



CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The roots of *Sophora exigua* Craib were collected from Sanamchaikade, Chachoengsao province, Thailand. Authentication was achieved by comparison with the herbarium specimens at Forest Herbarium, Royal Forest Department, Ministry of Agriculture and Cooperative, Phahonyothin Road, Bangkok, Thailand.

2. General Technigue

2.1 Thin-layer Chromatography (TLC)

- Adsorbent : Silica gel GF₂₅₄ (E. Merck) 30 g/60 ml of distilled water
- Plate size : 5 x 20 cm, 10 x 20 cm, 20 x 20 cm
- Layer thickness : 0.25 mm
- Activation : Air-dried for 15 minutes and then at 110°C for 1 hour
- Technique : One way, ascending
- Solvent systems : a) chloroform : ethyl acetate (10:1)
b) petroleum ether : diethyl ether (1:2)
c) cyclohexane : diethyl ether (1:4)
d) benzene : acetone (7:3)
e) chloroform : ethanol (92:8)

Distance : 15 cm

Laboratory temperature : 24-33°C

Development : In saturated chromatographic tank

Detection : a) Ultraviolet light at wavelength 254 and 366 nm.

b) Anisaldehyde-sulphuric acid spraying reagent (0.5% methanolic solution of anisaldehyde with 5% sulfuric acid)

The Phenols give red spots after heating at 100-105°C for 2-4 minutes.

c) Ferric chloride spraying reagent (5% solution of ferric chloride in 0.5 N hydrochloric acid)

The phenols give blue or greenish spots as positive test.

d) Vanillin-hydrochloric acid spraying reagent (5% vanillin in ethanol with concentrated hydrochloric acid in the ratio 4:1, just prior to use.)

Catechins and proanthocyanins give red or purplish-red spots immediately after spraying and warming, whilst flavanones and dihydroflavonols do more slowly.

2.2 Column Chromatography (CC)

Column size : 9.3 x 45, 7.9 x 45, 4.6 x 45, 2.2 x 45 and 1.7 x 45 cm glass columns, depending on the quantity of sample to be separated.

- Adsorbent : Silica gel 60(0.040-0.063 mm, E. Merck)
- Packing method : a) Dry packing
b) Wet packing
- Sample loading : a) The sample was dissolved in small amount of organic solvent, mixed with a small quantity of adsorbent, then dried, triturated and added gently on the top of the column.
b) The sample was dissolved in small amount of organic solvent, and slowly added onto the top of the column.
- Solvent system : a) acetone
b) chloroform : ethanol (99.7: 0.3, 99.5: 0.5, 95:5, 9:1)
c) cyclohexane : diethyl ether (7:3)
d) hexane : acetone (8:2, 7:3)
e) petroleum ether : acetone (85:15, 3:1, 1:1)
f) petroleum ether : diethyl ether (65:35, 4:6, 3:7)
- Collection of eluate: Fractions of 3, 15, 20, 25, 30 and 50 ml were collected.
- Examination of eluate: Fractions were examined by TLC using ultraviolet light (254 and 366 nm), and followed by anisaldehyde-sulphuric acid spraying reagent or vanillin-hydrochloric acid spraying reagent. Those which showed similar patterns were combined together.

2.3 Physical Constant

2.3.1 Melting points were determined by Gallenkamp melting point apparatus. The values recorded were uncorrected.

2.3.2 Optical rotations were determined by Polax (No. 45014) polarimeter.

2.3.3 Elemental analysis were determined by Perkin Elmer 240 C elemental analyzer.

2.4 Spectroscopy

2.4.1 Ultraviolet absorption spectra were obtained with a Shimadzu UV 240 double beam spectrophotometer.

2.4.2 Infrared absorption spectra were obtained with a Perkin Elmer 283 spectrophotometer. The material were examined in potassium bromide disc.

2.4.3 Proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectra were obtained with a JEOL FX-90 spectrometer (90 MHz), a Varian XL-300 spectrometer (300 MHz), and a Bruker AM-500 spectrometer (500 MHz).

2.4.4 Carbon-13 nuclear magnetic resonance ($^{13}\text{C-NMR}$) spectra were obtained with a Varian XL-300 spectrometer (75 MHz), and a Bruker AM-500 spectrometer (125 MHz).

2.4.5 The two dimensional homonuclear shift correlation (COSY) spectra were obtained with a Bruker AM-500 spectrometer (500 MHz).

2.4.6 The two dimensional heteronuclear shift

correlation (HETCOR) spectra were obtained with a Bruker AM-500 spectrometer (125 MHz).

All nuclear magnetic resonance spectra were taken by using tetramethylsilane (TMS) as an internal reference. Chemical shifts were reported as value (ppm).

2.4.7 Mass spectra were obtained on a JEOL DX 300 mass spectrometry for EIMS. Operating at 70 eV with direct inlet system.

3. The Extraction and Isolation of Chemical Constituents from the Roots of *Sophora exigua* Craib.

3.1 Extraction

The dried, coarsely powdered roots of *Sophora exigua* Craib (1 kg) were extracted thoroughly by percolation with 95% ethanol (12 liters) for seven days. The percolate was evaporated under reduced pressure to yield black-brown syrupy mass (150 g). The syrupy mass was dissolved with 20% ethanol (250 ml), then defatted with petroleum ether (5 x 200 ml). The ethanolic-aqueous layer was further extracted with diethyl ether (5 x 200 ml). The combined diethyl ether extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a brown crude extract (83.7 g).

3.2 Isolation

The crude extract was divided into nine portions, and treated in the same manner. Each was

dissolved in diethyl ether, mixed with small amount of silica gel 60, dried, triturated and added to the top of silica gel column (9.3 x 45 cm). The column was eluted with petroleum ether : acetone (85 : 15, 3 : 1, 1 : 1), and acetone, then washed with methanol. Fifty ml fractions were collected and compared by TLC. The eluting solvents were altered to more polar systems when the difference patterns on TLC were not observed. Those fractions of similar TLC pattern were combined, concentrated and named from F-1 to F-5.

Table 3.1 Information of the isolation of *Sophora* crude extract by column chromatography.

Fraction number	Eluting solvent	Combined fraction
1-37	petroleum ether:acetone 85:15 3:1	F-1
38-69	3:1	F-2
70-132	3:1	F-3
133-160	1:1	F-4
161-175	acetone acetone	F-5

Only two combined fractions, F-2 and F-4 had been further investigated.

3.2.1 Isolation of Chemical Constituent from the Fraction F-2

The fraction F-2 (4.769 g) was shown by TLC (silica gel GF₂₅₄/chloroform : ethanol 99.5 : 0.5) to contain at least five chemical constituents (Fig. 3.2) which gave yellow and orange colour to anisaldehyde-sulphuric acid spraying reagent. It was dissolved in twenty ml of chloroform : ethanol (99.7:0.3) and added to the top of silica gel column (4.6 x 45 cm). The column was eluted with chloroform : ethanol (99.7:0.3 and 99.5:0.5). Thirty ml fractions were collected, examined by TLC and the similar fractions were combined to give the following portions (Fig. 3.2):-

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 3.2 Information of the isolation of F-2 by column chromatography

Fraction number	Eluting solvent	Combined fraction	Remarks
	chloroform:ethanol		
1-8	99.7:0.3	-	trace of substance
9-21	99.5:0.5	F-2A	containing at least three substances (1.0798 g)
22-48	99.5:0.5	-	containing traces of substances (0.7124 g)
49-62	99.5:0.5	F-2B	containing at least three substances and flavonoid SE-1 (1.1805 g)
63-88	99.5:0.5	F-2C	containing at least three substances and small amount of SE-1 (0.3087 g)
89-100	99.5:0.5	-	containing traces of substances (0.2002 g)

The substances were detected under UV light at 254 nm.

Only fraction F-2B (49-62) had been further investigated. It was shown by TLC (silica gel GF₂₅₄/chloroform : ethanol 99.5 : 0.5 and hexane : acetone 7 : 3) to contain at least three substances and one flavonoid, SE-1 (Fig. 3.2 and 3.3). It was dissolved in hexane : acetone (8:2) about ten ml added to the top of silica gel column (2.2 x 45 cm). The column was eluted with hexane : acetone (8:2 and 7:3). Twenty ml fractions were collected. The fractions were examined by TLC and the similar fractions were combined to give the following portions (Fig. 3.3) :-



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 3.3 Information of the isolation of F-2B by column chromatography.

Fraction number	Eluting solvent	Combined fraction	Remarks
	hexane:acetone		
1-16	8:2	-	trace of substance
17-29	8:2	F-2B ₁	containing S ₁ and S ₂ (26.8 mg)
30-60	8:2	F-2B ₂	containing S ₁ , S ₂ and small amount of SE-1 (72.9 mg)
61-70	7:3	F-2B ₃	containing SE-1 and small amount of S ₂ (441.1 mg)
71-100	7:3	F-2B ₄	containing small amount of SE-1 and S ₃ (109.7 mg)

Only fraction F-2B₃ (61-70) had been further investigated. The TLC pattern (silica gel GF₂₅₄/cyclohexane : diethyl ether 7:3) was shown in Fig. 3.4. F-2B₃ was added to the top of silica gel column (1.7 x 45 cm), then eluted

with cyclohexane : diethyl ether (7:3) and collected three ml for each fractions. The flavonoid SE-1 (213.0 mg) was isolated from the fractions 9-30. It was concentrated to small volume and obtained pale yellow needles which were purified by recrystallization.

3.2.2 Isolation of Chemical Constituent from the Fraction F-4

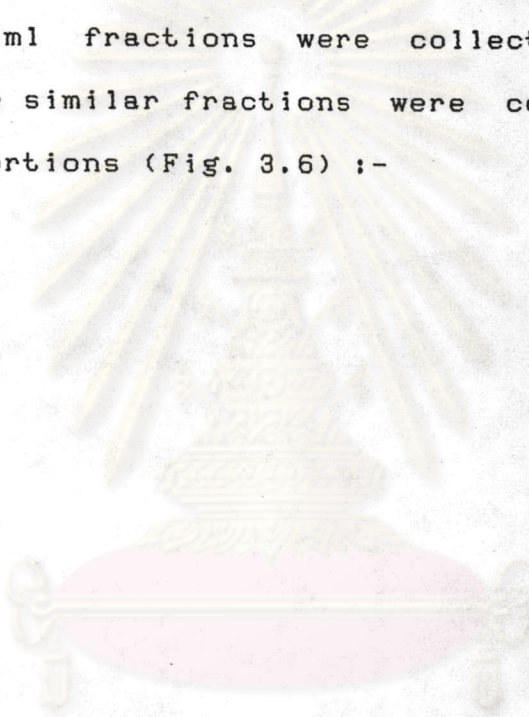
The fraction F-4 (7.740 g) was shown by TLC (silica gel GF₂₅₄/chloroform : ethanol 95:5) to contain at least six chemical constituents (Fig. 3.5) which gave orange and orange-brown colour to anisaldehyde-sulphuric acid spraying reagent. It was dissolved in twenty ml of chloroform : ethanol (95:5) and added to the top of silica gel column (7.9 x 45 cm). The column was eluted with chloroform : ethanol (95:5 and 9:1). Fifty ml fractions were collected. The fractions were examined by TLC and the similar fractions were combined to give the following portions (Fig. 3.5) :-

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 3.4 Information of the isolation of F-4 by column chromatography.

Fraction number	Eluting solvent	Combined fraction	Remarks
	chloroform:ethanol		
1-4	95:5	-	trace of substance
5-32	95:5	F-4A	containing at least four substances (0.8988 g)
33-64	95:5	F-4B	containing at least four substances (2.3590 g)
65-92	95:5	F-4C	containing at least five substances (1.4708 g)
93-133	95:5	F-4D	containing flavonoid SE-2 and at least three substances (2.1000 g)
134-146	9:1	F-4E	containing at least three substances (0.6475 g)

Only fraction F-4D (93-133) had been further investigated. F-4D, shown by TLC (silica gel GF₂₅₄/petroleum ether : diethyl ether 4:6), was comprised of at least five substances (Fig. 3.6). It was placed onto the top of silica gel column (4.6 x 45 cm). The column was eluted by the mixture of petroleum ether : diethyl ether (65:35, 4:6 and 3:7), with the volume of 350, 2050, and 400 ml, respectively. Twenty-five ml fractions were collected, and examined by TLC. The similar fractions were combined to give the following portions (Fig. 3.6) :-



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 3.5 Information of the isolation of F-4D by column chromatography

Fraction number	Eluting solvent	Combined fraction	Remarks
1-20	petroleum ether : diethyl ether 65:35, 4:6	-	trace of substance
21-37	4:6	F-4D ₁	containing T ₁ , T ₂ and small amount of SE-2 (179.7 mg)
38-75	4:6	F-4D ₂	containing SE-2 and small amount of T ₁ and T ₂ (964.0 mg)
76-112	4:6, 3:7	F-4D ₃	containing small amount of SE-2, T ₂ , T ₃ and T ₄ (61.5 mg)

Only fraction F-4D₂ (38-75) had been further investigated. It was shown by TLC (silica gel GF₂₅₄ / chloroform : ethanol 95 : 5) to contain one interesting flavonoid, SE-2, and the other two substances (T₁ and T₂) (Fig. 3.7). F-4D₂ was added to the top of silica gel column (2.2 x 45 cm), then eluted with chloroform : ethanol

(95 : 5), and fifteen ml fractions were collected. The flavonoid SE-2 was isolated from the fractions 24-56. It was concentrated to small volume and purified by crystallization to obtained white microcrystalline powder. (95.3 mg).

4. Characterization of the Isolated Flavonoids

The isolated flavonoids were characterized by the data of hRf values, melting point, specific rotation, colour reactions, elemental analysis, ultraviolet, infrared, nuclear magnetic resonance and mass spectra.

4.1 Characterization of SE-1

SE-1 was obtained as pale yellow needle crystals. It is slightly soluble in chloroform, diethyl ether and soluble in acetone, ethanol.

hRf value (Fig 3.8-3.12)

The hRf values given are obtained from the following systems :-

a) chloroform : ethyl acetate (10:1)	= 40.7
b) petroleum ether : diethyl ether (1:2)	= 63.0
c) cyclohexane : diethyl ether (1:4)	= 59.0
d) benzene : acetone (7:3)	= 59.3
e) chloroform : ethanol (92:8)	= 76.7

Melting point

169-170°C (uncorrected)

Specific optical rotation

$$[\alpha]_D^{25} = -100 \quad (c = 0.001 \text{ g/ml, ethanol})$$

Colour reaction

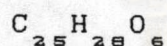
SE-1 gave bright pink colour with Shinoda test (magnesium-hydrochloric acid reaction) and dark green colour with 1% ferric chloride solution.

Elemental analysis

C	=	70.985 %
H	=	6.810 %
O	=	22.205 %

Molecular weight

424

Molecular formulaUltraviolet absorption spectral data

λ max (MeOH) :	291.5 (log ϵ = 4.30),		
	337.5 (log ϵ = 3.62) nm	(Fig 3.13)	
λ max (+AlCl ₃):	314.5, 392 nm	(Fig 3.14)	
λ max (+AlCl ₃ +HCl):	312.5, 386 nm	(Fig 3.14)	
λ max (+NaOH):	333.5 nm	(Fig 3.15)	
λ max (+NaOAc):	287.5, 333 nm	(Fig 3.16)	
λ max (+NaOAc+H ₃ BO ₃):	291.5, 335 nm	(Fig 3.16)	

Infrared absorption spectral data (KBr disc)

$\bar{\nu}$ max (cm^{-1}) : 3450 (O-H stretching), 2920, 1640 (C=O stretching), 1610 (C=C stretching), 1470, 1390, 1185, 1070 (Fig. 3.17).

Proton nuclear magnetic resonance spectral data

a) In the mixture of hexadeuterated acetone (CD_3COCD_3) and deuterated chloroform (CDCl_3) at 90 MHz and TMS as an internal reference (Fig. 3.18).

δ (ppm)	Proton	Multiplicity	J (Hz)
12.18	C(5)-OH	(1H) s	-
9.22	C(7)-OH	(1H) s	-
7.91	C(2',6')-OH	(2H) s	-
7.04	C(4')-H	(1H) t	8.2
6.48	C(3',5')-H	(2H) d	8.2
6.10	C(6)-H	(1H) s	-
6.02	C(2)-H	(1H) dd	12.1, 3.5
4.98	C(4'')-H	(1H) t	6.3
4.61	C(9'')-H Z^*	(1H) s	-
4.57	C(9'')-H E^{**}	(1H) s	-
3.44	C(3)-H E	(1H) dd	15.2, 12.1
2.65	C(1'')-H ₂	(2H) d	-
2.55	C(2'')-H	(1H) m	-
2.55	C(3)-H Z	(1H) dd	15.2, 3.5
2.05	C(3'')-H ₂	(2H) m	-
1.64	C(10'')-H ₃	(3H) s	-

δ (ppm)	Proton	Multiplicity	J. (Hz)
1.57	C(6'')-H ₃	(3H) s	-
1.49	C(7'')-H ₃	(3H) s	-

* = *cis*

** = *trans*

b) In tetradeuterated methanol (CD₃OD) at 300 MHz and TMS as an internal reference (Fig. 3.19).

δ (ppm)	Proton	Multiplicity	J (Hz)
6.98	C(4')-H	(1H) t	8.1
6.36	C(3',5')-H ₂	(2H) d	8.1
5.91	C(2)-H	(1H) dd	14.1, 3.0
5.91	C(6)-H	(1H) s	-
4.94	C(4'')-H	(1H) t	6.5
4.54	C(9'')-H <i>Z</i>	(1H) s	-
4.51	C(9'')-H <i>E</i>	(1H) s	-
3.91	C(3)-H <i>E</i>	(1H) dd	16.8, 14.1
2.52	C(1'')-H	(2H) d	-
2.44	C(2'')H	(1H) m	-
2.44	C(3)-H <i>Z</i>	(1H) dd	16.8, 3.0
1.97	C(3'')-H ₂	(2H) m	-
1.59	C(10'')-H ₃	(3H) s	-
1.53	C(6'')-H ₃	(3H) s	-
1.47	C(7'')-H ₃	(3H) s	-

Carbon-13 nuclear magnetic resonance spectral data

In hexadeuterated acetone (CD_3OCD_3) at 125 MHz and TMS as an internal reference (Fig 3.20).

<u>δ(ppm)</u>	<u>Carbon</u>	<u>Multiplicity</u>
198.75	C-4	s
165.22	C-7	s
163.09*	C-5	s
162.27*	C-9	s
157.74	C-2', 6'	s
149.18	C-8"	s
131.55	C-5"	s
130.71	C-4'	d
124.45	C-4"	d
111.74	C-1'	s
111.03	C-9"	t
108.27	C-3', 5'	d
107.84	C-8	s
103.14	C-10	s
96.18	C-6	d
73.66	C-2	d
47.77	C-2"	d
40.63	C-3	t
31.89	C-3"	t
27.72	C-1"	t
25.76	C-6"	q
19.03	C-10"	q
17.79	C-7"	q

* Values may be interchanged

Two dimensional nuclear magnetic resonance spectrometry

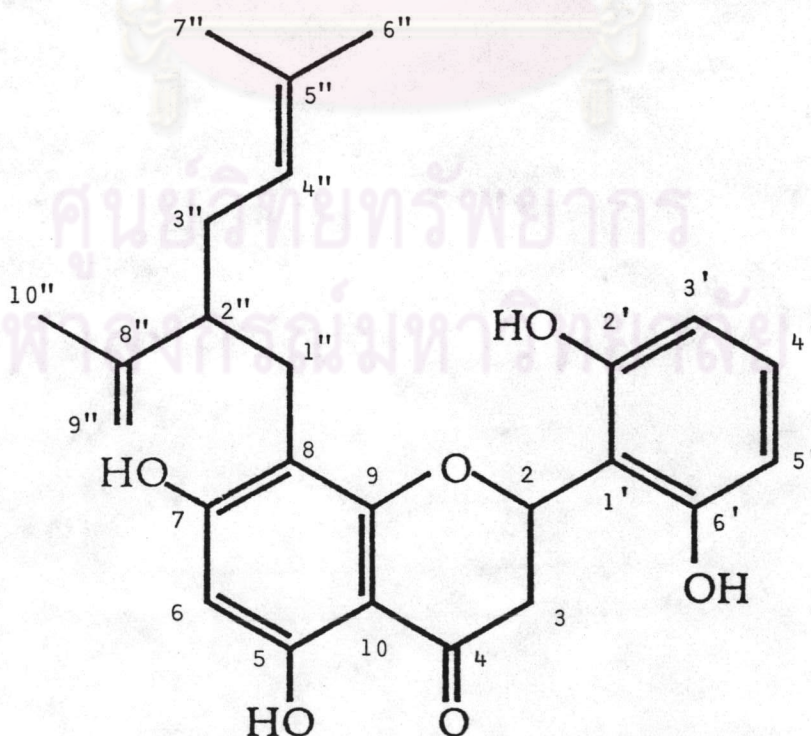
The two dimensional NMR spectra (COSY and HETCOR) of SE-1 in hexadeuterated acetone and TMS as internal reference are shown in Fig. 3.21 and 3.22, respectively.

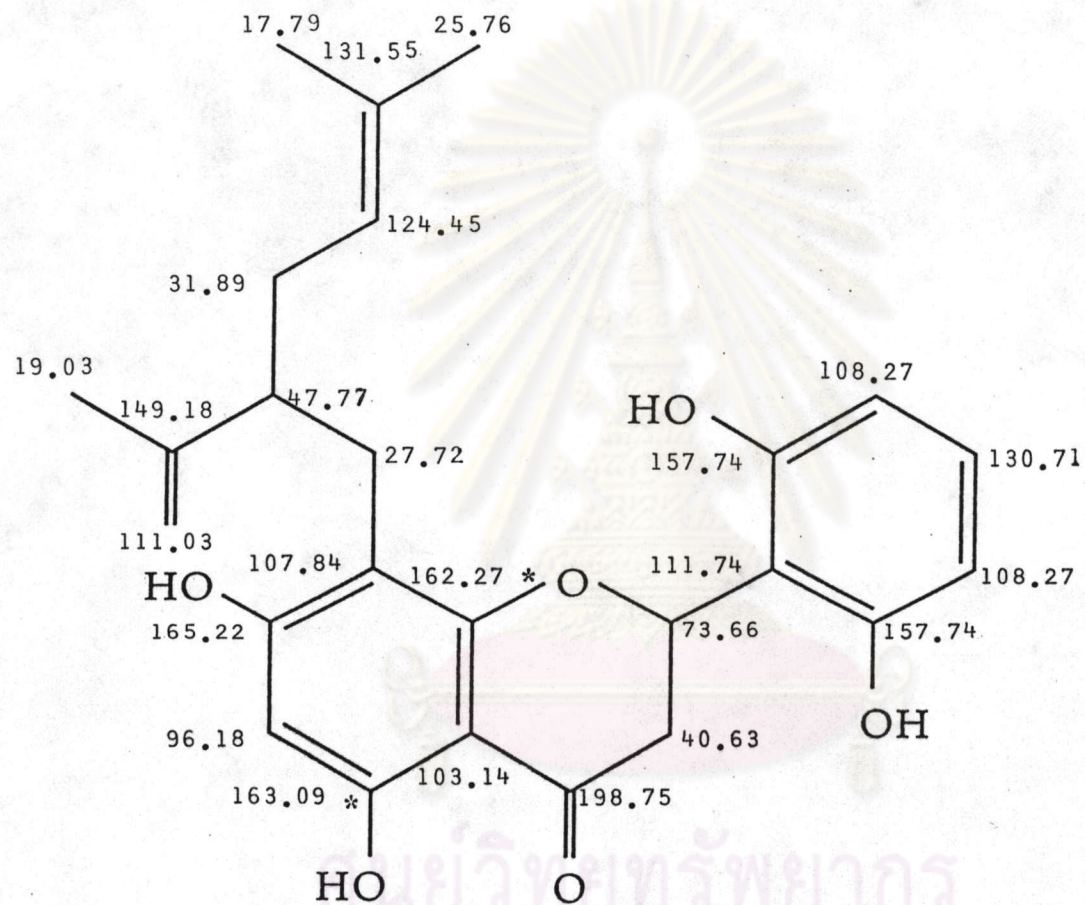
Mass spectral data

El, m/z (% relative intensity)

425 ($M^+ + 1$, 4.19), 424 (M^+ , 14.21), 406 (5.42), 302 (15.98), 301 (83.32), 284 (19.54), 283 (100.00), 219 (10.77), 166 (10.30), 165 (58.69), 138 (6.48), 136 (4.35), 123 (8.64), 69 (10.25), 55 (8.91), 41 (16.93). (Fig. 3.23)

The results were concluded for the chemical structure of SE-1 as 8-lavandulyl-5,7,2',6'-tetrahydroxyflavanone, a new flavanone. The structure is shown below.





(* = Values may be interchanged)

^{13}C -NMR assignment of SE-1. (In hexadeuterated acetone)

4.2 Characterization of SE-2

SE-2 was obtained as white microcrystalline powder. It was soluble in acetone, ethanol and methanol.

hRf value (Fig 3.8-3.12)

The hRf values given are obtained from the following systems :-

a) chloroform : ethyl acetate (10:1)	= 2.7
b) petroleum ether : diethyl ether (1:2)	= 20.3
c) cyclohexane : diethyl ether (1:4)	= 20.7
d) benzene : acetone (7:3)	= 34.0
e) chloroform : ethanol (92:8)	= 21.0

Melting point

234-235°C (decomposed)

Specific optical rotation

$[\alpha]_D^{25} - 100$ (c = 0.001 g/ml, ethanol)

Colour reaction

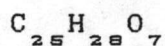
SE-2 gave negative result to Shinoda test (magnesium-hydrochloric acid reaction) and dark green colour to 1% ferric chloride solution.

Elemental analysis

C	=	67.925 %
H	=	6.430 %
O	=	25.645 %

Molecular weight

440

Molecular formulaUltraviolet absorption spectral data

λ max (MeOH) :	292.5 (log ϵ = 4.32),
	335 (log ϵ = 3.59) nm (Fig. 3.24)
λ max (+AlCl ₃):	311, 360.5 nm (Fig. 3.25)
λ max (+AlCl ₃ +HCl):	311, 361 nm (Fig. 3.25)
λ max (+NaOH):	329 nm (Fig. 3.26)
λ max (+NaOAC):	293, 329 nm (Fig. 3.27)
λ max (+NaOAC+H ₃ BO ₃):	292, 334 nm (Fig. 3.27)

Infrared absorption spectral data (KBr disc)

$\bar{\nu}$ max (cm⁻¹) : 3360 (O-H stretching), 2940, 1635 (C=O stretching), 1600 (C=C stretching), 1473, 1450, 1310, 1205, 1155, 1110, 1080, 885 (Fig. 3.28).

Proton nuclear magnetic resonance spectral data

a) In hexadeuterated acetone (CD₃COCD₃) at 500 MHz and TMS as an internal reference (Fig 3.29).

δ (ppm)	Proton	Multiplicity	J (Hz)
12.62	C(5)-OH	(1H) s	-
9.77	C(7)-OH	(1H) s	-
8.60	C(2',6')-OH	(2H) s	-
8.43	C(4')-OH	(1H) s	-

δ (ppm)	Proton	Multiplicity	J (Hz)
6.01	C(3',5')-H	(2H) s	-
5.96	C(6)-H	(1H) s	-
5.87	C(2)-H	(1H) dd	14.1, 2.8
5.04	C(4'')-H	(1H) t	6.3
4.59	C(9'')-H <i>Z</i>	(1H) s	-
4.55	C(9'')-H <i>E</i>	(1H) s	-
3.91	C(3)-H <i>E</i>	(1H) dd	17.0, 14.5
2.61	C(1'')-H ₂	(2H), m	-
	C(2'')-H	(1H)	
2.44	C(3)-H <i>Z</i>	(1H) dd	17.3, 2.7
2.09	C(3'')-H ₂	(2H) m	-
1.70	C(10'')-H ₃	(3H) s	-
1.61	C(6'')-H ₃	(3H) s	-
1.54	C(7'')-H ₃	(3H) s	-

b) In tetradeuterated methanol (CD₃OD) at 300 MHz and TMS as an internal reference (Fig. 3.30)

δ (ppm)	Proton	Multiplicity	J (Hz)
5.89	C(3',5')-H	(2H) s	-
5.89	C(6)-H	(1H) s	-
5.80	C(2)-H	(1H) dd	14.1, 2.9
5.04	C(4'')-H	(1H) t	6.2
4.60	C(9'')-H <i>Z</i>	(1H) s	-
4.54	C(9'')-H <i>E</i>	(1H) s	-
3.93	C(3)-H <i>E</i>	(1H) dd	17.5, 14.1
2.61	C(1'')-H	(2H) d	-

δ (ppm)	Proton		Multiplicity	J (Hz)
2.60	C(2'')-H	(1H)	m	-
2.39	C(3)-H Z	(1H)	dd	17.3, 2.9
2.07	C(3'')-H	(2H)	m	-
1.70	C(10'')-H _s	(3H)	s	-
1.63	C(6'')-H _s	(3H)	s	-
1.56	C(7'')-H _s	(3H)	s	-

Carbon-13 nuclear magnetic resonance spectral data

In tetradeuterated methanol (CD₃OD) at 75 MHz and TMS as an internal reference (Fig 3.31).

δ (ppm)	Carbon	Multiplicity
199.99	C-4	s
166.28	C-7	s
163.50 [*]	C-5	s
163.22 [*]	C-9	s
160.28	C-4'	s
158.20	C-2', 6'	s
149.64	C-8''	s
131.87	C-5''	s
124.90	C-4''	d
111.28	C-9''	t
108.80	C-8	s
104.38	C-1'	s
103.15	C-10	s
95.88	C-3', 5'	d
95.26	C-6	d

<u>δ(ppm)</u>	<u>Carbon</u>	<u>Multiplicity</u>
73.89	C-2	d
48.00	C-2"	d
41.35	C-3	t
32.51	C-3"	t
27.67	C-1"	t
25.91	C-6"	q
18.97	C-10"	q
17.90	C-7"	q

* Values may be interchanged

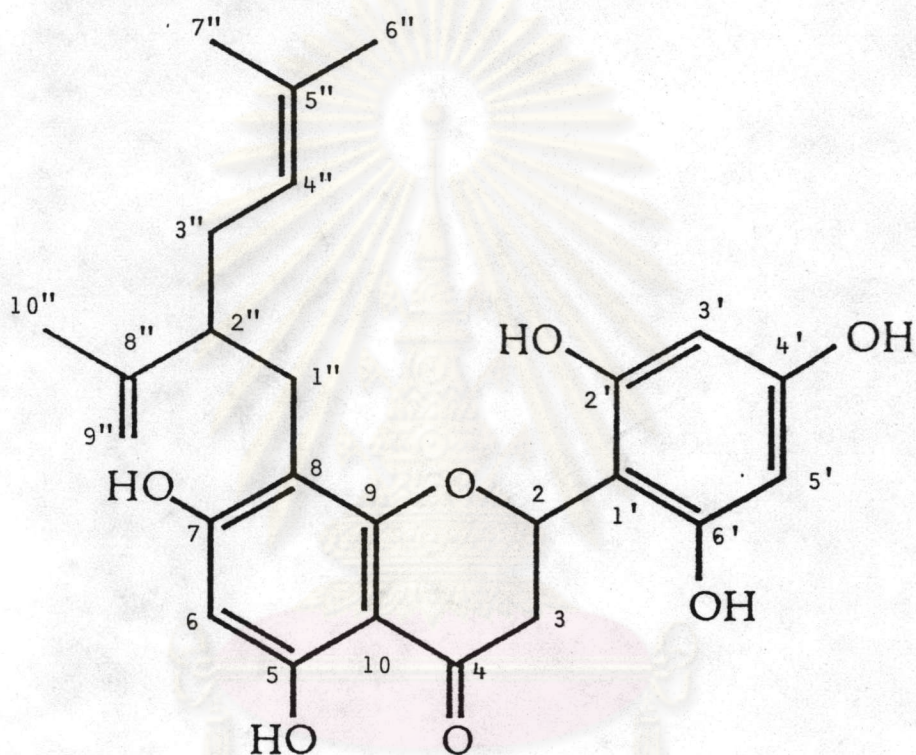
Two dimensional nuclear magnetic resonance spectrometry

The two dimensional NMR spectra (COSY and HETCOR) of SE-2 in hexadeuterated acetone and TMS as an internal reference are shown in Fig. 3.32 and 3.33, respectively.

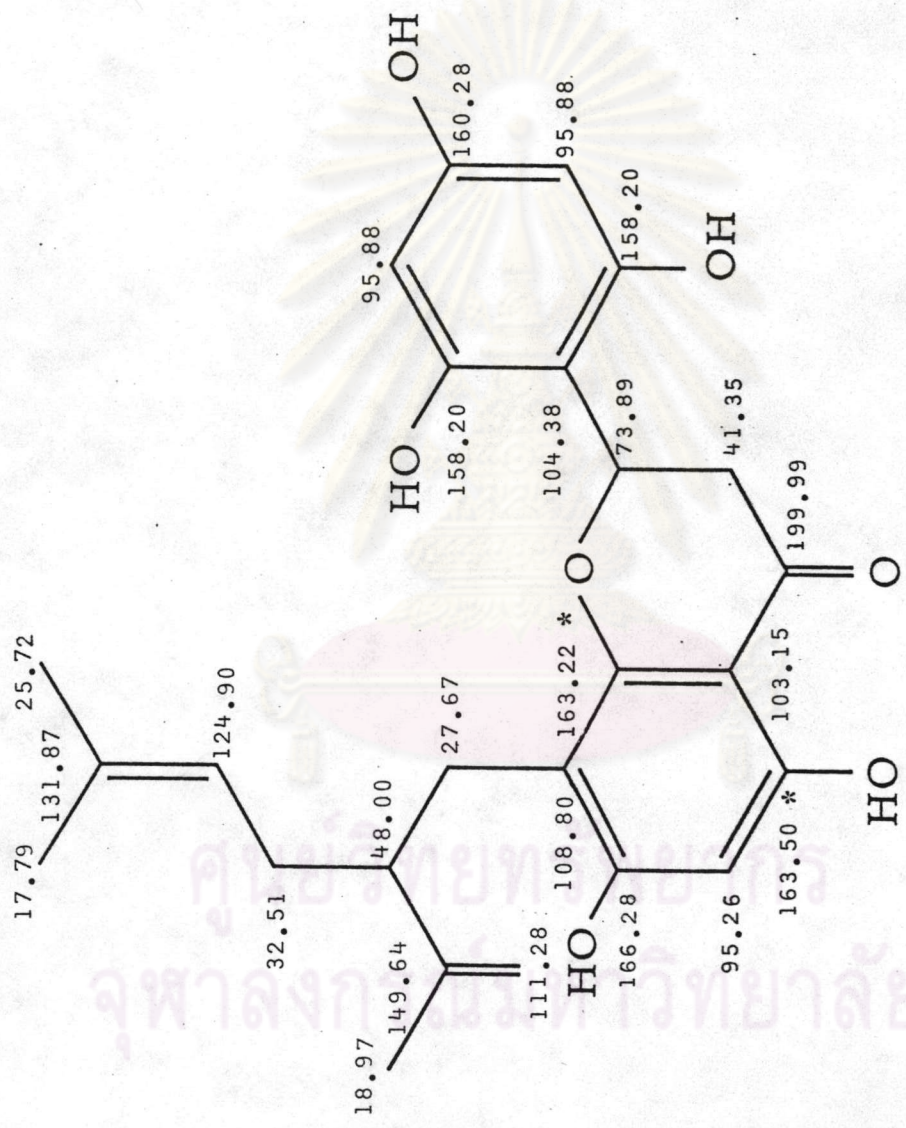
Mass spectral data

EI, m/z (% relation intensity)
 441 ($M^+ + 1$, 2.28), 440 (M^+ , 9.62), 422 (5.71),
 317 (30.65), 299 (81.99), 219 (7.66), 191 (45.59), 165
 (32.57), 152 (5.56), 139 (19.16), 126 (76.63), 124 (77.59),
 109 (100.00), 81 (48.66), 69 (53.45), 67 (88.70), 55 (31.03),
 44 (44.83), 41 (74.90) (Fig. 3.34).

The results were concluded for the chemical structure of SE-2 as 8-lavandulyl-5,7,2',4',6'-pentahydroxyflavanone, a new flavanone. The structure is shown below.



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



^{13}C -NMR assignment of SE-2. (In tetradeuterated methanol)