color	Reference
Gibbs reaction	Absence of *p*-substitution for OH	Blue	(1)
$ZrOCl_2$-Citric acid test	Flavonols(3-OH)	Yellow	(2)
Gossypetin reaction	*p*-Quinol type	Reddish brown	(3)
$SrCl_2/NH_3$ test	*ortho* Diphenol group	Yellow∼brown ∼green∼black	(4)
Baryta water test	5,6,7-hydroxyl group	Green	

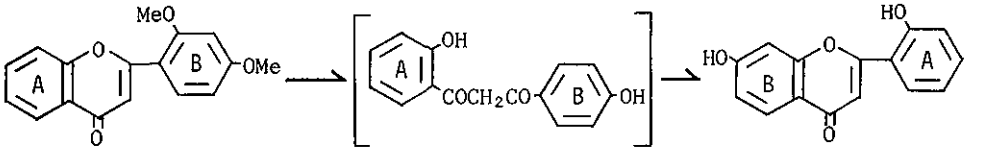
Alkaline degradation of flavonoids with 30 - 50 per cent potassium hydroxide solution or in a sealed tube with 30 per cent alcoholic sodium hydroxide or by fusion produces a phenol for A ring and a substituted benzoic acid from B ring, although acetophenones may sometimes be isolated or identified chromatographically.



It is noted that 5,8-oxygenated flavonoids are rearranged along with demethylation by hydrogen iodide. This rearrangment afford 5,6-hydroxy derivatives and generally is called Wessely-Moser rearrangment[5].



Also, 2'-methoxyflavonoids are rearranged with hydrogen iodide[6].



2. Chromatography

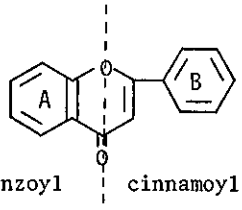
Paper chromatography is available for a method of separation and identification[7]. The best developing solvents is *n*-butanol-acetic acid-water(4:1:2). With dilute acetic acid, the aglycone of planar flavonoids will not move(except isoflavone), while O-glycosides, C-glycosides, and flavan derivative move slowly. The detection of most flavonoids on paper is relatively easy since they are all visible under ultraviolet light(except flavans) and can be seen yellow color with fuming ammonia.

The thin layer chromatography has been used with these compounds. Flavonoids can be separated on plates of silica gel, using chloroform-acetic acid(5:2)[8], chloroform-methanol(15:1)[9], ethyl acetate-petroleum ether(3:1, and 1:1)[10], ethanol-chloroform(1:3, and 1:1)[10], ethyl acetate-methyl ethyl ketone-formic acid-water(5:3:1:1)[11], and benzene-pyridine-formic acid(36:9:5)[12].

The studies on gas chromatography of flavonoid compounds was carried out by N. Narasimhachari, and E. von Rudloff[13]. In this studies, the methyl ethers of 36 flavones were separated with a silicone liquid polymer(SE-30) as the statinary phase.

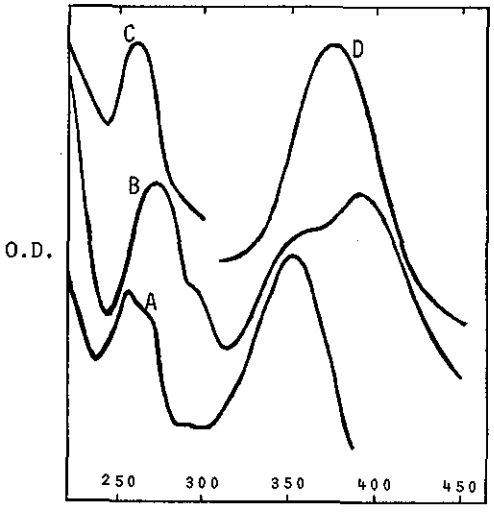
3. UV spectra

Ultraviolet spectra are useful in determining the structure of flavonoids. The spectra of flavonoids exhibit two major absorption peaks in region 240 - 400 nm. These two peaks are commonly referred to as Band I (usually 300 - 380 nm), and Band II (usually 240 - 280 nm). Band I is considered to be associated with absorption due to the ring B cinnamoyl system, and Band II with absorption involving the A ring benzoyl system[14]. The Band II of isoflavones generally lies in the



range 250 - 275 nm while that of flavanones is in the range 275 - 290 nm[15]. In general, the Band I of flavones show intense absorption at 320 - 350 nm, whereas flavonols maxima are at 340 - 380 nm.

Diagnostic reagents have been developed which, on being added to solutions of flavonoid, may cause shifts in the position of the maxima[16]. These include sodium ethylate, sodium acetate, aluminium chloride, and a boric acid-solid sodium acetate mixture. These reagents are best available for the determination of the position of hydroxyl groups in UV spectral studies. In the case of flavone glycosides, a positive shift with sodium acetate in Band I indicates that the 7-hydroxyl is not glycosylated and likewise a positive aluminium chloride shift in Band II shows that the 5-hydroxyl is free. The Band II maximum shifts with borate only if the flavone has been hydroxyl groups in the 3'-, and 4'-positions so that while the 5-, and 7-glucoside of luteolin exhibit bathochromic shifts of 20 nm, the 3'-, and 4'-glucosides do not respond to this reagent. The use of these reagents for structural analysis is described by L. Jurd[14], and T. J. Mabry et al.[17].



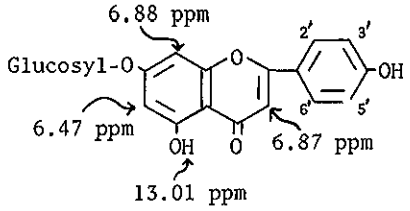
Absorption spectrum of luteolin-7-glucoside.

Curve A, 95% EtOH ; curve B, EtOH-$AlCl_3$; curve C, EtOH-NaOAc ; curve D, EtOH-H_3BO_3-NaOAc.

Infrared spectral determinations have been mainly used with flavones for "fingerprint" identification, rather than for structural determination, because interpretation is generally difficult, and shifts are unpredicatable . However, the useful two ways, lacking a 5-hydroxyl group and unsubstituted B ring, are reported by H. Wagner[18].

4. PMR spectra

The structure analysis of flavonoids is now established with the application of proton nuclear magnetic resonance (PMR) spectroscopy. Many flavonoid agycones are mostly soluble in deuteriochloroform ($CDCl_3$) for direct PMR analysis. However, most natural flavonoids are occurred as glycoside, therefore have low solubility in $CDCl_3$. Accordingly, most workers were limited to the PMR analysis of the more soluble methyl, ethyl, and acetyl derivatives. In 1964, A. C. Wass et al.[19] investigated the usefulness of trimethylsilyl (TMS) ether derivatives for obtaining PMR spectra of flavonoids which were otherwise insolble in $CDCl_3$. At about the same time hexadeuteriodimethylsulfoxide (DMSO-d_6) was introduced as a solvent for direct PMR analysis of flavonoids by T. J. Batterham, and R. J. Highet[20]. They were examined the spectral signals of the aromatic, olefinic , and hydroxyl protons of 41 flavonoids. The chemical shifts of typical flavonoid, cosmosiin (apigenin-7-glucoside), in DMSO-d_6, shows below.



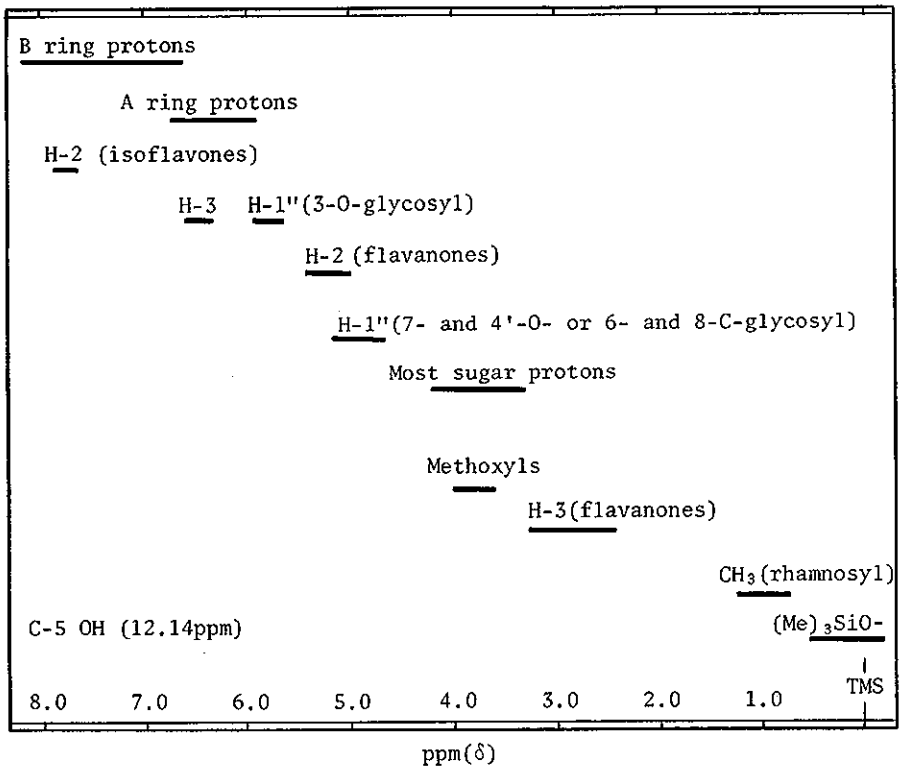
2', 6' H : 7.98 ppm

3', 5' H : 6.98 ppm

In flavonoids with the common 5,7-dioxygenation pattern the C-6 and C-8 protons occur as doublets (J=2.5 Hz) and are therefore readily distinguished

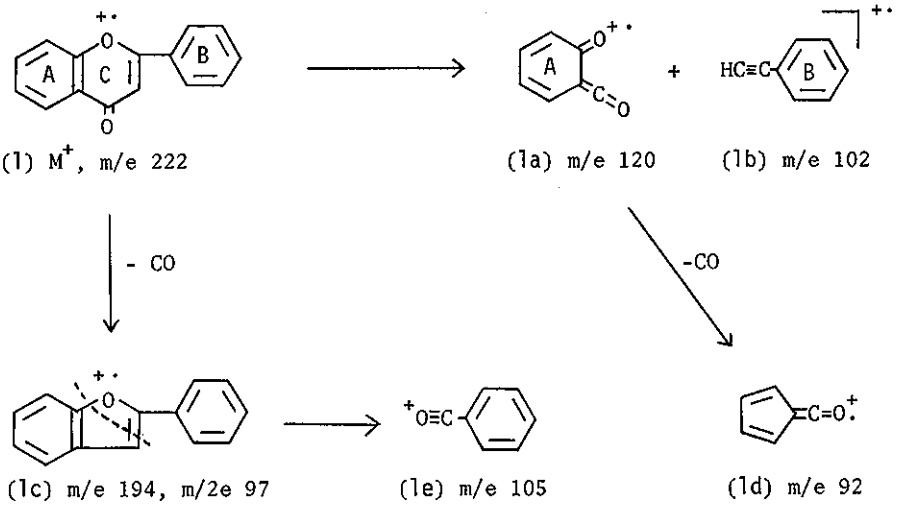
from the C-3 proton singlet. 5,6,7- or 5,7,8-trioxygenated flavonoids have deservedly one A ring proton at 8- or 6-position. C-8 proton signal is observed at 6.77 ppm and C-6 proton signal is at 6.46 ppm, therefore both trioxygenation pattern are differentiated easily[21]. But, DMSO-d_6 has a number of disadvantages which are the recovery of the flavonoid inconvenient ,rapidly absorb atomospheric moisture, and penetrate skin tissue carrying with any dissolved substances. Recently, T. J. Marbry et al.[22], published for PMR determination of TMS ether of flavonoid compounds, together with chromatographical and UV spectral identification of that. Approximate chemical shift ranges for the protons in TMS-flavonoids are showed in Fig. 2.

Fig. 2.



5. Mass spectra

The mass spectra of flavones are characterized by intense molecular ion (the base peak in most case), indicative of a stable heterocyclic system with no facile bond-cleavages available. The major fragmentation pathway for the parent compound, flavone(1), is the retro-Diels-Alder reaction, giving an abundant A ring fragment(1a, m/e 120) which further fragments by the usual loss of CO(1 → 1c) and a less abundant B ring fragment, the phenylacetylene radical ion(1b, m/e 102)[23), 24)].

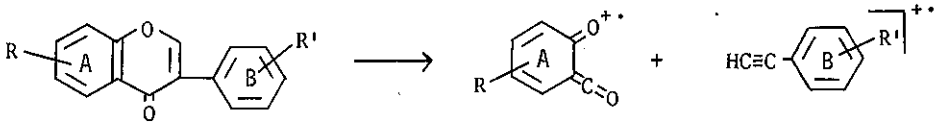


(1) M^+, m/e 222 (1a) m/e 120 (1b) m/e 102

(1c) m/e 194, m/2e 97 (1e) m/e 105 (1d) m/e 92

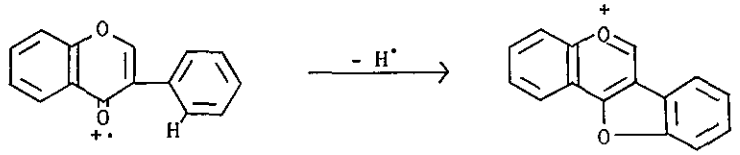
Loss of CO from the molecular ion is also marked, leading to the phenylbenzofuran ion(1c, m/e 194) which is associated with a considerable doubly-charged ion at m/2e 97.

The spectra of simple isoflavones resemble those of the isomeric flavones in that the retro-Diels-Alder reaction is a very important fragmentation process, and as in flavones the charge distribution on the two fragments



depends on the substitution of A and B ring[25].

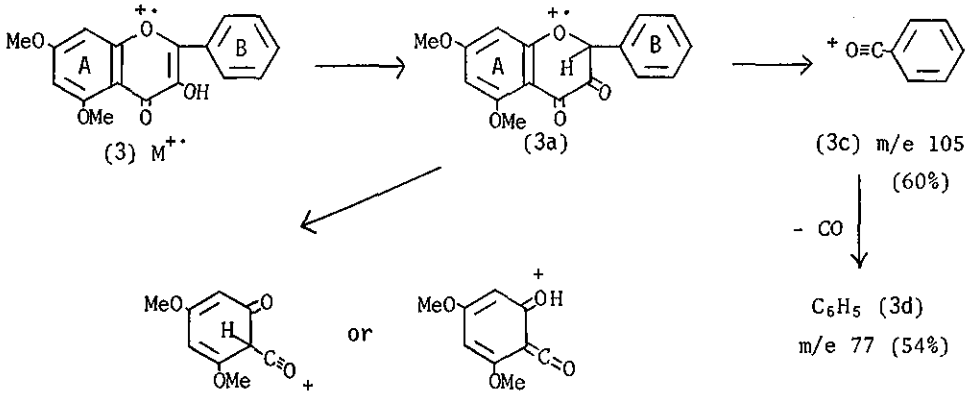
Isoflavones are also characterized by a rather intense M - 1 ion which is the base peak in the spectra of isoflavon(2) and 7-methoxyisoflavone.



(2) M^+, m/e 222 (2a) m/e 221 (100%)

It has been suggested that the lost hydrogen atom originates from the C_2' position as this would result in formation of a resonance-stabilized oxonium ion(2a) whose stability is reflected in the lack of further fragmentation[25].

Ion due to the retro-Diels-Alder reaction are either very weak or not observed at all in the mass spectra of flavonols. However, hydrogen-transfer reactions are important and may be most readily rationalized if the molecular ion exists in the diketo form (3a for 3). The retro-Diels-Alder

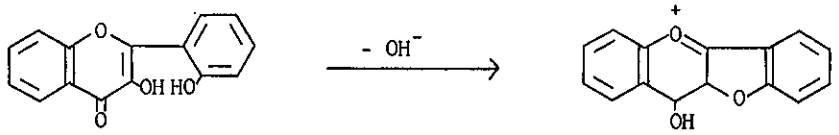


(3) $M^{+\cdot}$ (3a) (3c) m/e 105 (60%) C_6H_5 (3d) m/e 77 (54%)

(3b) m/e 181 (100%)

reaction with hydrogen transfer to A ring is generally observed and here results in the ion 3b. The benzoyl ion, 3c, is an important fragment possibly formed by further fragments as expected by loss of CO and formation of phenyl ion 3d. Ions corresponding to 3c and 3d are a characteristic feature

of spectra of flavonols[24]. The presence of the C_2'-hydroxyl group in 2'-hydroxyflavonol(4) results in the very facil loss of OH^-, most probably with formation of the stable hydroxychromenyl ion 4a. Deuterium labeling indicates that both the hydroxyl group in 4 are equally involved in loss of OH[26].



(4) M^+, m/e 254(82%) (4a) m/e 237(100%)

More extensive substitution as in quercetin[24] and isorhamnetin[27] increases the stability of the molecular ion (base peak) and relatively little fragmentation is observed.

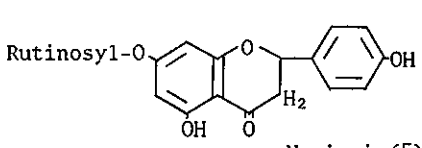
6. Chemical taxonomy

The development of the studies on plants components has been due to the investigation of the empirical plants for disease, in particular, the studies which had been started as alkaloidal researches gave rise to many effective compounds from the plant source. A finding of an available constituent from one species, lead to further research in the same genera source.

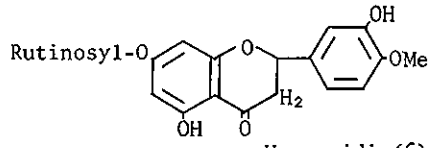
These studies gave rise to chemical and taxonomical workes and lead up to chemical taxonomy. The chemical taxonomy is plant classification to take advantage of difference on the constituents in plants, i. e., chemotaxonomy.

The flavonoids universally occurred in most plants, recently, were used to the plant classification, as taxonomic markers.

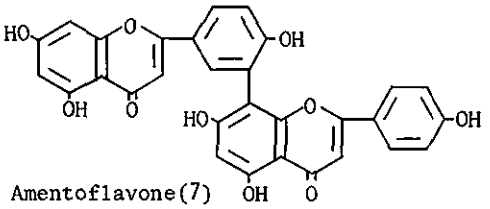
Flavanone glycosides(5, 6) in the genus *Citrus* and bisflavones(7, 8, 9) in



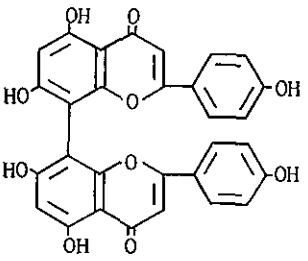
Naringin(5)



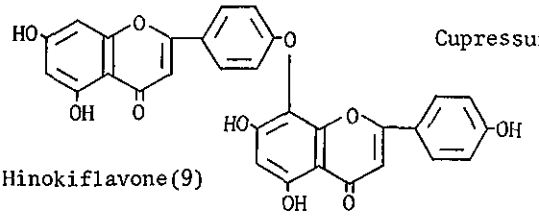
Hesperidin(6)



Amentoflavone(7)



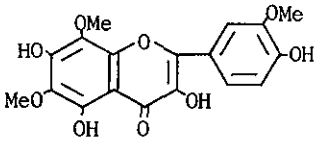
Cupressuflavone(8)



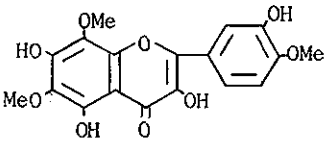
Hinokiflavone(9)

the *Coniferae* are characteristic components on the plant classification.

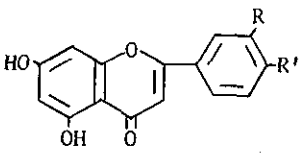
The common citrus fruits of commerce are particularly rich in flavones and *flavanones. The flavones present fall into three classes :* (1) *fully* methylated derivatives and the corresponding 5-demethyl compound, (2) partially methylated flavonols such as limocitrol(10) and isolimocitrol (11), and (3) common flavones such as apigenin(12), luteolin(13), diosmetin (14), and chrysoeriol(15)[28].



Limocitrol(10)



Isolimocitrol(11)



	Apigenin(12)	Luteolin(13)	Diosmetin(14)	Chrysoeriol(15)
R	H	OH	OMe	OH
R'	OH	OH	OH	OMe

In recent years, "Chemotaxonomy of the Leguminosae" in which the distribution of flavonoids[29] are mentioned, were published by J. B. Harborne et. al.. In this review, we wish to express the distribution of flavonoids in the genus *Cirsium(Compositae)*, *Chrysosplenium(Saxifraga)*, and *Iris (Iridaceae)* as following paper.

The genus *Cirsium* has been occurred about 100 species in japan, 28 species were examined with respect to flavonoid components. This genus may be fall into nine classes from the point of view of flavonoid components as follow. The last *C. setosum var. subulatum (Breea setosa)* must be put out from this genus, because flavonol is occurred.

Table 3.

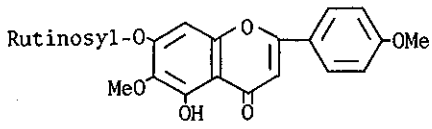
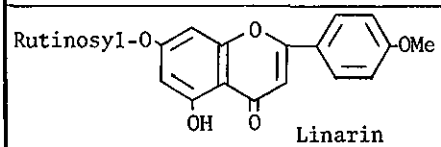
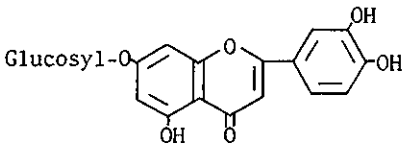
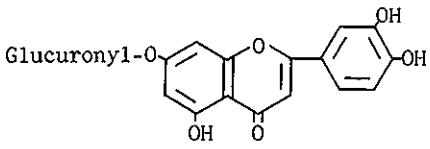
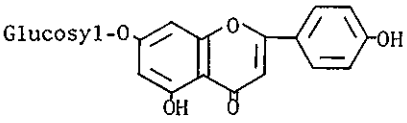
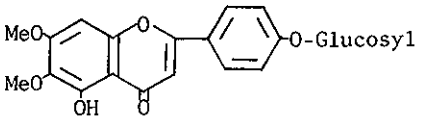
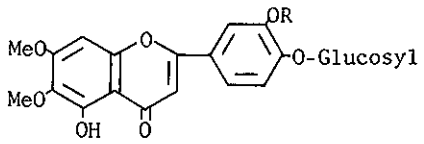
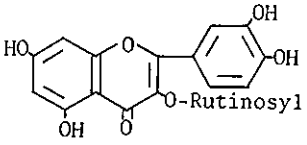
Containning flavonoids	*Cirsium* species	Reference
 Pectolinarin	*C. microspicatum*	(30)
	var. kiotense	(33)
	C. babanum var. otayae	(30)
	C. japonicum	(30)
	C. kagamontanum	(31)
	C. inundatum	(31)
	C. dipsacolepis	(33)
	C. brevicaule	(33)
	C. yezoense	(35)
	C. pendulum	(35)
 Linarin	*C. purpuratum*	(30)
	C. spinosum	(33)
Pectolinarin and linarin	*C. kamtschaticum*	(35)
	C. pectinellum	(35)

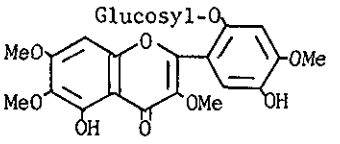
Table 3 (continued)

 Luteolin-7-glucoside	*C. matsumurae*	(33)
	var. pubescenc	(31)
	C. yakushimense	(33)
	C. buergeri	(33)
	C. nipponicum var. yoshinoi	(33)
	C. gyojanum	(34)
	C. lucens	(34)
	C. suffultum	(35)
 Luteolin-7-glucuronide	*C. sieboldii*	(34)
 Rhoifolin	*C. bitchuense*	(34)
 Cirsimarin	*C. maritimum*	(32)
	C. aomorense	(33)
 R=Me Cirsilineol-4'-glucoside R=H Cirsiliol-4'-glucoside	*C. lineare*	(36)
 Rutin	*C. setosum var. subulatum* (*Breea setosa*)	(33)

14 species and 9 varieties of the genus *Chrysosplenium* have been grown in japan. The studies on flavonoids were examined for 11 species, and its results were fall into two classes : (1) flavonols were occurred, and (2) flavonoids were not occurred. The former class was characterized by containing 3-OMe flavonoids as following structures.

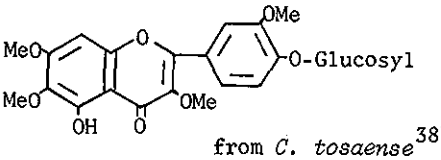
(1)

Chrysosplenoside A



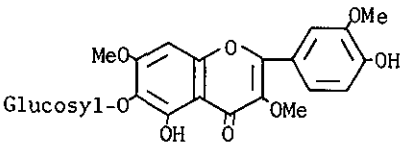
from *C. grayanum*[37] and *C. rhabdospermum*[42]

Chrysosplenoside B



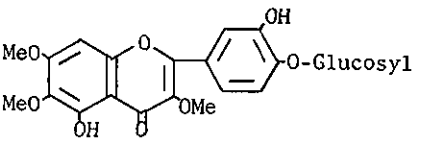
from *C. tosaense*[38] and *C. japonicum*[39]

Chrysosplenoside C



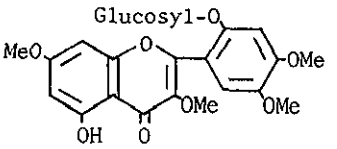
from *C. maximowizii*[40]

Chrysosplenoside D



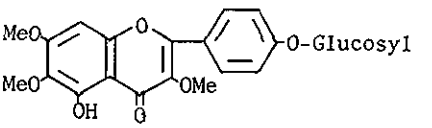
from *C. japonicum*[39]

Chrysosplenoside E



from *C. grayanum*[41] and *C. rhabdospermum*[42]

Pendulin



from *C. flagelliferum*[37]

(2)

C. album[42]

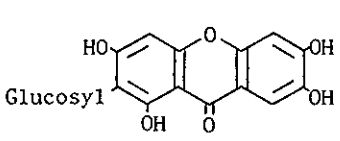
C. pilosum Maxim. *var. spaerospermum*[42]

C. fauriei[42]

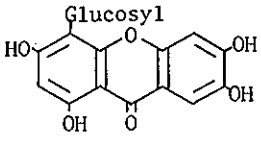
C. fauriei var. kiotense[42]

C. macrostemon[42]

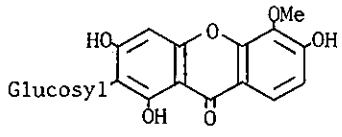
In the genus *Iris*, isoflavones and glycoxanthones from the rhizomes and leaves have been well known. Within the *Iris*, mangiferin(16) has been found in most species of the sub-section *Pogoniris*(which includes the garden form *I. germanica*), and insubsections *Apogon*(*I. pseudacorus*, *I. kaempferi*, and *I. unguicularis*), *Pardanthopsis*(*I. dichotoma*), and *Oncocyclus*(*I. sari*). These occurrences are in the leaves or petals, but it has been found curiously in high concentration in the rhizome of *I. unguicularis*.
Two other glycoxanthones, isomangiferin(17) and irisxanthone[43] (18) have been detected in *I. florentina*[43] and *I. unguicularis*[44] where they occurr with mangiferin.



Mangiferin(16)

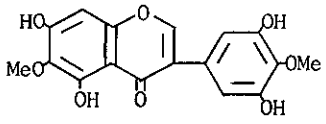


Isomangiferin(17)

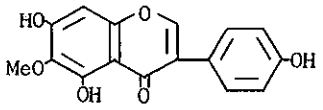


Irisxanthone(18)

Irigenin[45] (19) as isoflavone was first isolated from the rhizome of the cultivated *Iris*, and the related tectorigenin[46] (20) from *I. tectorum* in 1927.

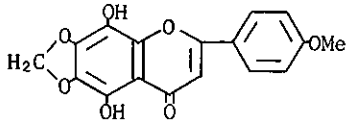


Irigenin(19)

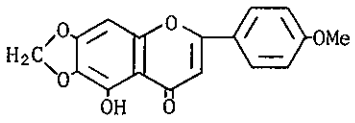


Tectorigenin(20)

In the most recently, two new flavones which were named kanzakiflavone-1 (21)[47] and -2(22)[48] have been found from the rhizomes of *I. unguicularis*.



Kanzakiflavone-1(21)



Kanzakiflavone-2(22)

The distribution of isoflavones in the *Iris* is showen as follows.

From *I. tectorum*

Tectorigenin(20) as 7-glucoside[46]

Iristectorigenin A(23) as 7-glucoside[49]

Iristectorigenin B(24)[50]

From *I. florentina*

Irigenin(19) as 7-glucoside[45]

Iristectorigenin B(24)[51]

Irisolone(25)[51] and as 4'-bioside[53]

Irisflorentin(26)[51]

Iriflogenin(27) as 4'-glucoside[52]

Irilone(28) as 4'-glucoside[53]

From *I. nepalensis*

Irigenin(19)[54]

Irisolone(25)[54]

Irisolidone(29)[55]

From *I. pallida*

Irigenin(19) as 7-glucoside[56]

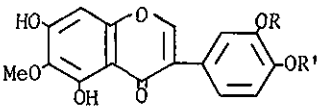
From *I. kumaonensis*

Irigenin(19) as 7-glucoside[57]

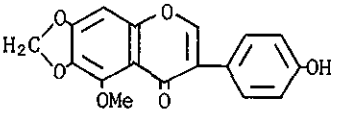
From *I. germanica*

Tectorigenin(20) as 7-glucoside[58]

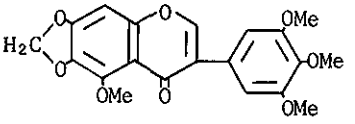
Homotectorigenin(30) as 7-glucoside[58]



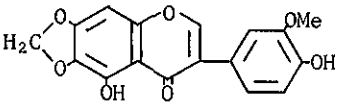
	R	R'
(23)	H	Me
(24)	Me	H



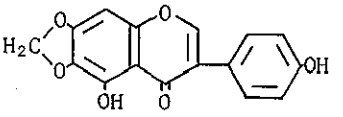
Irisolone(25)



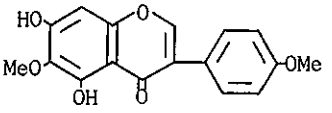
Irisflorentin(26)



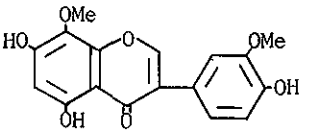
Iriflogenin(27)



Irilone(28)



Irisolidone(29)



Homotectorigenin(30)

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