CHAPTER IV

DISCUSSION

The present work, using chromatographic technique, has led to the isolation of two new flavanones, 8-lavandulyl-5,7,2',6'-tetrahydroxyflavanone and 8-lavandulyl-5,7,2',4',6'-pentahydroxyflavanone, from the diethyl ether extract of the roots of Sophora exigua Craib (Leguminosae). The structure elucidations were based on the data from UV, IR, NMR and Mass spectra.

The first component, SE-1, gave bright pink colour with Shinoda test and dark green colour with ferric chloride solution. That meant SE-1 was a polyhydroxy flavonoid.

In the mass spectrum of SE-1 (Fig. 3.23), the molecular ion peak at m/z 424, corresponding to the elemental analysis, established the molecular formula as $C_{25}H_{28}O_{6}$.

In the IR spectrum (Fig.3.17), the absorption bands at 3450 and 1640 cm^{-1} suggested the presence of the hydroxyl group and the chelated carbonyl group, respectively. The feature of UV spectrum (Fig. 3.13) showed the characteristic of a flavanone chromophore. The bathochromic shift (21 nm)
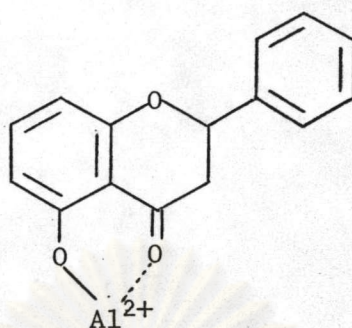
acid-stable AlCl$_3$ complex

of the band II in the UV spectrum with the presence of AlCl$_3$/HCl (Fig. 3.14) is effected from the acid-stable AlCl$_3$ complex between the C-4 keto function and the C-5 hydroxyl groups of the flavanone. Whilst, the bathochromic shift (42 and 41.5 nm) of the band II in the UV spectra on the addition of NaOH (Fig. 3.15) and NaOAc (Fig. 3.16) indicated the presence of a free C-7 hydroxyl group which the electron delocalization can be occurred (Fig. 4.1) (Mabry, Markham, and Thomas, 1970; Markham, 1982). From these data, SE-1 was considered to be a 5,7-dihydroxyflavanone derivative.

The ^{1}H-NMR spectrum of SE-1 (in the mixture of hexadeuterated acetone and deuterated chloroform) (Fig. 3.18) showed the singlet signals of one chelated hydroxyl and other three hydroxyl groups at 12.18, 9.22, and 7.91 (double intensity) respectively, which these signals were absent when the solvent was changed to tetradeuterated
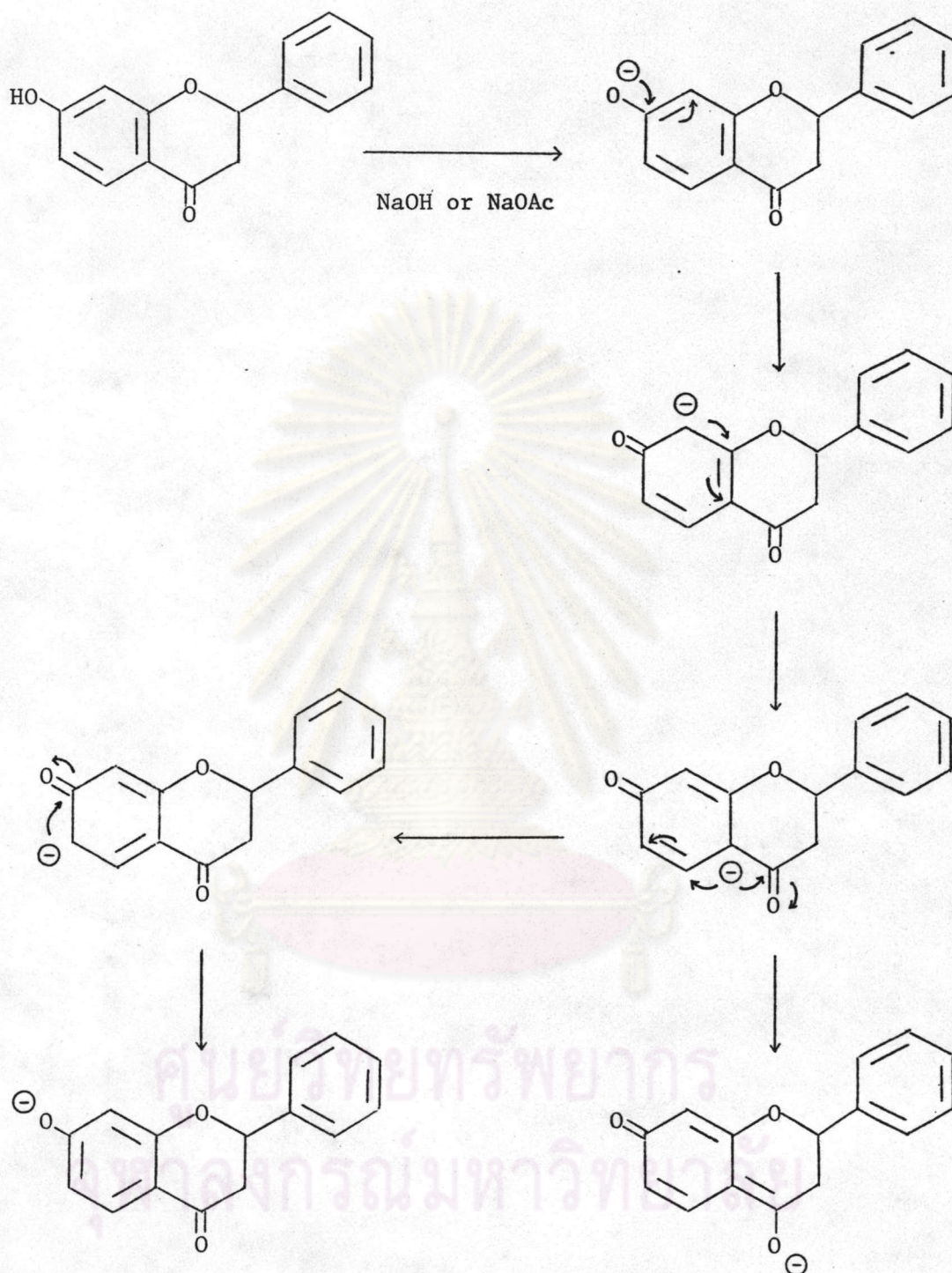
NaOH or NaOAc

Figure 4.1   Electron delocalization of the flavanone in the
basic medium.

methanol (Fig. 3.19). The most down field signal at δ 12.18 was certainly due to C(5)-OH, which chelated with the carbonyl group. The signal at δ 7.91 showed two equivalent hydroxyl groups which must be in the B ring. So the rest hydroxyl signal at δ 9.22 was attributed to the C(7)-OH.

In ^{1}H-NMR determination which tetradeuterated methanol was used as solvent (Fig 3.19), the substitution positions of the aromatic B ring was deduced from the *ortho* coupling of the phenyl proton signals at δ 6.98 (1H, t, J = 8.1 Hz) and 6.36 (2H, d, J = 8.1 Hz). These two signals were due to C(4')-H, and C(3')-H together with C(5')-H respectively. So the B ring was considered to have dihydroxy substitution at the positions 2' and 6'. The other aromatic proton signal at δ 5.91 was due to C(6)-H. The three signals at δ 5.91 (1H, dd, J = 14.1, 3.0 Hz), 3.91 (1H, dd, J = 16.8, 14.1 Hz), and 2.44 (1H, dd, J = 16.8, 3.0 Hz) were due to the ABX type grouping, one proton at C(2) and two at C(3). The rest signals were due to protons of a lavandulyl group which comprised three methyl groups [two at C(5"), and one at C(8")] showed the signals at δ 1.47, 1.53, and 1.59, two methylene groups [C (3") and (1")] at δ 1.97 and 2.52, two methine groups [C (2") and C (4")] at δ 2.44 and 4.94, and two methylene protons of C(9") at δ 4.51 and 4.54.

The two dimensional ^{1}H-NMR (COSY) was useful to distinguish two methyl groups at C(5") from the other at C

(8"). By this way, the signal at δ 1.59, which showed the correlation to the signals of C(9")-H, was due to the methyl protons at C(8"), and the ones at δ 1.53 and 1.47 which showed the correlation to the signals of C(4")-H and C(3")-H, were due to the protons of two methyl groups at C(5").

The ^{13}C-NMR assignment was mainly based on the ^{13}C-^{1}H HETCOR spectrum. All signals of carbons possesing attached proton could be assigned in accordance with the ^{1}H-NMR assignment, except two methyl groups at C(5") which must be determined by comparing their ^{13}C chemical shifts. Because of the steric effect (or γ effect), the high field signal at δ 17.79 should be assigned to Z-C(7"), and the lower field signal at δ 25.72 to E-C(6"). So these methyl protons could be unambiguously assigned from the correlation in the ^{13}C-^{1}H HETCOR spectrum, as the signal at δ 1.47 was due to C(7")-H and the one at δ 1.53 was due to C(6")-H.

The structure was confirmed by the fragmentation at m/z 301 [M^{+}-123 (C_9H_{15})] and 123 which suggested the presence of a lavandulyl group. So it is no doubt to find the fragment at m/z 409 [M^{+}-15 (CH_3)] also. The fragment at m/z 136 ($C_8H_8O_2$), due to the retro Diels-Alder cleavage of the flavanone, suggested that the B ring of SE-1 has two hydroxyl groups. And the fragment at m/z 165 [301-136 ($C_8H_8O_2$)] suggested that the A ring has one lavandulyl and two hydroxyl groups.

$-CH_3$

$C_{24}H_{25}O_6$

m/z 409

HO

$-H_2O$

$C_{25}H_{26}O_5$

m/z 406

A

B

HO

HO

OH

$C_{25}H_{28}O_6$

M^+ m/z 424

RDL

CH_2

HO

A

B

HO

OH

OH

m/z 301

m/z 123

HO

A

OH

C=O

m/z 288

B

$H_2C=HC$

HO

OH

m/z 136

$-H_2O$

$C_{16}H_{11}O_5$

m/z 283
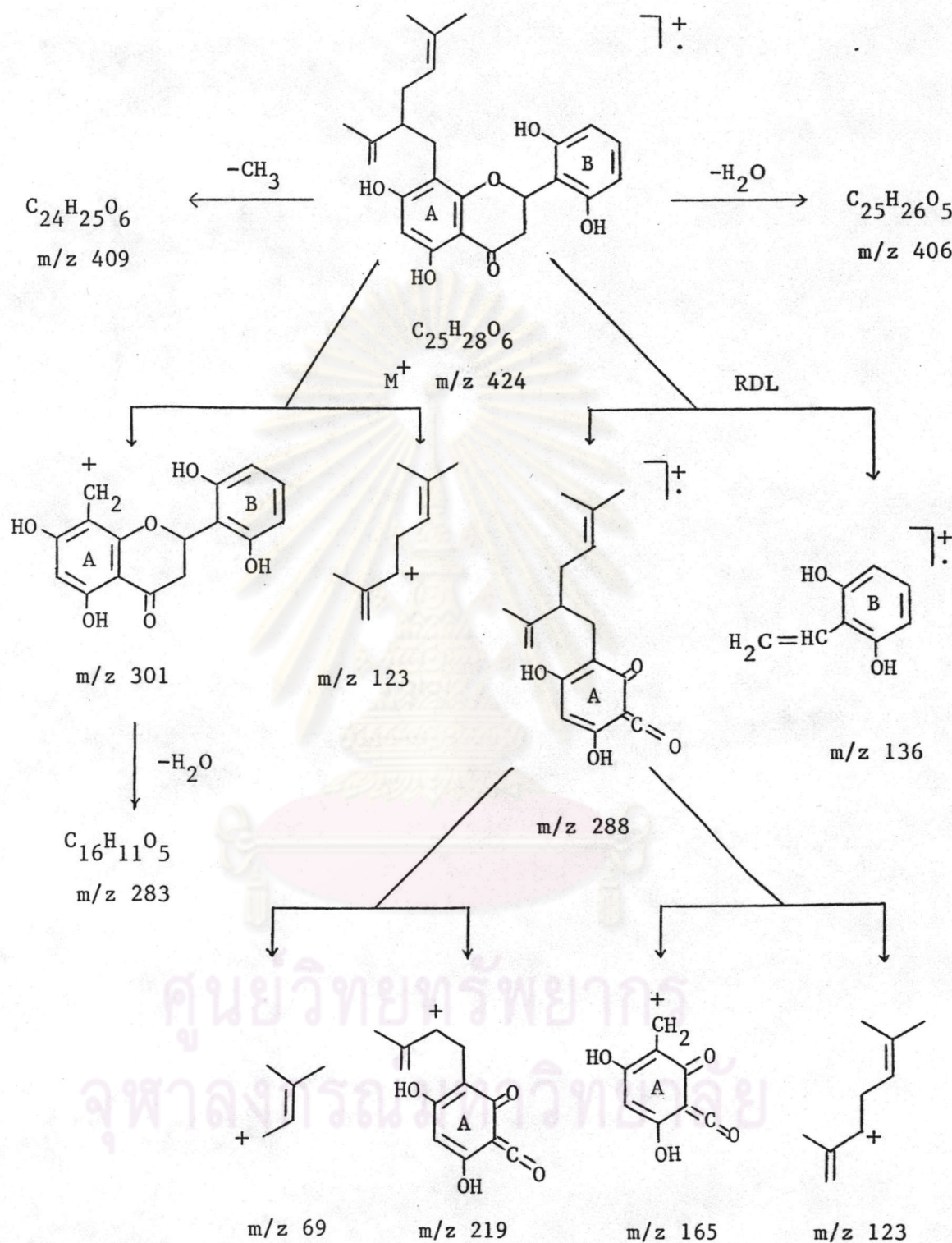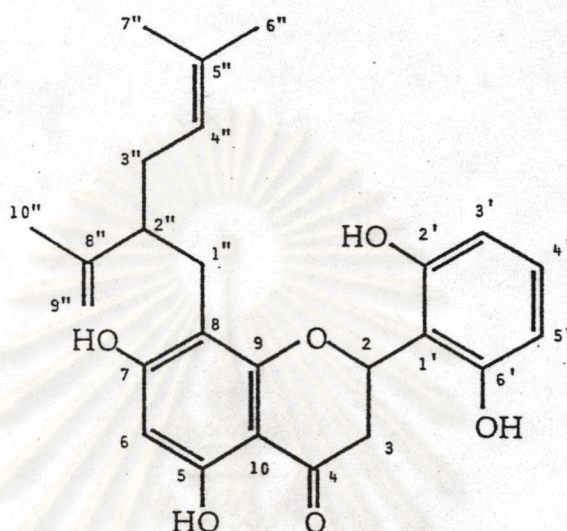
HO

A

OH

C=O

CH_2

HO

A

OH

C=O

m/z 69

m/z 219

m/z 165

m/z 123

Figure 4.2   Mass fragmentation of SE-1

From all of these informations, SE-1 is characterized
as 8-lavandulyl-5,7,2',6'-tetrahydroxyflavanone, the structure
of which is shown below :-

The second isolated compound from *Sophora exigua*
Craib root is SE-2. It gave negative result to Shinoda
test, dark green colour with ferric chloride solution
and the feature of UV spectrum (Fig. 3.24) showed the
flavanone chromophore. The IR spectrum (Fig. 3.28) also
showed the absorption bands of the hydroxyl group and the
chelated carbonyl group at 3440 and 1635 cm^{-1}, respectively.
As the same as SE-1, the UV spectra with varied shift
reagents (Fig. 3.25-3.27) exhibited the presence of 5,7-
dihydroxyflavanone moiety.

From the mass spectrum of SE-2 (Fig. 3.34), the
molecular ion peak at m/z 440, corresponding to the
elemental analysis, established the molecular formula as

$C_{25}H_{28}O_7$. The ^{1}H-NMR of SE-2, using hexadeuteroacetone as solvent (Fig. 3.29) showed signals in the same manner as those of SE-1, except the low field signals at δ 8.43 and 6.01. There are singlets representing one proton of the hydroxyl group and two symmetric protons of the aromatic B ring, respectively, instead of three adjacent symmetric protons of the B ring in SE-1. The ^{13}C-NMR of SE-2 also showed the signals in the same manner as SE-1, except those due to C(4'), C(1'), and C(3',5') which shifted to the higher field region at δ 160.28, 104.38, and 95.88, respectively. These shifts resulted from the shielding effect of the hydroxyl group adding to C-4'. The hydroxy substitution pattern in B ring can be confirmed by comparing the calculated chemical shifts of B ring carbon atoms. The effect of hydroxy substituent on the chemical shift of B ring carbon atoms could be estimated from the differences of those of pinocembrin (5,7-dihydroxyflavanone) and naringenin (5,7,4'-trihydroxyflavanone) (Table 4.1, Fig. 4.3) (Markham and Chari, 1982).

Table 4.1   The ^{13}C chemical shift and the effect of hydroxy substituent on the
^{13}C chemical shift in B ring of flavanone derivatives.

δ (PPM) ^{13}C-Position	Pinocembrin (5,7-dihydroxyflavanone)	Naringenin (5,7,4'-trihydroxyflavanone)	Difference (position to the hydroxyl group)
1'	138.0	128.9	-9.1 (p)
2'	126.5	128.2	+1.7 (m)
3'	128.5	115.2	-13.3 (o)
4'	128.5	157.8	+29.3 (ι)
5'	128.5	115.2	-13.3 (o)
6'	126.5	128.2	+1.7 (m)

<div align="center">

+1.7(m)   -13.3(σ)

R——B——OH

-9.1(ρ)   +29.3(ℓ)

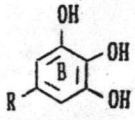+1.7(m)   -13.3(σ)
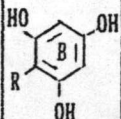
</div>
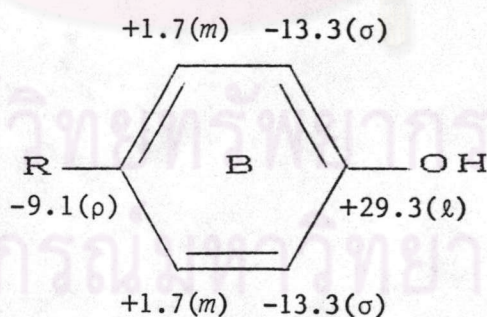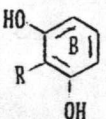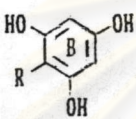
Figure 4.3   Shifts induced by introduction of a hydroxy group

in B ring

195

Table 4.2 The predicted ^{13}C chemical shifts in B ring of 8-lavandulyl-5,7,2',4',6'-pentahydroxyflavanone and 8-lavandulyl-5,7,3',4',5'-pentahydroxyflavanone and the observed ^{13}C chemical shifts in B ring of SE-1 and SE-2.

^{13}C Position	(observed from SE-1)	(predicted)	(predicted)	(predicted)	(observed from SE-2)
		8-Lavandulyl-5,7-dihydroxyflavanone derivatives with different hydroxy substitution patterns in B ring (ppm)			
1'	111.736	102.636 ((111.736+(-9.1)))	138.336 (111.736-(2x-13.3))	132.636 (138.336+(2x1.7)+(-9.1))	103.419
2'	157.735	159.435 (157.735+1.7)	126.735 (157.735-29.3-1.7)	106.035 (126.735+(-13.3)+1.7+(-9.1))	157.918
3'	108.272	94.972 (108.272+(-13.3))	130.672 (108.272-(-13.3)-(-9.1))	148.372 (130.672+29.3+(-13.3)+1.7)	95.943
4'	130.708	160.008 (130.708+29.3)	127.308 (130.708-(2x1.7))	130.008 (127.308+29.3+(2x-13.3))	159.435
5'	108.272	94.972 (108.272+(-13.3))	130.672 (108.272-(-13.3)-(-9.1))	148.372 (130.672+29.3+(-13.3)+1.7)	95.943
6'	157.735	159.435 (157.735+1.7)	126.735 (157.735-29.3-1.7)	106.035 (126.735+(-13.3)+1.7+(-9.1))	157.918

Using the principle of additivity of hydroxy substituent effect (Table 4.1, Fig. 4.3) and ^{13}C chemical shifts in B ring of SE-1, predicted ^{13}C chemical shifts in B ring of 8-lavandulyl-5,7,2',4',6'-pentahydroxyflavanone and 8-lavandulyl-5,7,3',4',5'-pentahydroxyflavanone could be compared with the observed ^{13}C chemical shifts in B ring of SE-2 (Table 4.2)

From these information, it can be concluded that SE-2 is 8-lavandulyl-5,7,2',4',6'-pentahydroxyflavanone, the structure of which is shown below :-
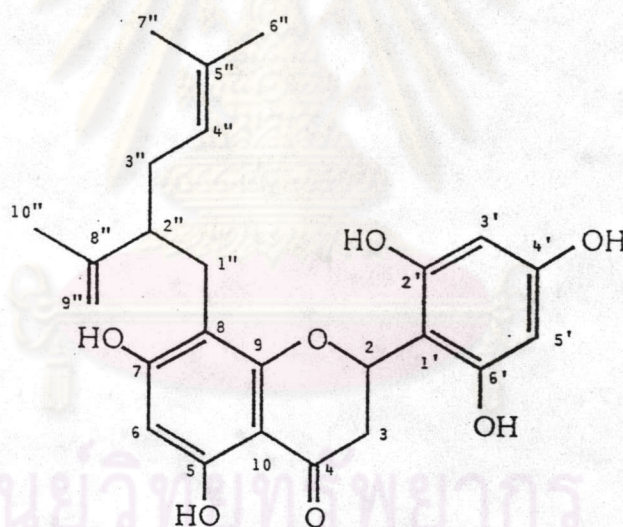
Both flavonoids isolated in this investigation have a lavandulyl group substitution. This is agree with a few chemotaxonomic points of view. The first one is that most of flavonoids with C_5 or C_{10} side chain were found in leguminous plants. According to the data survey of flavanone distribution in the genus Sophora in table 2.8, though flavanones are classified to be the natural minor

group by Bohm (1982), *Sophora* in one of the genus which enriched of various kinds of flavanone. In addition, it can be notified that the lavandulyl group may be one of the chemotaxonomic marker of the genus *Sophora*. So, this recent work supports the classification of genus *Sophora* in family Leguminosae and the chemotaxonomic significance agree with the morphological classification. These suggest that the contained flavonoids are useful taxonomic markers and will contribute to the establishment of chemotaxonomy on the genus *Sophora* after further accumulation of data on their occurrences and distributions.

The substitution pattern at C-2' and C-6' in the B ring of flavonoids is very rare in nature. Some of these are (±)-5,2'-dihydroxy-7,8,6'-trimethoxyflavanone, and (±)-5,2'-dihydroxy-6,7,6'-trimethoxyflavanone from the roots of *Scutellaria discolor* Colebr. and (2S)-5,7,2',6'-tetrahydroxyflavanone, (2R,3R)-3,5,7,2',6'-pentahydroxyflavanone, and skullcapflavone II (5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavone from *Scutellaria baicalensis* Georgi (Kimura et al, 1982; Tomimori et al, 1985; Wollenweber, 1982). So both SE-1 (8-lavandulyl-5,7,2',6'-tetrahydroxyflavanone) and SE-2 (8-lavandulyl-5,7,2',4',6'-pentahydroxyflavanone) are naturally uncommon flavanones.