CHAPTER IV

RESULTS AND DISCUSSION

The fresh aerial (EU9) and subterranean (EU10) parts of this plant (10 kg each) were separately ground and extracted at room temperature with 95%ethanol. The extracts were concentrated *in vacuo* and then were partitioned with solvents having different polarity. The percentage yield of all of the extracts which was calculated based on fresh plant weight is shown in Table 4.1.

Table 4.1 The amount and percentage yield of extracts from *C. odorata*

Solvents of partition	Weight (g) and %yield*	
	EU9	EU10
Dichloromethane	-	19 (0.19%)
Hexane	87 (0.87%)	-
90%methanol	105 (1.05%)	-
Butanol	38 (0.38%)	26 (0.26%)
Water	384 (3.84%)	182 (1.82%)

*The percentage yield was calculated on a fresh weight basis

The Antibacterial Activity Results

The essential oil and some fractions from both aerial parts and subterranean parts showed antibacterial activity against *S. aureus* and *E. coli* (Table 4.2). The antibacterial activity of the essential oil was similar to the result of Inya-Agha, S.I. (12), however, their chemical constituents may be different. This is the first report of the antibacterial activity of the subterranean parts of this plant. Fraction EU9W and EU10W, which were the major fractions of the crude extract, did not show activity against *S. aureus* and *E. coli*. This result indicated that the partition method was an advantageous procedure to eliminate a large amount of inactive material which, in turn, reduced cost and time to find out the active compounds.

Table 4.2 The antibacterial activity results of *C. odorata* fractions

Test samples	Diameter of inhibition zone (mm)[a]	
	S. aureus	*E. coli*
Essential oil	11.0	7.0
EU9H	6.5	6.5
EU9M	9.0	6.5
EU9B	9.5	inactive
EU9W	inactive	inactive
EU10C	10.5	7.5
EU10B	8.5	inactive
EU10W	inactive	inactive

[a] the diameter of paper disc = 6.0 mm

Chemical Components Analysis of the Essential Oil

The identification of oil constituents was performed by a comparison of mass spectra with literature data (NIST and NISTREP) and by a comparison of their retention indices (RI) with those in the literature (136-137). Twenty-two components were identified in the order of their elution on the BP-5 capillary column used for GC-FID analysis (Table 4.3). A typical gas chromatogram of the essential oil of *C. odorata* is presented in Figure 4.

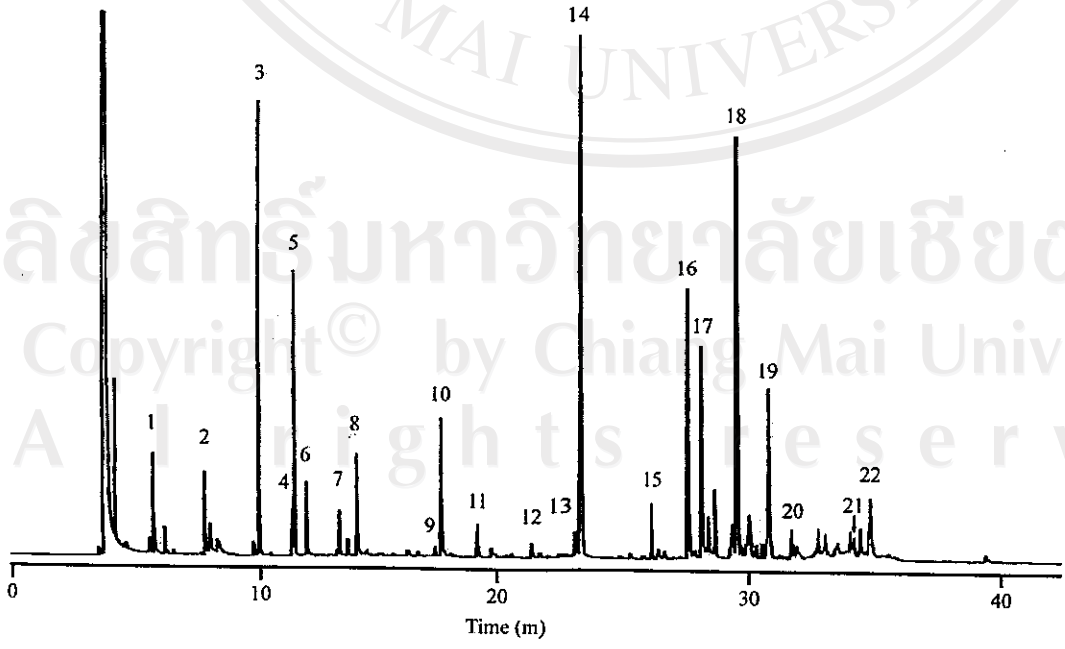


Figure 4. The gas chromatogram of the essential oil of *C. odorata*

Table 4.3 The volatile components in aerial parts of *C. odorata*

Peak No.	Compounds	RA[a] (%)	RI[b] (Exp.)	RI[c] (Lit.)	MW[d]	Identification[e]	Previous citation
1	2,3-Dihydro-4-methylfuran	1.58		-	84	2	(138)
2	2-Hexenal	1.61	854	-	98	2	(139)
3	α-Pinene	8.36	934	939[T]	136	1,2	(12,43-47)
4	Sabinene	0.92	973	976[T]	136	1,2	(43-45,47)
5	β-Pinene	5.64	977	980[T]	136	1,2	(43-47)
6	Myrcene	1.47	992	991[T]	136	1,2	(43-47)
7	Limonene	1.01	1029	1031[T]	136	1,2	(12,43-47)
8	*trans*-Ocimene	2.22	1049	1050[T]	136	1,2	(43,45-46)
9	Geijerene isomer	0.31	1138	-	162		(46-47)
10	Geijerene	3.08	1144	1144[T]	162	1,2	(43-47)
11	Terpinen-4-ol	0.80	1183	1177[T]	154	2	(43-46)
12	1,2,3,6-Tetramethyl-bicyclo[2.2.2]octa-2,5-diene	0.37	1244	-	162	2	-
13	Mellitene	0.83	1289	-	162	2	(140)
14	Pregeijerene	17.56	1295	1288[T]	162	1,2	(43,45,47)
15	α-Copaene	1.60	1380	1376[T]	204	1,2	(43-47)
16	β-Caryophyllene	7.33	1426	1418[T]	204	1,2	(12,43-47)
17	unknown	6.51	1445	?	160	?	
	Vestitenone			1443[T]	178	1,2	(141)
18	Germacrene D	11.13	1489	1480[T]	204	1,2	(45,47)
19	δ-Cadinene	4.85	1530	1524	204	1,2	(43-47)
20	4-Ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-[1*S*-(1α,3α,4α)]-cyclohexane methanol	0.82	1560		222	2	-
21	Epi-α-cadinol	0.58	1640	1640[T]	222	1,2	(142)
22	Bulnesol	2.90	1668	1666	222	1,2	(143)

[a] RA, relative area (peak area relative to total peak area);
[b] RI, programmed temperature retention indices as determined on BP-5 column using a homologous series of *n*-hydrocarbons;
[c] RI values from literature data, with T, denoting programmed temperature values;
[d] molecular weight from GC-MS (CI) data;
[e] 1, based on retention index; 2, based on comparison of mass spectra.

The major components of the essential oil were identified as pregeijerene (17.6%), germacrene D (11.1%), α-pinene (8.4%), β-caryophyllene (7.3%), vestitenone (6.5%), β-pinene (5.6%), δ-cadinene (4.9%), geijerene (3.1%), bulnesol (2.9%), and *trans*-ocimene or (*E*)-β-ocimene (2.2%). Most of them are monoterpenes and sesquiterpenes except vestitenone. The minor components were 2-hexenal (1.6%), α-copaene (1.6%), 2,3-dihydro-4-methylfuran (1.6%), myrcene (1.5%), limonene (1.0%), sabinene (0.9%), mellitene (0.8%), 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-[1*S*-(1α,3α,4α)]-cyclohexane methanol (0.8%), terpinen-4-ol (0.8%), epi-α-cadinol (0.6%), 1,2,3,6-tetramethyl-bicyclo-[2.2.2]octa-2,5-diene (0.4%), and a geijerene isomer (0.3%). Some monoterpenes and sesquiterpenes such as α-pinene, β-pinene, germacrene D, β-caryophyllene and myrcene have shown antimicrobial and antifungal activities (144-146) which might be useful for medical purposes. Eight compounds, 2,3-dihydro-4-methylfuran, 2-hexenal, 1,2,3,6-tetramethyl-bicyclo-[2.2.2]octa-2,5-diene, mellitene, vestitenone, 4-ethenyl-α,α,4-trimethyl-3-(1-methyl ethenyl)-[1*S*-(1α,3α,4α)]-cyclohexane methanol, epi-α-cadinol and bulnesol, had not been reported previously in the essential oil of *C. odorata*. The chemical structures of some identified components from the essential oil are shown in Figure 5.

One other significant component which was assigned as peak no. 17 (see Table 4.3) remains unknown. The tentative molecular weight from the mass spectral data (EI+) was 160, but it is possible that this peak arises from a facile loss of water from a molecular ion peak which could not be detected. The mass spectral fragmentation pattern and retention index could not be matched to any reported data for essential components. Further studies are required to identify this component. The major constituents of the essential oil of *C. odorata* collected from different places are compared in Table 4.4. The compounds are listed in order of their retention indices on a DB5 column and percentage compositions are given.

Table 4.4 The comparison of major components of essential oil of *C. odorata*

Compounds	Thailand	Nigeria (12)	Cameroon (43)	Congo 2.(43)	Vietnam (44)	Ivory Coast (45)	Assam, India* (46)	Ivory Coast (47)
α-pinene	8.3	19.3	14.3	8.5	2.6	18.8	18.8	21.2
Sabinene	0.9	-	1.2	1.4	0.6	-	-	1.76
β-pinene	5.7	-	8.0	5.9	2.6	10.5	7.2	10.1
Myrcene	1.5	-	2.4	t	0.9	2.3	0.8	2.4
Limonene	1.0	10.2	1.4	1.7	t	1.4	1.0	1.1
(*Z*)-β-ocimene	-	-	0.8	-	t	-	1.3	1.0
(*E*)-β-ocimene	2.2	-	5.2	-	t	3.7	-	3.4
p-cymene	-	1.6	-	22.2	-	-	-	t
Geijerene	3.1	-	9.0	4.2	42.5	4.7	7.8	11.7
(+)-camphor	-	15.5	-	-	-	-	-	-
Pregeijerene	17.6	-	25.1	14.8	-	14.3	-	19.6
Thymyl acetate	-	-	-	15.8	-	-	-	-
α-copaene	1.6	-	1.4	1.1	1.0	2.3	2.1	0.9
β-cubebene	-	-	-	-	12.5	-	13.0	-
β-caryophyllene	7.3	7.1	4.3	9.8	7.4	6.5	10.2	3.5
γ-muurolene	-	-	9.8	1.7	-	-	1.9	0.3
Germacrene D	11.1	-	-	-	-	8.2	-	9.5
Cadinene	-	19.1	-	-	-	-	-	-
γ-cadinene	-	-	t	0.8	-	0.3	0.7	-
δ-cadinene	4.9	14.5	1.6	t	2.1	3.8	3.4	1.7
β-caryophyllene oxide	-	-	t	0.9	-	-	-	t

t ≤ 0.5%, *chemical constituents from the flowers of *C. odorata*

The chemical constituents of the essential oil of *C. odorata* from Thailand is apparently very similar to the one from the Ivory Coast (45). However, the antibacterial result of the plant from Thailand showed activity against both Gram-positive, *S. aureus* and Gram-negative, *E. coli* while that from the Ivory Coast showed no effect on *S. aureus* but it exhibited a notable activity towards the Gram-negative species.

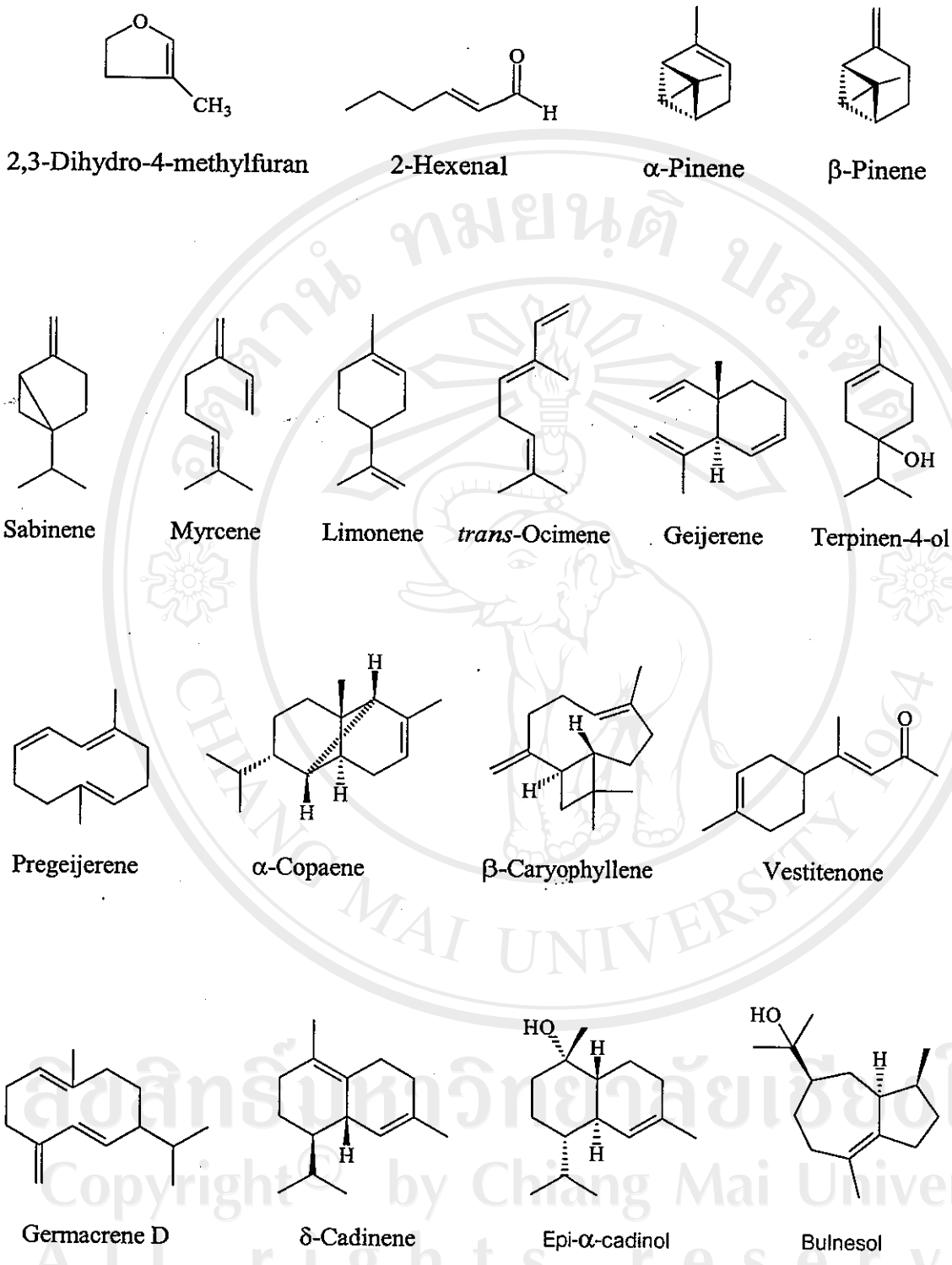
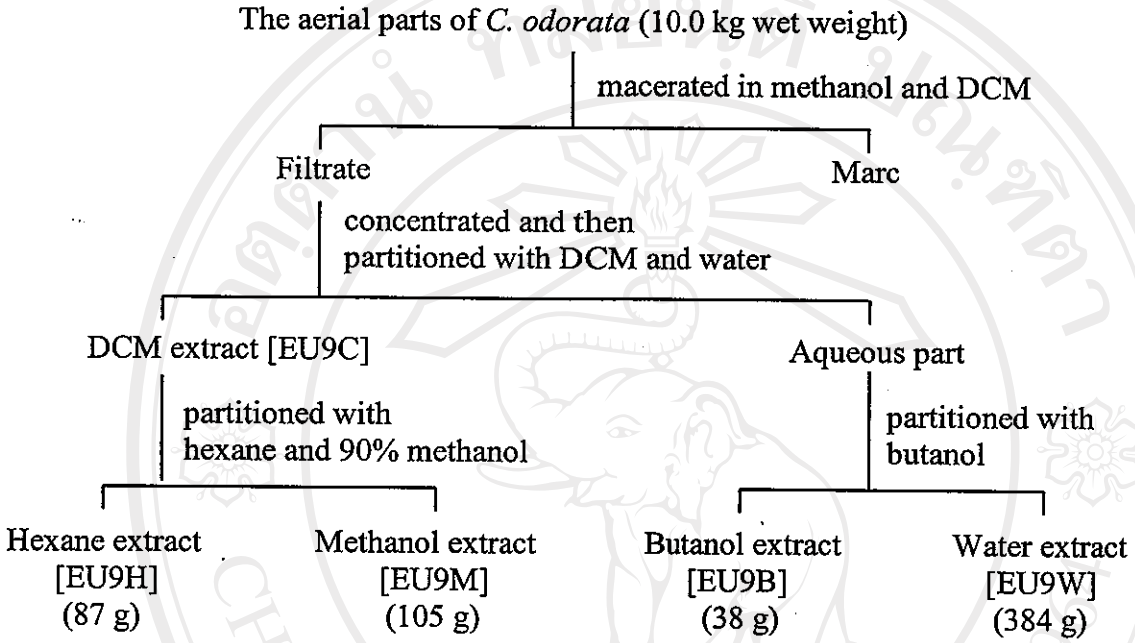


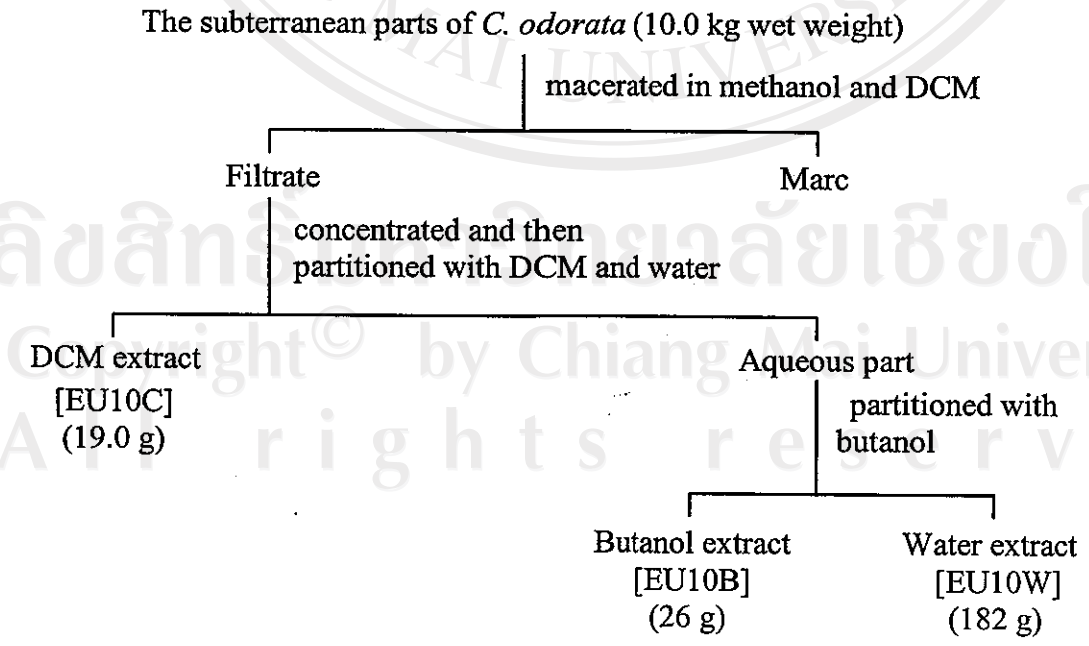
Figure 5. The structures of some identified components of the essential oil

Other Biological Activities of the Crude Extracts

According to the isolation procedures, Scheme 1-2 (below), the extracts were tested for their activities and the results are shown in Table 4.5-4.10.



Scheme 1. Extraction scheme of the aerial parts *C. odorata*



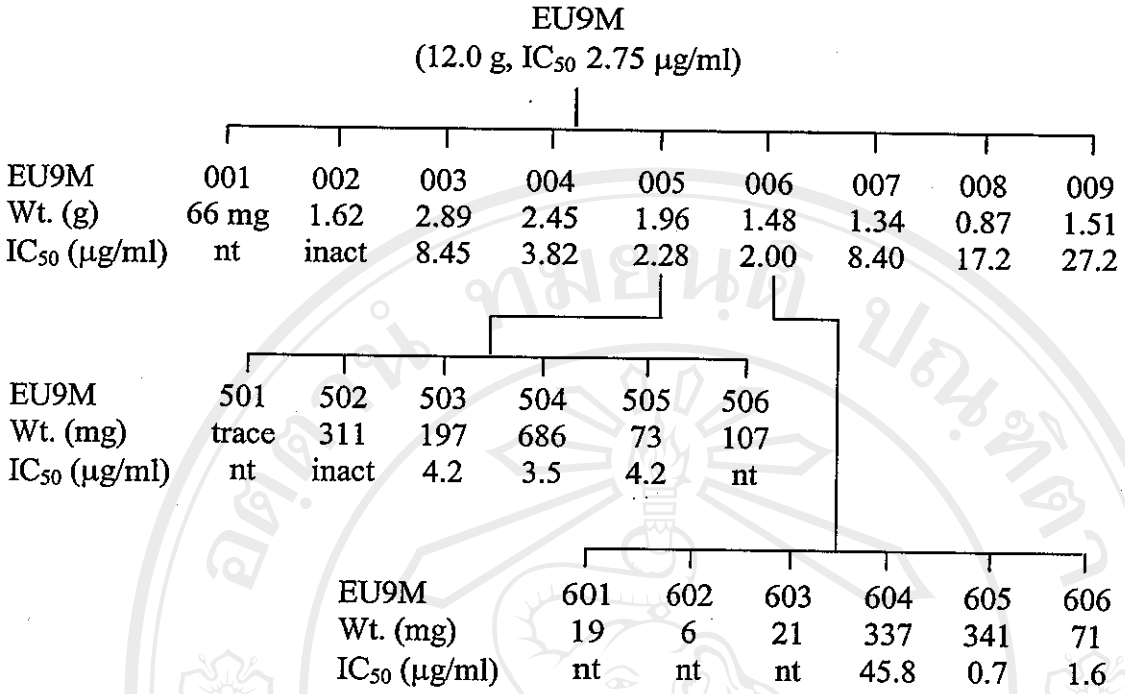
Scheme 2. Extraction scheme of the subterranean parts *C. odorata*

Table 4.5 The anti-herpes simplex virus type 1 of EU9 and EU10 extracts

Samples	Cytotoxicity against vero cell (IC_{50} ; µg/ml)	Anti-HSV-1 activity	Anti-HSV-1 activity (IC_{50} ; µg/ml)
EU9C	> 50	active	1.74
EU9B	> 50	active	27.04
EU9W	> 50	inactive	-
EU10C	> 50	moderately active	-
EU10B	> 50	inactive	-
EU10W	> 50	inactive	-

Anti-herpes simplex virus type 1 activity was determined simultaneously with cytotoxicity against vero cells, in which the maximum concentration of sample tested was at the concentration of 50 µg/ml. The extract which showed less than 25% inhibition at non-toxic concentrations was determined as inactive whereas the extract which showed 25-35% inhibition was assigned to be weakly active. If the extract showed 35-50% inhibition, it would be assigned to be moderately active, and if the extract showed activity more than 50% inhibition, it would be determined as active. None of the extracts showed toxicity against vero cells (IC_{50} > 50 µg/ml) but two of them, EU9C and EU9B, showed anti-HSV-1 activity with IC_{50} value of 1.74 and 27.04 µg/ml, respectively.

According to the bioassay result, fraction EU9C was further partitioned between 90% methanol and hexane to obtain fraction EU9M and EU9H, respectively. Fraction EU9M which showed activity against HSV-1 with the IC_{50} value of 2.75 µg/ml was further fractionated using vacuum and flash column chromatography. The fractionation scheme and the anti-herpes simplex virus type 1 activity result of the isolated fractions from EU9M are shown in Scheme 10.



Scheme 10. The isolation scheme and anti-herpes simplex virus type 1 activity of some fractions of EU9M

Table 4.6 The cytotoxicity against human cancer cell lines of EU9 and EU10 extracts

Samples	Anticancer (BC)		Anticancer (KB)		Anticancer (NCI-H187)	
	Activity	IC_{50} ; μg/ml	Activity	IC_{50} ; μg/ml	Activity	IC_{50} ; μg/ml
EU9C	inactive	-	inactive	-	inactive	-
EU9B	inactive	-	inactive	-	inactive	-
EU9W	inactive	-	inactive	-	inactive	-
EU10C	inactive	-	inactive	-	inactive	-
EU10B	inactive	-	inactive	-	inactive	-
EU10W	inactive	-	inactive	-	inactive	-

The extracts were tested against three human cancer cell lines including KB (human mouth carcinoma), BC (human breast cancer) and NCI-H187 (small cell lung cancer). The maximum concentration of sample was tested at the concentration of 20 μg/ml. None of the extracts showed cytotoxicity against the three human cancer cell lines at the concentration of 20 μg/ml and were determined to be inactive.

Table 4.7 The antimalarial activity of EU9 and EU10 extracts against *P. falciparum*

Samples	Activity (EC_{50} ; μg/ml)
EU9C	inactive
EU9B	inactive
EU9W	inactive
EU10C	9.39
EU10B	inactive
EU10W	inactive

According to the bioassay result, only one fraction, EU10C showed antimalarial activity against *Plasmodium falciparum* K1 strain with an EC_{50} of 9.39 μg/ml. In Nigerian ethnomedicine (6), a decoction of leaves of this plant was used to cure malaria, but the extract from the aerial parts, EU9, did not show antimalarial activity. Interestingly, the extract from the subterranean parts, EU10C, showed activity against *P. falciparum* and this is the first report of *in vitro* antimalarial activity of *C. odorata*. Fraction EU10C was further fractionated using vacuum and flash column chromatography. The fractionation scheme and the antimalarial activity result of the isolated fractions from EU10C are shown in Scheme 11.

EU10C
(9.5 g, EC_{50} 9.39 μg/ml)

EU10C	001	002	003	004	005	006	007	008	009	010
Wt.(mg)	420	40	100	1.4 g	-	320	2.6 g	1.1 g	1.7 g	390
EC_{50}(μg/ml)	32.5	nt	inact	inact	inact	11.8	2.93	6.9	inact	inact

EU10C	701	702	703	704	705	706	707	708	709
Wt.(mg)	90	60	655	70	179	64	35	62	53
EC_{50}(μg/ml)	inact	inact	inact	inact	inact	6.6	6.3	5.9	6.2

EU10C	710	711	712	713	714	715	716	717
Wt.(mg)	64	130	156	266	92	96	117	32
EC_{50}(μg/ml)	3.5	3.4	2.7	1.9	2.8	3.2	3.1	inact

Scheme 11. The isolation scheme and antimalarial activity of some fractions of EU10C

Table 4.8 The antituberculosis activity of EU9 and EU10 extracts

Samples	Results against TB at 200 μg/ml	MIC (μg/ml)
EU9C	active	100
EU9B	inactive	-
EU9W	active	-
EU10C	inactive	200
EU10B	inactive	-
EU10W	inactive	-

Fractions with less polarity, obtained from partitioning with DCM, showed activity against *Mycobacterium tuberculosis* H37Ra including EU9C and EU10C. This was also the first report on antituberculosis activity of this plant but there has been no ethnobotanical data on this plant curing this disease.

Table 4.9 The anti-inflammatory activity of EU9 and EU10 extracts

Samples	Activity against COX-1	Activity against COX-2
EU9C	inactive	inactive
EU9B	active %inhibition = 48	active %inhibition = 51
EU9W	inactive	inactive
EU10C	inactive	inactive
EU10B	inactive	inactive
EU10W	inactive	inactive

The samples were tested for their anti-COX-2 activity at a concentration of 10^{-5} g/ml and any positive samples (samples that inhibited more than 50%) were further tested for anti-COX-1 activity. The result showed that only fraction EU9B exhibited anti-COX-1 and COX-2 activity with the 48% and 51% inhibition, respectively. As a result, this fraction did not show selective inhibition of COX-2 over COX-2.

According to the brine shrimp lethality activity result, only fraction EU9M showed weakly toxicity with the LD_{50} of 850 μg/ml while other fractions were inactive (LD_{50} > 1000 μg/ml).

Spectral Data of Isolated Compounds

The isolated compounds were characterized by their spectroscopic data including NMR, MS and IR spectra.

1. Compound EU9M021

hrcims *m/z* : 287.0918 (MH^+, calcd. 287.0919 for $C_{16}H_{15}O_5$)

ir (KBr) : ν cm^{-1}; 3320, 1640, 1582, 1523, 1455, 1344, 1254, 1147, 1073, 1024, 843, 810 (Figure 26, page 142)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 27, page 143)

12.04 (1H, s), 7.37 (2H, d, J = 8.4 Hz), 6.95 (2H, d, J = 8.4 Hz), 5.99 (1H, d, J = 2.4 Hz), 5.97 (1H, d, J = 2.4 Hz), 5.65 (1H, brs), 5.37 (1H, dd, J = 12.9, 3.0 Hz), 3.83 (3H, s), 3.11 (1H, dd, J = 17.1, 12.9 Hz), 2.79 (1H, dd, J = 17.1, 3.0 Hz)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 28, page 144)

195.9(C), 164.2(C), 164.1(C), 163.1(C), 159.9(C), 130.2(C), 127.6(2xCH), 114.2(2xCH), 103.2(C), 96.6(CH), 95.4(CH), 79.0(CH), 55.4(CH_3), 43.2(CH_2)

2. Compound EU9M028

hrcims *m/z* : 301.1062 (MH^+, calcd. 301.1075 for $C_{17}H_{17}O_5$)

ir (KBr) : ν cm^{-1}; 1640, 1582, 1528, 1368, 1310, 1278, 1260, 1211, 1161, 1086, 830, 797 (Figure 35, page 151)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 36, page 152)

12.03 (1H, s), 7.38 (2H, d, J = 8.4 Hz), 6.96 (2H, d, J = 8.4 Hz), 6.07 (1H, d, J = 2.4 Hz), 6.04 (1H, d, J = 2.4 Hz), 5.37 (1H, dd, J = 12.9, 3.0 Hz), 3.84 (3H, s), 3.81 (3H, s), 3.11 (1H, dd, J = 17.1, 12.9 Hz), 2.79 (1H, dd, J = 17.1, 3.0 Hz)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 37, page 153)

195.8(C), 167.8(C), 164.0(C), 162.7(C), 159.9(C), 130.3(C), 127.6(2xCH), 114.2(2xCH), 103.1(C), 95.0(CH), 94.2(CH), 79.0(CH), 55.7(CH_3), 55.4(CH_3), 43.3(CH_2)

3. Compound EU9M029

hrcims *m/z* : 345.1333 (MH^+, calcd. 345.1338 for $C_{19}H_{21}O_6$)

ir (KBr) : ν cm^{-1}; 1624, 1558, 1508, 1459, 1345, 1293, 1252, 1172, 1015, 832 (Figure 45, page 161)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 46, page 162)

13.76 (1H, s), 7.84 (2H, s), 7.60 (2H, d, *J* = 8.7 Hz), 6.94 (1H, d, *J* = 8.7 Hz), 6.29 (1H, s), 3.93 (3H, s), 3.90 (3H, s), 3.86 (3H, s), 3.84 (3H, s)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 47, page 163)

192.6(C), 162.5(C), 161.4(C), 159.8(C), 154.8(C), 143.3(CH), 135.2(C), 130.1(2xCH), 128.0(C), 124.0(CH), 114.4(2xCH), 108.7(C), 96.6(CH), 61.9(CH), 61.3(CH_3), 56.1(CH_3), 55.4(CH_2)

4. Compound EU9M044

hrcims *m/z* : 317.1015 (MH^+, calcd. 317.1025 for $C_{17}H_{17}O_6$)

ir (in absolute ethanol) : ν cm^{-1}; 3369, 1640, 1582, 1513, 1459, 1300, 1254, 1163, 1088, 827 (Figure 55, page 171)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 56, page 172)

12.19 (1H, s), 7.37 (2H, d, *J* = 8.7 Hz), 6.95 (2H, d, *J* = 8.7 Hz), 6.47(1H, s), 6.11 (1H, s), 5.34 (1H, dd, *J* = 12.9, 3.0 Hz), 3.94 (3H, s), 3.83 (3H, s), 3.09 (1H, dd, *J* = 17.4, 12.9 Hz), 2.79 (1H, dd, *J* = 17.4, 3.0 Hz)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 57, page 173)

196.6(C), 159.9(C), 158.5(C), 157.2(C), 154.2(C), 130.2(C), 128.2(C), 127.6(2xCH), 114.1(2xCH), 103.0(C), 94.5(CH), 79.0(CH), 61.0(CH_3), 55.4(CH_3), 43.3(CH_2)

5. Compound EU9M055

hrcims *m/z* : 317.1014 (MH^+, calcd. 317.1025 for $C_{17}H_{17}O_6$)

ir (in absolute ethanol) : ν cm^{-1}; 3450, 1627, 1528, 1470, 1372, 1297, 1253, 1190, 1157, 1138, 1083, 1009, 845 (Figure 64, page 180)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 65, page 181)

11.19 (1H, s), 7.48 (2H, d, *J* = 8.4 Hz), 6.99 (2H, d, *J* = 8.4 Hz), 6.12 (1H, d, *J* = 2.1 Hz), 6.06 (1H, d, *J* = 2.1 Hz), 5.04 (1H, d, *J* = 12.0 Hz), 4.57(1H, dd, *J* = 12.0, 1.5 Hz), 3.84 (3H, s), 3.82 (3H, s), 3.46 (1H, d, *J* = 1.5 Hz)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 66, page 182)

195.8(C), 168.6(C), 163.5(C), 162.8(C), 160.3(C), 128.8(CH, 2 carbon atoms), 128.0(C), 114.1(CH, 2 carbon atoms), 100.8(C), 95.4(CH), 94.6(CH), 83.2(CH), 72.4(CH), 55.9(CH_3), 55.4(CH_3)

6. Compound EU9M058

hrcims *m/z* : 299.0918 (MH^+, calcd. 299.0919 for $C_{17}H_{15}O_5$)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 74, page 190)

12.81 (1H, s), 7.85 (2H, d, J = 9.0 Hz), 7.02 (2H, d, J = 9.0 Hz), 6.58 (1H, s), 6.49 (1H, d, J = 2.4 Hz), 6.37 (1H, d, J = 2.4 Hz), 3.90 (3H, s), 3.88 (3H, s)

7. Compound EU9M067

hrcims *m/z* : 331.1181 (MH^+, calcd. 331.1181 for $C_{18}H_{19}O_6$)

ir (in absolute ethanol) : ν cm^{-1}; 2932, 1639, 1579, 1513, 1464, 1291, 1258, 1167, 1112, 1013, 827, 763 (Figure 75, page 191)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 76, page 192)

11.87 (1H, s), 7.38 (2H, d, J = 8.7 Hz), 6.96 (2H, d, J = 8.7 Hz), 6.10 (1H, s), 5.37 (1H, dd, J = 13.2, 3.0 Hz), 3.87 (3H, s), 3.844 (3H, s), 3.839 (3H, s), 3.11 (1H, dd, J = 17.4, 13.2 Hz), 2.79 (1H, dd, J = 17.4, 3.0 Hz)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 77, page 193)

196.4(C), 160.8(C), 159.9(C), 158.6(C), 154.9(C), 130.4(C), 130.2(C), 127.6 (CH, 2 carbon atoms), 114.2(CH, 2 carbon atoms), 103.1(C), 91.6(CH), 79.3(CH), 60.9(CH_3), 56.2(CH_3), 55.4(CH_3), 43.3(CH_2)

8. Compound EU9M068

hrcims *m/z* : 331.1197 (MH^+, calcd. 331.1182 for $C_{18}H_{19}O_6$)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 85, page 201)

12.02 (1H, s), 6.988 (1H, d, J = 1.8 Hz), 6.985 (1H, dd, J = 8.4, 1.8 Hz), 6.90 (1H, d, J = 8.4 Hz), 6.08 (1H, d, J = 2.1 Hz), 6.06 (1H, d, J = 2.1 Hz), 5.36 (1H, dd, J = 12.9, 3.0 Hz), 3.92 (3H, s), 3.91 (3H, s), 3.81 (3H, s), 3.12 (1H, dd, J = 17.4, 12.9 Hz), 2.80 (1H, dd, J = 17.4, 3.0 Hz)

9. Compound EU9M089

hreims *m/z* : 286.0823 (M^+, calcd. 286.0841 for $C_{16}H_{14}O_5$)

ir (KBr) : ν cm^{-1}; 3541, 3460, 3148, 1619, 1572, 1512, 1454, 1344, 1306, 1256, 1207, 1162, 1082, 843, 762, 729 (Figure 86, page 202)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 87, page 203)

12.01 (1H, s), 7.32 (2H, d, *J* = 8.4 Hz), 6.88 (2H, d, *J* = 8.4 Hz), 6.07 (1H, d, *J* = 2.4 Hz), 6.04 (1H, d, *J* = 2.4 Hz), 5.35 (1H, dd, *J* = 12.9, 3.0 Hz), 3.80 (3H, s), 3.09 (1H, dd, *J* = 17.4, 12.9 Hz), 2.78 (1H, dd, *J* = 17.4, 3.0 Hz)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 88, page 204)

195.9(C), 167.8(C), 163.9(C), 162.7(C), 156.0(C), 130.4(C), 127.9(CH, 2 carbon atoms), 115.6(CH, 2 carbon atoms), 103.1(C), 95.1(CH), 94.2(CH), 78.9(CH), 55.7(CH_3), 43.2(CH_2)

10. Compound EU9M101

hreims *m/z* : 330.0746 (M^+, calcd. 330.0740 for $C_{17}H_{14}O_7$)

ir (KBr) : ν cm^{-1}; 3410, 1668, 1620, 1599, 1503, 1372, 1330, 1297, 1256, 1227, 1157, 1032, 1003, 805, 762 (Figure 95, page 211)

^{1}H-NMR (300 MHz, in dimethylsulfoxide-d_6) : δ ; (Figure 96, page 212)

12.41 (1H, s), 7.71 (1H, d, *J* = 2.1 Hz), 7.68 (1H, dd, *J* = 8.4, 2.1 Hz), 7.08 (1H, d, *J* = 8.4 Hz), 6.72 (1H, d, *J* = 2.4 Hz), 6.35 (1H, d, *J* = 2.4 Hz), 3.86 (3H, s), 3.84 (3H, s)

^{13}C-NMR (75 MHz, in dimethylsulfoxide-d_6) : δ ; (Figure 97, page 213)

175.8(C), 164.7(C), 160.1(C), 155.9(C), 149.2(C), 146.6(C), 146.0(C), 136.2(C), 123.1(C), 119.6(CH), 114.6(CH), 111.6(CH), 103.9(C), 97.4(CH), 91.8(d), 56.0(q), 55.6(q)

11. Compound EU9M104 (EU9M063)

hreims *m/z* : 316.0940 (M^+, calcd. 316.0947 for $C_{17}H_{16}O_6$)

ir (KBr) : ν cm^{-1}; 3384, 2948, 1648, 1510, 1573, 1344, 1287, 1193, 1147, 1076, 1024, 865, 809, 761, 728 (Figure 108, page 224)

^{1}H-NMR (300 MHz, in chloroform-d) : δ ; (Figure 109, page 225)

12.01 (1H, s), 7.04 (1H, d, J = 1.8 Hz), 6.93 (1H, dd, J = 8.4, 1.8 Hz), 6.88 (1H, d, J = 8.4 Hz), 6.07 (1H, d, J = 2.1 Hz), 6.04 (1H, d, J = 2.1 Hz), 5.32 (1H, dd, J = 12.9, 3.0 Hz), 3.91 (3H, s), 3.80 (3H, s), 3.07 (1H, dd, J = 17.1, 12.9 Hz), 2.78 (1H, dd, J = 17.1, 3.0 Hz)

^{13}C-NMR (75 MHz, in chloroform-d) : δ ; (Figure 110, page 226)

195.7(C), 167.8(C), 163.9(C), 162.7(C), 146.8(C), 145.8(C), 131.4(C), 118.1(CH), 112.6(CH), 110.6(CH), 103.1(C), 95.1(CH), 94.2(CH), 78.9(CH), 56.1(CH_3), 55.7(CH_3), 43.3(CH_2)

12. Compound EU9M112

hreims *m/z* : 284.0689 (M^+, calcd. 284.0685 for $C_{16}H_{12}O_5$)

ir (KBr) : ν cm^{-1}; 3351, 3163, 1660, 1602, 1569, 1503, 1434, 1370, 1297, 1235, 1174, 1161, 1031, 828 (Figure 119, page 235)

^{1}H-NMR (300 MHz, in dimethylsulfoxide-d_6) : δ ; (Figure 120, page 236)

12.90 (1H, s), 8.02 (2H, d, J = 8.7 Hz), 7.10 (2H, d, J = 8.7 Hz), 6.86 (1H, s), 6.49 (1H, d, J = 2.1 Hz), 6.19 (1H, d, J = 2.1 Hz), 3.85 (3H, s)

^{13}C-NMR (75 MHz, in dimethylsulfoxide-d_6) : δ ; (Figure 121, page 237)

181.5(C), 164.1(C), 163.0(C), 162.1(C), 161.2(C), 157.1(C), 128.1(CH, 2 carbon atoms), 122.7(C), 114.4(CH, 2 carbon atoms), 103.6(C), 103.4(CH), 98.8(CH), 93.9(CH), 55.5(CH_3)

13. Compound EU9M135

hrcims *m/z* : 303.0873 (MH^+, calcd. 303.0869 for $C_{16}H_{15}O_6$)

ir (KBr) : ν cm^{-1}; 3462, 3384, 1628, 1510, 1469, 1373, 1288, 1257, 1206, 1148, 1084, 1010, 829 (Figure 128, page 244)

^{1}H-NMR (300 MHz, in acetone-d_6) : δ ; (Figure 129, page 245)

11.67 (1H, brs), 8.53 (1H, brs), 7.42 (2H, d, J = 8.4 Hz), 6.90 (2H, d, J = 8.4 Hz), 6.08 (1H, d, J = 2.1 Hz), 6.05 (1H, d, J = 2.1 Hz), 5.11 (1H, d, J = 12.0 Hz), 4.74 (1H, d, J = 3.6 Hz), 4.68 (1H, dd, J = 12.0, 3.6 Hz), 3.86 (3H, s)

^{13}C-NMR (75 MHz, in acetone-d_6) : δ ; (Figure 130, page 246)

198.3(C), 169.0(C), 164.4(C), 163.7(C), 158.6(C), 130.1(CH, 2 carbon atoms), 128.8(C), 115.7(CH, 2 carbon atoms), 101.9(C), 95.6(CH), 94.5(CH), 84.3(CH), 73.1(CH), 56.3(CH_3)

14. Compound EU9M138

hrcims *m/z* : 303.0874 (MH^+, calcd. 303.0869 for $C_{16}H_{15}O_6$)

ir (in absolute ethanol) : ν cm^{-1}; 3386, 1647, 1513, 1469, 1347, 1274, 1157, 1088, 808 (Figure 138, page 254)

^{1}H-NMR (300 MHz, in acetone-d_6) : δ ; (Figure 139, page 255)

12.17 (1H, s), 7.05 (1H, s), 6.97 (2H, s), 5.97 (1H, d, J = 2.4 Hz), 5.95 (1H, d, J = 2.4 Hz), 5.43 (1H, dd, J = 12.6, 3.0 Hz), 3.86 (3H, s), 3.15 (1H, dd, J = 17.1, 12.6 Hz), 2.75(1H, dd, J = 17.1, 3.0 Hz)

^{13}C-NMR (75 MHz, in acetone-d_6) : δ ; (Figure 140, page 256)

196.8(C), 167.0 (C), 165.0(C), 164.0(C), 148.4(C), 147.3 (C), 132.6(C), 118.6 (CH), 114.2(CH), 112.1 (CH), 103.1(C), 96.7(CH), 95.7(CH), 79.7(CH), 56.2(CH_3), 43.4(CH_2)

15. Compound EU9M147

hrcims *m/z* : 303.0876 (MH^+, calcd. 303.0869 for $C_{16}H_{15}O_6$)

ir (in absolute ethanol) : ν cm^{-1}; 3432, 1636, 1513, 1469, 1255, 1166, 828 (Figure 148, page 264)

^{1}H-NMR (300 MHz, in acetone-d_6) : δ ; (Figure 149, page 265)

11.70 (1H, brs), 7.51 (2H, d, J = 8.7 Hz), 6.99 (2H, d, J = 8.7 Hz), 6.00 (1H, d, J = 2.4 Hz), 5.96 (1H, d, J = 2.4 Hz), 5.12 (1H, d, J = 11.7 Hz), 4.66 (1H, d, J = 11.7 Hz), 3.83 (3H, s)

^{13}C-NMR (75 MHz, in acetone-d_6) : δ ; (Figure 150, page 266)

197.8(C), 167.5(C), 164.7(C), 163.8(C), 160.8(C), 130.0(C), 129.9(2xCH), 114.3(2xCH), 101.4(C), 97.0(CH), 95.9(CH), 84.0(CH), 73.0(CH), 55.5(CH_3)

Structure Elucidation of the Isolated Compounds

1. Compound EU9M021

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 287.0918 which represented MH^+ for $C_{16}H_{15}O_5$ comparing to the calculated mass at *m/z* 287.0919. The ^{13}C-NMR spectrum of EU9M021 in chloroform-d (Figure 28) showed 14 carbons peaks which represented 16 carbon atoms. The carbons were determined as one methyl, one methylene, seven methine and seven quaternary by High Sensitive Quantum Coherence (HSQC) (Figure 31) and Distortionless Enhancement by Polarization Transfer (DEPT) (Figure 29) experiments. These data established its tentative molecular formula as $C_{16}H_{14}O_5$.

The ^{1}H-NMR spectrum of EU9M021 in chloroform-d (Figure 27) showed the characteristic signals of a flavanone including three groups of doublets of doublets at δ5.37, 3.11 and 2.79 ppm which represented one proton at C-2 and two protons at C-3. The *para*-monosubstitution on ring B was indicated by the signals of two groups of doublets at δ7.37 and 6.95 ppm. The *meta*-disubstitution on ring A was indicated by the signals of two groups of doublets at δ5.97 and 5.99 ppm together with a singlet at δ12.04 ppm which was the resonance signal of the hydroxyl group at C-5. The last singlet at δ3.83 ppm was integrated to be a methoxy group which was substituted on the aromatic ring (see the numbering system in Figure 6).

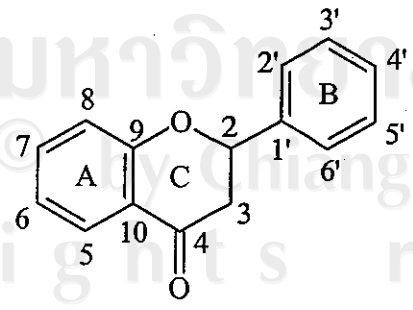


Figure 6. The flavanone skeleton and its numbering system

The ^{1}H-^{1}H COSY spectrum of EU9M021 (Figure 30) showed the correlations among the protons having geminal, vicinal and allylic coupling as summarized in Table 4.10. The following vicinal couplings were observed : the proton at δ5.37 (H-2_{ax}) to two protons at δ3.11 and 2.79 (H-3_{ax} and H-3_{eq}) and two protons at δ7.37 (H-2′ and H-6′) to two protons at δ6.95 (H-3′ and H-5′). These data suggested a flavanone with the *para*-monosubstitution at ring B. An allylic coupling was also observed between two olefinic protons at δ5.99 (H-6) and 6.97 (H-8).

Table 4.10 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M021

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	79.0	*ax* 5.37	dd	H-3_{ax} (12.9), H-3_{eq} (3.0)
3	43.2	*ax* 3.11	dd	H-3_{eq} (17.1), H-2_{ax} (12.9)
		eq 2.79	dd	H-3_{ax} (17.1), H-2_{ax} (3.0)
4	195.9	-	-	-
5	164.2	-	-	-
6	96.6	5.99	d	H-8 (2.4)
7	163.1	-	-	-
8	95.4	5.97	d	H-6 (2.4)
9	164.1	-	-	-
10	103.2	-	-	-
1′	130.2	-	-	-
2′	127.6	7.37	d	H-3′ (8.7)
3′	114.2	6.95	d	H-2′ (8.7)
4′	159.9	-	-	-
5′	114.2	6.95	d	H-6′ (8.7)
6′	127.6	7.37	d	H-5′ (8.7)
5-OH	-	12.04	s	-
7-OH	-	5.65	s	-
4′-OMe	55.4	3.83	s	-

The assignment of the position of the methoxy group and connection of the structure was finally accomplished by using a Heteronuclear Multiple Bond Coherence (HMBC) experiment (Figure 32-33). The position of the methoxy group was confirmed by the Nuclear Overhauser Effect Difference Spectrum (NOEDS) (Figure 34). The NOEDS showed correlations between protons through space by which the irradiated protons would affect the intensity of correlated proton signals. Compound EU9M021, mp 179-180°C, was therefore identified as the known 5,7-dihydroxy-4′-methoxyflavanone (isosakuranetin) which has been found in this species previously (25, 31).

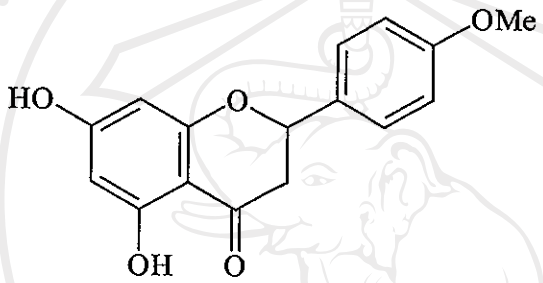


Figure 7. The chemical structure of compound EU9M021

2. Compound EU9M028

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 301.1062 which represented MH^+ for $C_{17}H_{17}O_5$ comparing the calculated mass of 301.1075. The ^{13}C-NMR spectrum of EU9M028 in chloroform-d (Figure 37) showed 15 carbon peaks which represented 17 carbon atoms. The carbons were determined as two methyl, one methylene, seven methine and seven quaternary carbons by HSQC (Figure 40) and DEPT (Figure 38) experiments. These data established its tentative molecular formula as $C_{17}H_{16}O_5$.

The ^{1}H-NMR spectrum of EU9M028 in chloroform-d (Figure 36) showed the characteristic peaks of a flavanone including three groups of doublets of doublets at δ5.37, 3.11 and 2.79 ppm which represented one proton at C-2 and two protons at C-3. The *para*-monosubstitution on ring B was indicated by the signals of two groups of

doublets at δ7.38 and 6.96 ppm. The *meta*-disubstitution on ring A was indicated by the signals of two groups of doublets at δ6.07 and 6.04 ppm together with a singlet at δ12.03 ppm which was the resonance signal of the hydroxyl group at C-5. The last two singlets at δ3.81 and 3.84 ppm integrated for methoxy groups which were substituted on an aromatic ring.

The ^{1}H-^{1}H COSY spectrum of EU9M028 (Figure 39) which showed the correlations among the protons having geminal, vicinal and allylic coupling is summarized in Table 4.11. The vicinal couplings were observed as follows : the proton at δ5.37 ppm (H-2_{ax}) to two protons at δ3.11 and 2.79 ppm (H-3_{ax} and H-3_{eq}), and two protons at δ7.38 ppm (H-2′ and H-6′) to two protons at δ6.96 (H-3′ and H-5′). These data suggested a flavanone with a *para*-monosubstituted aryl group. An allylic coupling was also observed between two olefinic protons at δ6.07 ppm (H-6) and 6.04 ppm (H-8).

The assignment of the position of the methoxyl group and completion of the structure was finally accomplished by using a HMBC experiment (Figure 41-42). The NOEDS of compound EU9M028 (Figure 43-44) showed the correlations between methoxyl groups at δ3.81 and H-6 and another at δ3.84 and H-3′ indicating 7-OMe and 4′-OMe, respectively.

Compound EU9M028 was therefore identified as the known 5-hydroxy-7,4′-dimethoxyflavanone (sakuranetin-4′methyl ether) which has also been found previously in this species (25, 35).

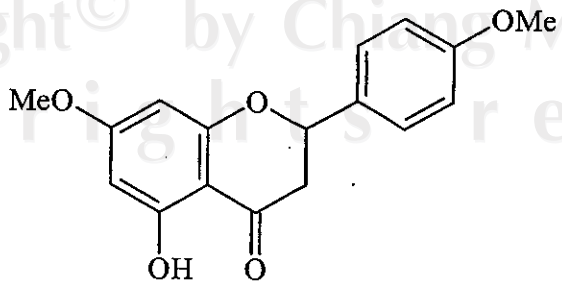


Figure 8. The chemical structure of compound EU9M028

Table 4.11 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M028

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	79.0	*ax* 5.37	dd	H-3$_{ax}$ (12.9), H-3$_{eq}$ (3.0)
3	43.3	*ax* 3.11	dd	H-3$_{eq}$ (17.1), H-2$_{ax}$ (12.9)
		eq 2.79	dd	H-3$_{ax}$ (17.1), H-2$_{ax}$ (3.0)
4	195.8	-	-	-
5	164.0	-	-	-
6	95.0	6.07	d	H-8 (2.4)
7	167.8	-	-	-
8	94.2	6.04	d	H-6 (2.4)
9	162.7	-	-	-
10	103.1	-	-	-
1′	130.3	-	-	-
2′	127.6	7.38	d	H-3′ (8.4)
3′	114.2	6.96	d	H-2′ (8.4)
4′	159.9	-	-	-
5′	114.2	6.96	d	H-6′ (8.4)
6′	127.6	7.38	d	H-5′ (8.4)
5-OH	-	12.03	s	-
7-OMe	55.7	3.81	s	-
4′-OMe	55.4	3.84	s	-

3. Compound EU9M029

The high resolution chemical ionization mass spectrum exhibited the molecular ion peak at *m/z* 345.1333 which was consistent with the MH^+ for $C_{19}H_{21}O_6$. The ^{13}C-NMR spectrum of EU9M029 in chloroform-d (Figure 47) showed 17 carbon peaks which represented 19 carbon atoms. The carbons were determined as four methyl, seven methine and eight quaternary by HSQC (Figure 50) and DEPT (Figure 48) experiments. These data established its tentative structure molecular formula as $C_{19}H_{20}O_6$.

The ^1H-NMR spectrum of EU9M029 in chloroform-d (Figure 46) showed two *trans*-olefinic protons (H-α and H-β) at δ7.84 ppm as a singlet rather than the usual doublet. One phenolic proton appeared as a singlet at δ13.76 ppm which indicated that the hydroxyl group is *ortho* to the carbonyl group. The *para*-substitution on ring B was indicated by the signals of two groups of doublets at δ7.60 and 6.94 ppm. One proton appeared as a singlet at δ6.29 ppm indicating the tetrasubstitution on ring A. The signals at δ3.93, 3.90, 3.86 and 3.84 ppm, each 3H, were due to the protons of the four methoxyl groups which were substituted on the aromatic rings.

Data from the ^1H-^1H COSY spectrum of EU9M029 (Figure x) is summarized in Table 4.12. Only two vicinal couplings were observed as follows : two protons at δ7.60 ppm (H-2 and H-6) to two protons at δ6.94 ppm (H-3 and H-5). These data suggested a chalcone with the *para*-monosubstituted ring B.

The assignment of the position of the methoxyl groups and connection of the structure was finally accomplished by using the HMBC experiment (Figure 51-52). The position of the methoxyl groups was confirmed by the NOEDS (Figure 53-54). Compound EU9M029, mp 140-141°C, was therefore identified as the known 2′-hydroxy-4,4′,5′,6′-tetramethoxychalcone (odoratin) which has also been found previously in this species (25, 31, 34).

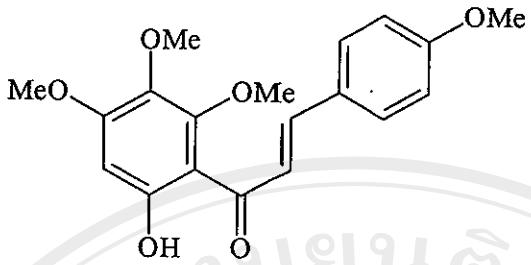


Figure 9. The chemical structure of compound EU9M029

Table 4.12 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M029

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
C=O	192.6	-	-	-
α	124.0	7.84	s	-
β	143.3	7.84	s	-
1	128.0	-	-	-
2	130.1	7.60	d	H-3 (8.7)
3	114.4	6.94	d	H-2 (8.7)
4	161.4	-	-	-
5	114.4	6.94	d	H-6 (8.7)
6	130.1	7.60	d	H-5 (8.7)
1′	108.7	-	-	-
2′	162.5	-	-	-
3′	96.6	6.29	s	-
4′	159.8	-	-	-
5′	135.2	-	-	-
6′	154.8	-	-	-
2′-OH	-	13.76	s	-
4-OMe	55.4	3.86	s	-
4′-OMe	56.1	3.90	s	-
5′-OMe	61.3	3.84	s	-
6′-OMe	61.9	3.93	s	-

4. Compound EU9M044K

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 317.1015 which represented MH^+ for $C_{17}H_{17}O_6$. The ^{13}C-NMR spectrum of EU9M044 in chloroform-d (Figure 57) showed 15 carbon peaks which represented 17 carbon atoms. The carbons were determined as two methyl, one methylene, six methine and eight quaternary carbons by HSQC (Figure 60) and DEPT (Figure 58) experiments. These data established its tentative molecular formula as $C_{17}H_{16}O_6$.

The ^1H-NMR spectrum of EU9M044 in chloroform-d (Figure 56) displayed a pair of singlets assigned to two methoxyl groups at δ3.83 and 3.94, an aromatic proton singlet at δ6.11, a pair of doublets at δ7.37 and 6.95 with a coupling constant of 8.7 Hz for protons in a 1,4-disustituted benzene. The characteristic of ABX system indicated a flavanone including the protons at δ5.34 ppm (1H, dd, J_{AX} = 3.0 Hz and J_{BX} = 12.9 Hz) for H-2_{ax} signal, δ3.09 ppm for H-3_{ax} signal and δ2.79 ppm for H-3_{eq} signal. Two phenolic hydroxyl groups were also present as evidenced by the singlet signals at δ12.19 and δ6.47.

The ^1H-^1H COSY spectrum of EU9M044 (Figure 59) which showed the correlations among the protons having geminal, vicinal and allylic coupling is summarized in Table 4.13. The vicinal couplings were observed as follows : the proton at δ5.37 ppm (H-2_{ax}) to two protons at δ3.11 and 2.79 ppm (H-3_{ax} and H-3_{eq}), and two protons at δ7.37 ppm (H-2′ and H-6′) to two protons at δ6.95 (H-3′ and H-5′). These data suggested a flavanone with a *para*-substituted aromatic substitution on ring B.

The assignment of the position of the methoxyl group and completion of the structure was finally accomplished by using HMBC experiment (Figure 10, 61-62). The NOEDS (Figure 63) indicated the substitution of the methoxyl group at δ3.83 ppm on ring B. No NOE evidence was observed when the proton at δ6.11 ppm (H-8) was irradiated and also no NOE evidence of the proton at δ6.11 ppm when the two

methoxyl protons at δ3.83 and δ3.94 were irradiated. Thus, the methoxyl group at δ3.94 should be present at C-6 (Figure 11).

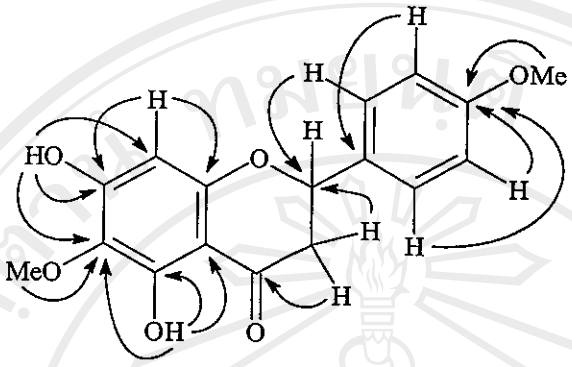


Figure 10. The long-range correlations in the HMBC spectrum

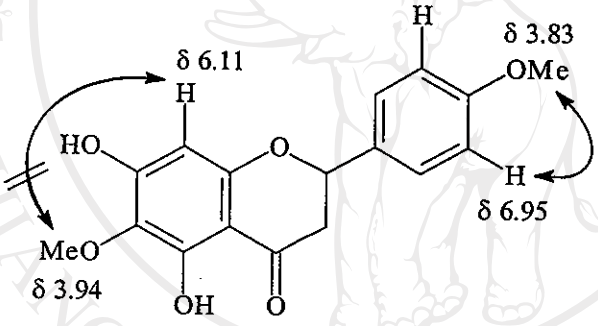
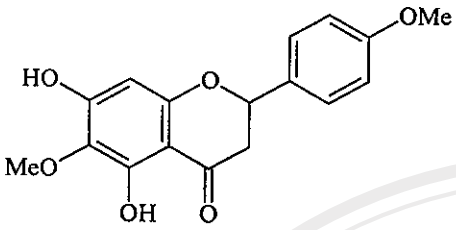
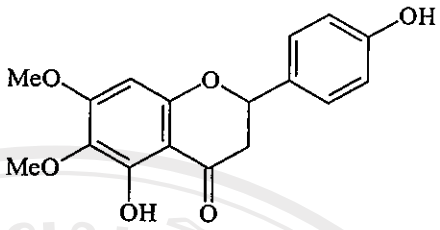


Figure 11. The correlations in the NOEDS of EU9M044

Compound EU9M044 was therefore identified as 5,7-dihydroxy-6,4′-dimethoxyflavanone, a new flavanone derivative (mp 171.0-172.0°C; $[\alpha]_D^{25}$ +26°, *c* 0.43, acetone). The comparison of ^{1}H and ^{13}C-NMR data with other known 5,7,8-substituted and 5,6,7-substituted flavanones is shown in Table 4.14. The chemical shift of C-5 in 5-dihydroxy-7-methoxyflavanone or 5,7-dihydroxyflavanone normally appeared at 164.0±0.5 ppm, but the signal for C-5 in compound EU9M044 appeared at δ154.2 which implied the substitution of the methoxyl group at C-6; similar assignments have been reported for 5,4′-dihydroxy-6,7-dimethoxyflavanone (147).



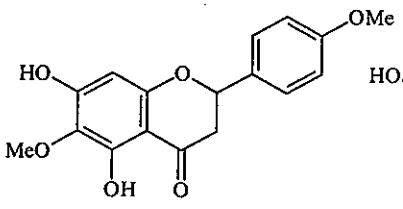
EU9M044



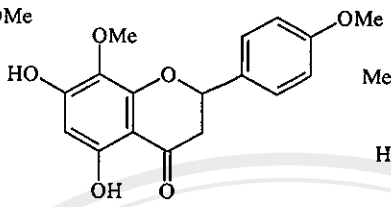
5,4′-dihydroxy-6,7-dimethoxyflavanone

Table 4.13 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M044

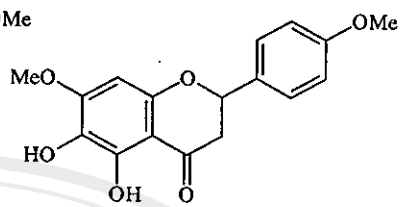
	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	79.0	*ax* 5.34	dd	H-3$_{ax}$ (12.9), H-3$_{eq}$ (3.0)
3	43.3	*ax* 3.09	dd	H-3$_{eq}$ (17.4), H-2$_{ax}$ (12.9)
		eq 2.79	dd	H-3$_{ax}$ (17.4), H-2$_{ax}$ (3.0)
4	196.6	-	-	-
5	154.2	-	-	-
6	128.2	-	-	-
7	157.3	-	-	-
8	94.5	6.11	s	-
9	158.5	-	-	-
10	103.0	-	-	-
1′	130.2	-	-	-
2′	127.6	7.37	d	H-3′ (8.7)
3′	114.1	6.95	d	H-2′ (8.7)
4′	159.9	-	-	-
5′	114.1	6.95	d	H-6′ (8.7)
6′	127.6	7.37	d	H-5′ (8.7)
5-OH	-	12.19	s	-
7-OH	-	6.47	s	-
6-OMe	61.0	3.94	s	-
4′-OMe	55.4	3.83	s	-



EU9M044



[1]Amaro-Luis, J.M. (80)
[2]Wollenweber, E. (148)



[3]Bernini,R.(149)

Table 4.14 The ^{1}H and ^{13}C-NMR data of 5,6,7- and 5,7,8-substituted flavanones

position	EU9M044		[1]#		[2]#		[3]	
	δC (ppm)	δH (ppm)	δC (ppm)	δH (ppm)	δC (ppm)	δH (ppm)	δC (ppm)	δH (ppm)
1	-	-	-	-	-	-	n.a.	n.a.
2	79.0	5.34	79.1	5.35	78.5	5.54	n.a.	n.a.
3	43.3	3.09	43.2	2.90	42.0	3.28	n.a.	n.a.
		2.79		2.90		2.76	n.a.	n.a.
4	196.6	-	196.7	-	196.5	-	n.a.	n.a.
5	154.2	-	158.7*	-	154.3	-	n.a.	n.a.
6	128.2	-	130.6	6.10	128.5	5.97	n.a.	n.a.
7	157.3	-	160.2	-	160.0	-	n.a.	n.a.
8	94.5	6.11	94.6	-	95.8	-	n.a.	n.a.
9	158.5	-	154.1	-	158.6	-	n.a.	n.a.
10	103.0	-	103.1	-	101.8	-	n.a.	n.a.
1′	130.2	-	129.8	-	130.7	-	n.a.	n.a.
2′	127.6	7.37	127.6	7.37	128.2	7.46	n.a.	n.a.
3′	114.1	6.95	114.3	6.94	114.0	6.99	n.a.	n.a.
4′	159.9	-	157.5*	-	159.5	-	n.a.	n.a.
5′	114.1	6.95	114.3	6.94	114.0	6.99	n.a.	n.a.
6′	127.6	7.37	127.6	7.37	128.2	7.46	n.a.	n.a.
5-OH	-	12.19	-	11.76	-	11.92	n.a.	n.a.
7-OH	-	6.47	-	-	-	10.69	n.a.	n.a.
6/8-OMe	61.0	3.94	60.8	3.93	60.4	3.77	n.a.	n.a.
4′-OMe	55.4	3.83	55.4	3.82	55.2	3.60	n.a.	n.a.

* asterisk, indicates that assignments may be reversed
# deuterated solvent for NMR spectrum of [1] was $CDCl_3$, [2] was DMSO-d_6

5. Compound EU9M055

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 317.1014 which was assigned to the MH^+ for $C_{17}H_{17}O_6$ comparison on the basis of the calculated mass at *m/z* 317.1025. The ^{13}C-NMR spectrum of EU9M055 in chloroform-d (Figure 66) showed 15 carbon peaks which represented 17 carbon atoms. The carbons were determined as two methyl, eight methine and seven quaternary carbons by HSQC (Figure 69) and DEPT (Figure 67) experiments. These data established its tentative structure molecular formula as $C_{17}H_{16}O_5$.

The ^{1}H-NMR spectrum of EU9M055 in chloroform-d (Figure 65) exhibited a typical flavanone derivative AX system of H-2$_{ax}$ and H-3$_{ax}$ with the signal at δ5.04 ppm (H-2$_{ax}$, J_{AX} = 12.0 Hz) and δ4.57 ppm (H-3$_{ax}$, J_{AX} = 12.0 Hz and $J_{3,\,OH}$ = 1.5 HZ), where the H-3$_{ax}$ signal was split further by coupling with the hydroxyl proton (δ3.46 ppm, J = 1.5 Hz, 3-OH) which was substituted at C-3. The large coupling constant (J_{AX} = 12.0 Hz) showed a *trans*-diaxial relationship and indicated a dihydroflavonol structure for compound EU9M055. The proton signals on ring B were similar to the signals of compound EU9M021, EU9M028 and EU9M044.

The correlations from the ^{1}H-^{1}H COSY spectrum of EU9M055 (Figure 68) are summarized in Table 4.15. Besides the coupling between H-2$_{ax}$ and H-3$_{ax}$, the other vicinal couplings were observed between two protons at δ7.48 ppm (H-2′ and H-6′) to two protons at δ6.99 (H-3′ and H-5′). These data suggested the dihydroflavonol with a *para*-substituted ring B. An allylic coupling was also observed between two olefinic protons at δ6.12 ppm (H-6) and 6.06 ppm (H-8).

The assignment of the position of the methoxyl groups and other structural connectivities was finally accomplished by using HMBC experiment (Figure 70-71). The NOEDS (Figure 72-73) indicated the position of the methoxyl groups including the methoxyl protons at δ3.82 ppm (7-OMe) and at 3.84 ppm (4′-OMe).

Compound EU9M055 was therefore identified as the known 3,5-dihydroxy-7,4′-dimethoxyflavanone (aromadendrin-7,4′-dimethyl ether) which was previously

found in this species (25) and in the stem bark of *Lannea coromandelica* (see ^{1}H-NMR data in Table 4.16) (150). Their ^{1}H-NMR data were also compared with data for 3,5-dihydroxy-7-methoxyflavanone (151) (Table 4.16), and, as expected, good correlations with respect to signals associated with ring A and B and their substituents were observed.

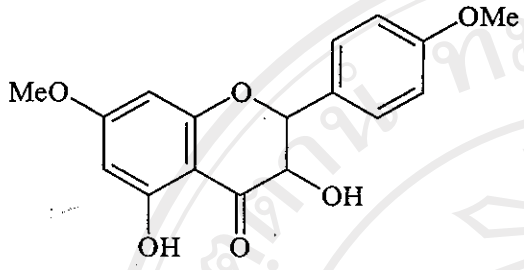
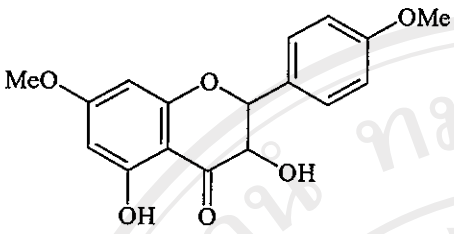


Figure 12. The chemical structure of compound EU9M055

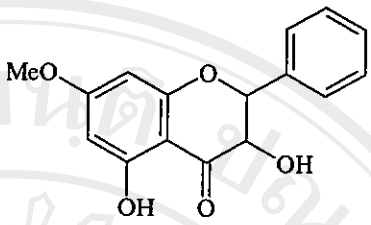
Table 4.15 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M055

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	83.2	5.04	d	H-3 (12.0)
3	72.4	4.57	dd	H-3 (12.0), 3-OH (1.5)
4	195.8	-	-	-
5	163.5	-	-	-
6	95.4	6.12	d	H-8 (2.1)
7	168.6	-	-	-
8	94.6	6.06	d	H-6 (2.1)
9	162.8	-	-	-
10	100.8	-	-	-
1′	128.0	-	-	-
2′	128.8	7.48	d	H-3′ (8.4)
3′	114.1	6.99	d	H-2′ (8.4)
4′	160.3	-	-	-
5′	114.1	6.99	d	H-6′ (8.4)
6′	128.8	7.48	d	H-5′ (8.4)
3-OH	-	3.46	d	H-3 (1.5)
5-OH	-	11.19	s	-
7-OMe	55.9	3.82	s	-
4′-OMe	55.4	3.84	s	-

Table 4.16 The proton and carbon NMR data comparison of compound EU9M055, 3,5-dihydroxy-7-methoxyflavanone[1] and 3,5-dihydroxy-7,4′-dimethoxy flavanone[2]



EU9M055 and [2]



[1]

position	EU9M055		[1] (151)[a]		[2] (151)[b]		[2] (150)[c]	
	δC (ppm)	δH (ppm)	δC (ppm)	δH (ppm)	δC (ppm)	δH (ppm)	δC[d] (ppm)	δH (ppm)
1	-	-	-	-	-	-	-	-
2	83.2	5.04	83.4	5.08	83.0	-	-	5.16
3	72.4	4.57	72.4	4.55	71.7	-	-	4.70
4	195.8	-	195.8	-	198.6	-	-	-
5	163.5	-	163.6	-	163.2	-	-	-
6	95.4	6.12	95.5	6.11	95.1	-	-	6.06
7	168.6	-	168.9	-	167.7	-	-	-
8	94.6	6.06	94.7	6.05	94.1	-	-	6.09
9	162.8	-	?	-	162.0	-	-	-
10	100.8	-	100.8	-	101.6	-	-	-
1′	128.0	-	136.1	-	129.6	-	-	-
2′	128.8	7.48	127.5	7.46	129.3	-	-	7.52
3′	114.1	6.99	128.7	7.46	113.8	-	-	7.00
4′	160.3	-	129.4	7.46	159.7	-	-	-
5′	114.1	6.99	129.4	7.46	113.8	-	-	7.00
6′	128.8	7.48	127.5	7.46	129.3	-		7.52
3-OH	-	3.46	-	3.46	-	-		4.75
5-OH	-	11.19	-	11.20	-	-		11.67
7-OMe	55.9	3.82	55.8	3.84	56.1	-		3.84
4′-OMe	55.4	3.84	-	-	55.3	-		3.86

[a] in $CDCl_3$ [b] in DMSO-d_6 [c] in acetone-d_6
[d] ^{13}C-NMR data was identical with those in (151)

6. Compound EU9M058

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 299.0918 which represented MH^+ for $C_{17}H_{15}O_5$ by comparison with the calculated mass of 299.0919. The ^{1}H-NMR spectrum of EU9M058 in chloroform-d (Figure 74) exhibited a singlet at δ12.81 (5-OH), a pair of doublets which showed vicinal coupling between the signals at δ7.85 ppm (J = 9.0 Hz, H-2′ and H-6′) and 7.02 ppm (J = 9.0 Hz, H-3′ and H-5′), another two doublets which showed allylic coupling between two olefinic protons at δ6.49 and 6.37 ppm (J = 2.4 Hz, H-6 and H-8), a singlet at δ6.58 ppm (H-3) and two singlets at δ3.88 and 3.90 ppm (assigned to the 7- and 4′-methoxyl groups). The proton chemical shift assignments of this compound are shown in Table 4.17. Because of the small amount of this compound, the complete NMR spectral data could not be obtained. However, the tentative structure of compound EU9M058 (Figure 13) was elucidated as 5-hydroxy-7,4′-dimethoxyflavone (apigenin-7,4′-dimethyl ether) which was previously found in the leaves of *Isodon flavidus* (see ^{1}H-NMR data comparison in Table 4.17) (152). This is the first isolation of this common flavone from *C. odorata*.

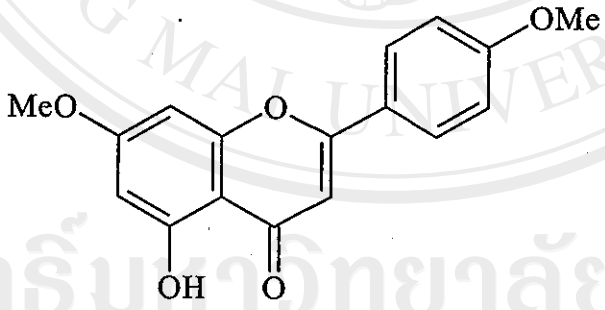


Figure 13. The tentative structure of compound EU9M058

Table 4.17 Proton chemical shift assignments and proton-proton correlations of compound EU9M058 comparing to 5-hydroxy-7,4′-dimethoxyflavone

	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)	^{1}H-NMR data of 5-hydroxy-7,4′-dimethoxyflavone (152)[c]
1	-	-	-	-
2	-	-	-	-
3	6.58	s	-	6.51 (s)
4	-	-	-	-
5	-	-	-	-
6	6.49[a]	d	H-8 (2.4)	6.41 (d, *J* = 2.4 Hz)
7	-	-	-	-
8	6.37[a]	d	H-6 (2.4)	6.30 (d, *J* = 2.4 Hz)
9	-	-	-	-
10	-	-	-	-
1′	-	-	-	-
2′	7.85	d	H-3′ (9.0)	7.78 (dd, *J* = 7.2, 2.4 Hz)
3′	7.02	d	H-2′ (9.0)	6.96 (dd, *J* = 7.2, 2.4 Hz)
4′	-	-	-	-
5′	7.02	d	H-6′ (9.0)	6.96 (dd, *J* = 7.2, 2.4 Hz)
6′	7.85	d	H-5′ (9.0)	7.78 (dd, *J* = 7.2, 2.4 Hz)
5-OH	12.81	s	-	-
7-OMe	3.90[b]	s	-	3.83[d]
4′-OMe	3.88[b]	s	-	3.84[d]

[a] indicates that assignments may be reversed between protons at δ6.49 and 6.37 ppm
[b] indicates that assignments may be reversed between protons at δ3.90 and 3.88 ppm
[c] used pyridine-d_5 as deuterated solvent
[d] did not indicate the substitution position

7. Compound EU9M067

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 331.1181 which represented MH^+ for $C_{18}H_{19}O_6$ by comparison with the calculated mass of 331.1181. The ^{13}C-NMR spectrum of EU9M067 in chloroform-d (Figure 77) showed 16 signals which represented 18 carbon atoms. The carbons were determined as three methyl, one methylene, six methine and eight quaternary by HSQC (Figure 80) and DEPT (Figure 78) experiments. These data established its tentative molecular formula as $C_{18}H_{18}O_6$.

The ^{1}H-NMR spectrum of EU9M067 in chloroform-d (Figure 76) showed the characteristic peaks of a 5-OH-flavanone including the singlet signal for the hydroxyl group at δ11.87 ppm and three groups of doublets of doublets at δ5.37, 3.11 and 2.79 ppm which were consistent with one proton at C-2 and two protons at C-3. The *para*-substituted aryl group on ring B was indicated by the signals of two groups of doublets at δ7.38 and 6.96 ppm. An aromatic proton singlet at δ6.10 ppm indicated trisubstitutiton on ring A. Three singlet signals (3H each) at δ3.87, 3.844 and 3.839 ppm were assigned to methoxyl groups that substituted aromatic rings.

The ^{1}H-^{1}H COSY spectrum of EU9M067 (Figure 79) which showed the correlations among the protons having geminal, vicinal and allylic coupling is summarized in Table 4.18. The vicinal couplings were observed as follows : the proton at δ5.37 ppm (H-2_{ax}) to two protons at δ3.11 and 2.79 ppm (H-3_{ax} and H-3_{eq}), and two protons at δ7.38 ppm (H-2′ and H-6′) to two protons at δ6.96 (H-3′ and H-5′). These data suggested a flavanone with ring B a *para*-substituted aryl group.

The assignment of the positions of the methoxyl groups and completion of the structure was finally accomplished by using the HMBC experiment (Figure 81-82). The NOEDS (Figure 83-84) confirmed the position of the methoxyl groups including the methoxyl groups assigned to the signals at δ3.87 ppm (7-OMe) and at 3.839 ppm (4′-OMe). The ^{1}H and ^{13}C-NMR spectral data of compound EU9M067 was identical with a known 5-hydroxy-6,7,4′-trimethoxyflavanone which was previously isolated

from *Sideritis gomerae* (153), but was found to be a metabolite of *C. odorata* for the first time.

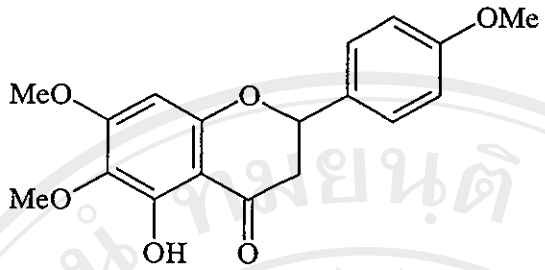


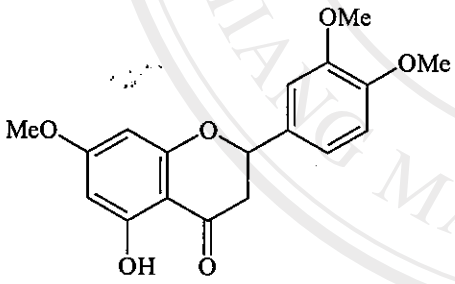
Figure 14. The chemical structure of compound EU9M067

Table 4.18 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M067

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	79.3	*ax* 5.37	dd	H-3$_{ax}$ (13.2), H-3$_{eq}$ (3.0)
3	43.3	*ax* 3.11	dd	H-3$_{eq}$ (17.4), H-2$_{ax}$ (13.2)
		eq 2.79	dd	H-3$_{ax}$ (17.1), H-2$_{ax}$ (3.0)
4	196.4	-	-	-
5	154.9	-	-	-
6	130.4	-	-	-
7	160.8	-	-	-
8	91.6	6.10	s	-
9	158.6	-	-	-
10	103.1	-	-	-
1′	130.2	-	-	-
2′	127.6	7.38	d	H-3′ (8.7)
3′	114.2	6.96	d	H-2′ (8.7)
4′	159.9	-	-	-
5′	114.2	6.96	d	H-6′ (8.7)
6′	127.6	7.38	d	H-5′ (8.7)
5-OH	-	11.87	s	-
6-OMe	60.9	3.844	s	-
7-OMe	56.2	3.87	s	-
4′-OMe	55.4	3.839	s	-

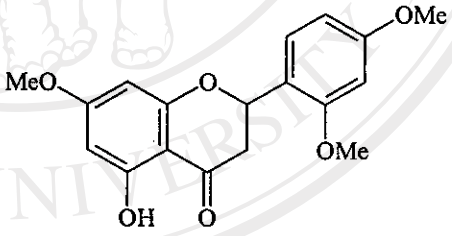
8. Compound EU9M068

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 331.1197 which represented MH^+ for $C_{18}H_{19}O_6$ (calculated mass 331.1182). The ^{1}H-NMR spectrum of EU9M068 in chloroform-d (Figure 85) exhibited the typical flavanone ABX system of H-2_{ax} and H-3_{ax} with the signals at δ5.36 ppm (H-2_{ax}; J_{AX} = 12.9, J_{BX} = 3.0 Hz), δ3.12 ppm (H-3_{ax}; J_{AB} = 17.4 Hz, J_{AX} = 12.9 Hz) and δ2.80 ppm (H-3_{eq}, J_{AB} = 17.4 Hz, J_{BX} = 3.0 Hz), a singlet signal at δ12.02 ppm (5-OH), a pair of doublets which showed allylic coupling between two olefinic protons at δ6.06 and 6.08 ppm (J = 2.1 Hz, H-6 and H-8), three aromatic protons on ring B at δ6.988 ppm (d, J = 1.8 Hz), 6.985 ppm (dd, J = 8.4, 1.8 Hz) and 6.90 ppm (d, J = 8.4 Hz) and three aromatic methoxyl groups at δ3.92, 3.91 and 3.81 ppm. The position of the methoxyl groups could be assigned into three types as below [1]-[3]. The available proton chemical shift assignments of compound EU9M068, [1] and [4] were compared and the results are shown in Table 4.19.



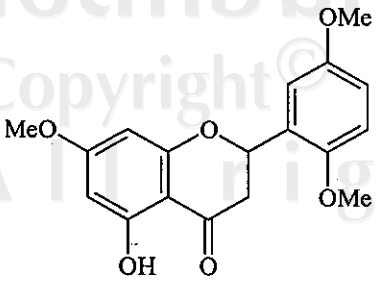
5-hydroxy-7,3′,4′-trimethoxyflavanone

[1]



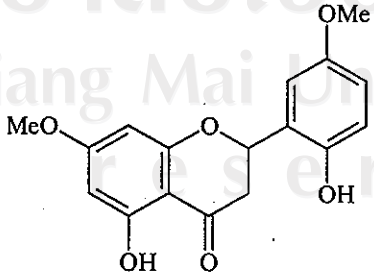
5-hydroxy-7,2′,4′-trimethoxyflavanone

[2]



5-hydroxy-7,2′,5′-trimethoxyflavanone

[3]



5,2′-dihydroxy-7,5′-dimethoxyflavanone

[4]

Table 4.19 Proton chemical shift assignments and proton-proton correlations of compound EU9M068 compared with 5-hydroxy-7,3′,4′-trimethoxy flavanone [1] and 5,2′-dihydroxy-7,5′-dimethoxyflavanone [4]

	δH (ppm), splitting pattern and coupling constant (Hz)		
	EU9M068	[1] (153)	[4] (38)
1	-	-	-
2	5.36, dd, J = 12.9, 3.0	5.36, dd, J = 13, 3	5.33, dd, J = 12.9, 3.0
3_{ax}	3.12, dd, J = 17.4, 12.9	3.11, dd, J = 17, 13	3.08, dd, J = 17.2, 12.9
3_{eq}	2.80, dd, J = 17.4, 3.0	2.79, dd, J = 17, 3	2.78, dd, J = 17.2, 3.0
4	-	-	-
5	-	-	-
6	6.06, d, J = 2.1	6.05, d, J = 2	6.04, d, J = 2.4
7	-	-	-
8	6.08, d, J = 2.1	6.07, d, J = 2	6.07, d, J = 2.4
9	-	-	-
10	-	-	-
1′	-	-	-
2′	6.988, d, J = 1.8 6.985, dd, J = 8.4, 1.8 6.90, d, J = 8.4	6.97, m	-
3′		-	6.88, d, J = 8.3
4′		-	6.93, dd, J = 8.3
5′		6.88, d, J = 9	-
6′		6.97, m	7.04, d, J = 1.8
5-OH	12.02, s	12.02, s	12.02, s
-OMe	3.81, s	3.80, s	3.80, s
-OMe	3.91, s	3.90, s	3.91, s
-OMe	3.92, s	3.91, s	-

Because of the small amount of this compound, the complete NMR spectral data could not be obtained. However, the ^{1}H-NMR spectral data of EU9M068 was very close to those of 5-hydroxy-7,3′,4′-trimethoxyflavanone [1]. Thus, the tentative structure of compound EU9M068 (Figure 15) was assigned as 5-hydroxy-7,3′,4′-trimethoxyflavanone (eriodictyol-7,3′,4′-trimethyl ether) which was previously found in *Sideritis infernalis* (153), but this flavanone had no been isolated previously from *C. odorata*.

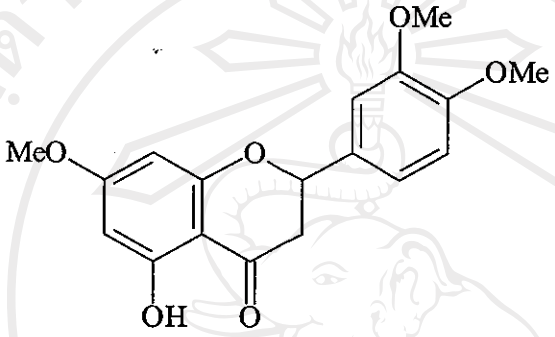


Figure 15. The tentative structure of compound EU9M068

9. Compound EU9M089

The high resolution electron impact mass spectrum exhibited a molecular ion peak at *m/z* 286.0823 which was consistent with M^{+} for $C_{16}H_{14}O_5$ on comparing the calculated mass of 286.0841. The ^{13}C-NMR spectrum of EU9M089 in chloroform-d (Figure 88) showed 14 carbon peaks which represented 16 carbon atoms. The carbons were determined as one methyl, one methylene, seven methine and seven quaternary carbons by HSQC (Figure 91) and DEPT (Figure 89) experiments. These data established its tentative molecular formula as $C_{16}H_{14}O_5$.

The ^{1}H-NMR spectrum of EU9M089 in chloroform-d (Figure 87) showed the characteristic peaks of a 5-OH-flavanone including the singlet signal at δ12.01 ppm (5-OH), three groups of doublets of doublets at δ5.35, 3.09 and 2.78 ppm which represented the ABX system of one proton at C-2 and two protons at C-3. The *para*-substituted aryl group on ring B was indicated by the signals of two groups of

doublets at δ7.32 and 6.88 ppm which showed vicinal coupling (J = 8.4 Hz). The *meta*-disubstitution on ring A was indicated by the signals of two groups of doublets at δ6.07 and 6.04 ppm which showed a *meta* coupling (J = 2.4 Hz). The last 3-proton singlet at δ3.80 ppm was consistent with an aromatic methoxyl group.

The ^{1}H-^{1}H COSY spectrum of compound EU9M089 (Figure 90) confirmed the following vicinal couplings : the proton at δ5.35 ppm (H-2_{ax}) to two protons at δ3.09 and 2.78 ppm (H-3_{ax} and H-3_{eq}), and two protons at δ7.32 ppm (H-2′ and H-6′) to two protons at δ6.88 (H-3′ and H-5′) (Table 4.20).

The assignment of the position of the methoxyl group and other structural connectivities were finally accomplished by using the HMBC experiment (Figure 92-93). The position of methoxyl group was confirmed by the NOEDS (Figure 94) as being in position 7. Compound EU9M089 was therefore identified as the known 5,4′-dihydroxy-7-methoxyflavanone (sakuranetin) which has been found previously in this species (36).

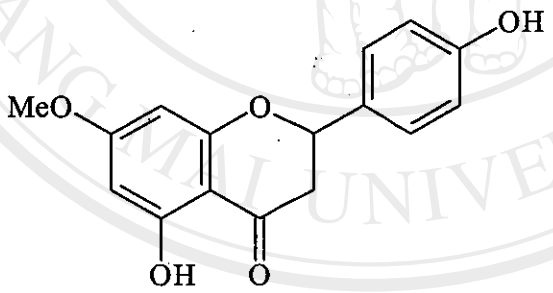


Figure 16. The chemical structure of compound EU9M089

Table 4.20 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M089

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	78.9	*ax* 5.35	dd	H-3$_{ax}$ (12.9), H-3$_{eq}$ (3.0)
3	43.2	*ax* 3.09	dd	H-3$_{eq}$ (17.4), H-2$_{ax}$ (12.9)
		eq 2.78	dd	H-3$_{ax}$ (17.4), H-2$_{ax}$ (3.0)
4	195.9	-	-	-
5	163.9	-	-	-
6	95.1	6.07	d	H-8 (2.4)
7	167.8	-	-	-
8	94.2	6.04	d	H-6 (2.4)
9	162.7	-	-	-
10	103.1	-	-	-
1′	130.4	-	-	-
2′	127.9	7.32	d	H-3′ (8.4)
3′	115.6	6.88	d	H-2′ (8.4)
4′	156.0	-	-	-
5′	115.6	6.88	d	H-6′ (8.4)
6′	127.9	7.32	d	H-5′ (8.4)
5-OH	-	12.01	s	-
7-OMe	55.7	3.80	s	-

10. Compound EU9M101

The high resolution electron impact mass spectrum exhibited the molecular ion peak at *m/z* 330.0746 consistent with the formula $C_{17}H_{14}O_7$ (calculated mass 330.0740). The ^{13}C-NMR spectrum of EU9M101 in dimethylsulfoxide-d_6 (Figure 97) showed 17 carbon peaks which represented 17 carbon atoms. The carbons were determined as two methyl, five methine and ten quaternary carbons by HSQC (Figure 100) and DEPT (Figure 98) experiments. These data established its molecular formula as $C_{17}H_{14}O_7$.

The ^{1}H-NMR spectrum of EU9M101 in DMSO-d_6 (Figure 96) showed three aromatic protons on ring B assigned to signals at δ7.08 ppm (d, $J_{5',6'}$ = 8.4 Hz; H-5′), δ7.68 ppm (dd, $J_{5',6'}$ = 8.4 Hz, $J_{2',6'}$ = 2.1 Hz; H-6′) and δ7.71 ppm (d, $J_{2',6'}$ = 2.1 Hz; H-2′). A *meta* coupling between two olefinic protons at δ6.72 ppm (d, $J_{6,8}$ = 2.4 Hz; H-8) and δ6.35 ppm (d, $J_{6,8}$ = 2.4 Hz; H-6) and the singlet signal at δ12.41 ppm (5-OH) indicated a 5,7-disustitution pattern on ring A. Two singlet signals at δ3.86 and 3.84 ppm were assigned as aromatic methoxyl groups. The other two singlet signals at δ9.55 and 9.51 ppm fulfilled the substitution of this compound and were assigned to two hydroxyl groups. The ^{1}H-^{1}H COSY spectrum of compound EU9M101 (Figure 99) confirmed these couplings and the results are summarized in Table 4.21.

The assignment of the position of the methoxyl group and completion of the structure was finally accomplished by using the HMBC experiment (Figure 101-102). The position of the methoxyl group was confirmed by the NOEDS (Figure 103-107). The NOE correlations are summarized in Figure 17. Compound EU9M101 was therefore identified as the known 3,5,3′-trihydroxy-7,4′-dimethoxyflavone (ombuin) which has also been found previously in this species (25).

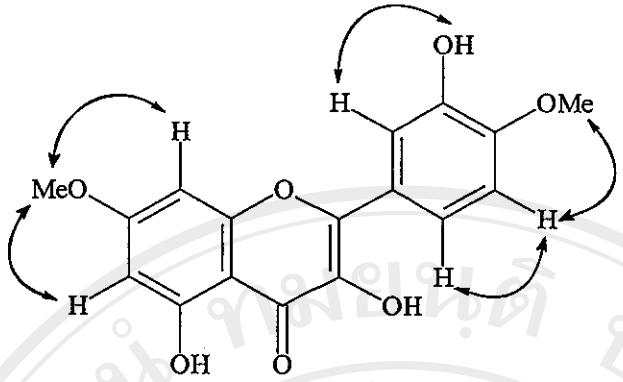


Figure 17. The correlations in the NOEDS of EU9M101

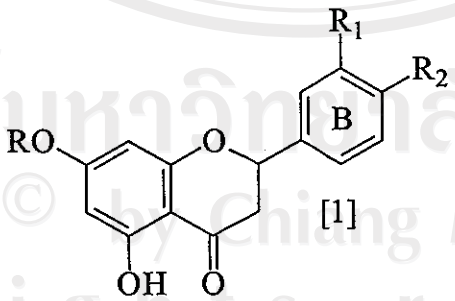
Table 4.21 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M101

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	146.6	-	-	-
3	136.2		-	-
4	175.8	-	-	-
5	160.1	-	-	-
6	97.4	6.35	d	H-8 (2.4)
7	164.7	-	-	-
8	91.8	6.72	d	H-6 (2.4)
9	155.9	-	-	-
10	103.9	-	-	-
1′	123.1	-	-	-
2′	114.6	7.71	d	H-6′ (2.1)
3′	146.0	-	-	-
4′	149.2	-	-	-
5′	111.6	7.08	d	H-6′ (8.4)
6′	119.6	7.68	dd	H-5′ (8.4), H-2′ (2.1)
3-OH	-	9.55	s	-
5-OH	-	12.41	s	-
3′-OH	-	9.31	s	-
7-OMe	56.0	3.80	s	-
4′-OMe	55.6	3.80	s	-

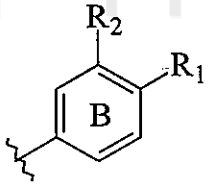
11. Compound EU9M104 (EU9M063)

The high resolution electron impact mass spectrum exhibited a molecular ion peak at *m/z* 316.0940 which represented M^+ for $C_{17}H_{16}O_6$ (calculated mass 316.0947). The ^{13}C-NMR spectrum of EU9M104 in chloroform-d (Figure 110) showed 17 carbon peaks which represented 17 carbon atoms. The carbons were determined as two methyl, one methylene, six methine and eight quaternary carbons by HSQC (Figure 113) and DEPT (Figure 111) experiments. These data established its molecular formula as $C_{17}H_{16}O_6$.

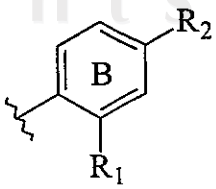
The ^{1}H-NMR spectrum of EU9M104 in chloroform-d (Figure 109) again showed the characteristic peaks of a 5-OH-flavanone including the singlet signal at δ12.01 ppm (5-OH) and the typical ABX system of the signal at δ5.32 ppm (dd, J = 12.9, 3.0 Hz; H-2_{ax}), δ3.07 ppm (dd, J = 17.1, 12.9 Hz; H-3_{ax}) and δ2.78 ppm (dd, J = 17.1, 3.0 Hz; H-3_{eq}). Another aromatic hydroxyl group was apparent from singlet at δ5.71 ppm. Two coupled olefinic protons (J = 2.1 Hz) indicated a *meta* coupling between protons at δ6.07 ppm (H-6) and δ6.04 ppm (H-8). Two intense singlet signals at δ3.91 and 3.80 ppm indicated the presence of two aromatic methoxyl groups. The other three aromatic protons showed the splitting pattern and coupling constants which could be demonstrated to be due to one of four possible structures as shown below [1]-[4] ($R_1 \neq R_2$).



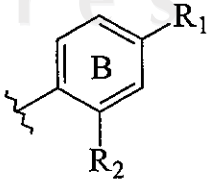
[1]



[2]



[3]



[4]

The results of the ^{1}H-^{1}H COSY spectrum of EU9M104 (Figure 112) are summarized in Table 4.22. The vicinal couplings were observed as follows : the proton at δ5.32 ppm (H-2_{ax}) to two protons at δ3.07 and 2.78 ppm (H-3_{ax} and H-3_{eq}).

The assignment of the position of the methoxyl group and finalization of the structure were finally accomplished by using the HMBC experiment (Figure 114-115) and from the NOEDS of compound EU9M101 (Figure 116-117). The latter showed the correlations between methoxyl groups at δ3.80 and H-6 and another at δ3.91 and H-5′ indicating a 7-OMe and 4′-OMe, respectively (Figure 18). The structure of compound EU9M104 was also unequivocally determined by single crystal x-ray crystallography undertaken by Professor A.H. White of the University of Western Australia. The crystal structure is shown in Figure 118 (Note : the crystal atom numbering is not the same as the systematic numbering).

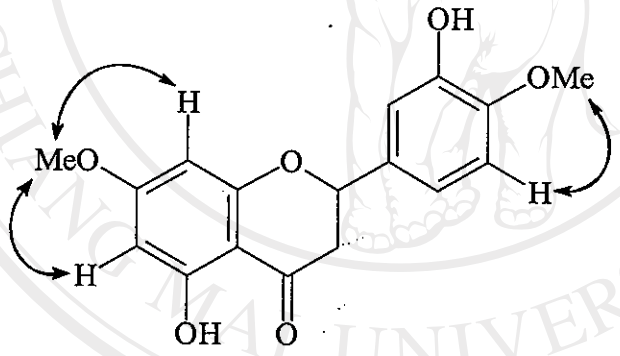


Figure 18. The correlations in the NOEDS of EU9M104

Compound EU9M104, (mp 160-161°C; $[\alpha]_D^{25}$ -33°, *c* 0.90, chloroform), was therefore identified as the known 5,3′-dihydroxy-7,4′-dimethoxyflavanone (persicogenin) which has also been found previously in this species (39).

Table 4.22 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M104

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	78.9	*ax* 5.32	dd	H-3$_{ax}$ (12.9), H-3$_{eq}$ (3.0)
3	43.3	*ax* 3.07	dd	H-3$_{eq}$ (17.1), H-2$_{ax}$ (12.9)
		eq 2.78	dd	H-3$_{ax}$ (17.1), H-2$_{ax}$ (3.0)
4	195.7	-	-	-
5	163.9	-	-	-
6	95.1	6.07	d	H-8 (2.1)
7	167.8	-	-	-
8	94.2	6.04	d	H-6 (2.1)
9	162.7	-	-	-
10	103.1	-	-	-
1′	130.1	-	-	-
2′	112.6	7.04	d	H-6′ (1.8)
3′	145.8	-	-	-
4′	146.8	-	-	-
5′	110.6	6.88	d	H-6′ (8.4)
6′	118.1	6.93	dd	H-5′ (8.4), H-2′ (1.8)
5-OH	-	12.03	s	-
3′-OH	-	5.71	s	-
7-OMe	55.7	3.81	s	-
4′-OMe	56.1	3.84	s	-

12. Compound EU9M112

The high resolution electron impact mass spectrum exhibited a molecular ion peak at *m/z* 284.0689 which represented M^+ for $C_{16}H_{12}O_5$ on comparing the calculated mass of 284.0685. The ^{13}C-NMR spectrum of EU9M112 in dimethyl-sulfoxide-d_6 (Figure 121) showed 14 carbon peaks which represented 16 carbon atoms; these were assigned as one methyl, seven methine and eight quaternary carbons by HSQC (Figure 124) and DEPT (Figure 122) experiments. These data established its tentative molecular formula as $C_{16}H_{12}O_5$.

The ^{1}H-NMR spectrum of EU9M112 in DMSO-d_6 (Figure 120) showed characteristic 5-OH-flavone peaks including a singlet signal at δ12.90 ppm (5-OH) and a singlet signal at δ6.86 ppm (H-3). Two coupled olefinic protons (J = 2.1 Hz) indicated a *meta* coupling between protons at δ6.19 ppm (H-6) and δ6.49 ppm (H-8). A singlet signal at δ3.85 ppm indicated a methoxyl group substituted on an aromatic ring. Two doublet signals at δ8.02 (2H, d, J = 8.7 Hz; H-2′ and H-6′) and δ7.10 ppm (2H, d, J = 8.7 Hz; H-3′ and H-5′) were also apparent..

The ^{1}H-^{1}H COSY spectrum of EU9M112 (Figure 123) confirmed vicinal couplings between the protons at δ7.10 ppm (H-3′ and H-5′) and the protons at δ8.02 ppm (H-2′ and H-6′) (Table 4.23).

The assignment of the position of the methoxyl group and other structural connectivities were finally accomplished by using the HMBC experiment (Figure 125-126). The NOEDS of compound EU9M112 (Figure 127) showed the correlation between the methoxyl group at δ3.85 (at C-4′) and the protons at δ7.10 ppm (H-3′ and H-5′).

Compound EU9M112 was therefore identified as the known 5,7-dihydroxy-4′-methoxyflavone (acacetin) which has been found previously in this species (39).

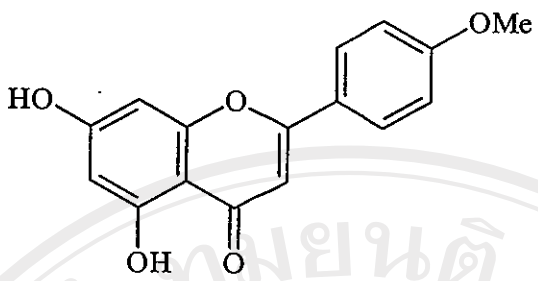


Figure 19. The chemical structure of compound EU9M112

Table 4.23 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M112

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	164.1	-	-	-
3	103.4	6.86	s	-
4	181.5	-	-	-
5	163.9	-	-	-
6	98.8	6.19	d	H-8 (2.1)
7	163.0	-	-	-
8	93.9	6.49	d	H-6 (2.1)
9	157.1	-	-	-
10	103.6	-	-	-
1′	122.7	-	-	-
2′	128.1	8.02	d	H-3′ (8.7)
3′	114.4	7.10	d	H-2′ (8.7)
4′	162.1	-	-	-
5′	114.4	7.10	d	H-6′ (8.7)
6′	128.1	8.02	d	H-5′ (8.7)
5-OH	-	12.90	s	-
4′-OMe	55.5	3.85	s	-

13. Compound EU9M135

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 303.0873 consistent with MH^+ for $C_{16}H_{15}O_6$ (calculated mass 303.0869). The ^{13}C-NMR spectrum of EU9M135 in acetone-d_6 (Figure 130) showed 14 peaks which represented 16 carbon atoms. The carbons were determined as one methyl, eight methine and seven quaternary carbons by HSQC (Figure 133) and DEPT (Figure 131) experiments. These data established its tentative molecular formula as $C_{16}H_{15}O_6$.

The ^{1}H-NMR spectrum of EU9M135 in acetone-d_6 (Figure 129) exhibited resonance signals that were similar to those of compound EU9M055. The typical AX system of H-2$_{ax}$ and H-3$_{ax}$ appeared at δ5.11 ppm (H-2$_{ax}$; J_{AX} = 12.0 Hz) and δ4.68 ppm (H-3; J_{AX} = 12.0 Hz and $J_{3,OH}$ = 3.6 HZ), where the H-3$_{ax}$ signal was split further by coupling with the hydroxyl proton (δ4.74, d, J = 3.6 Hz; 3-OH) which was substituted at C-3. The large coupling constant (J_{AX} = 12.0 Hz) showed a *trans*-diaxial relationship and indicated a dihydroflavonol structure for compound EU9M135. The proton signals at δ7.42 ppm (2H, d, J = 8.4 Hz; H-2′ and H-6′) and δ6.90 ppm (2H, d, J = 8.4 Hz; H-3′ and H-5′) indicated *para*-monosubstitution on ring B.

The correlations from the ^{1}H-^{1}H COSY spectrum of EU9M135 (Figure 132) are summarized in Table 4.24. The assignment of the position of the methoxyl groups and connection of the structure was finally accomplished by use of the HMBC experiment (Figure 134-135). The NOEDS (Figure 136-137) indicated the 7-position for the methoxyl group at δ3.86 ppm. The NOE correlations are shown in Figure 20.

Compound EU9M135 was therefore identified as the known 3,5,4′-trihydroxy-7-methoxyflavanone (aromadendrin-7-dimethyl ether) which was previously found in the stem bark of *Afzelia bella* (154), but has not been reported previously from *C. odorata*.

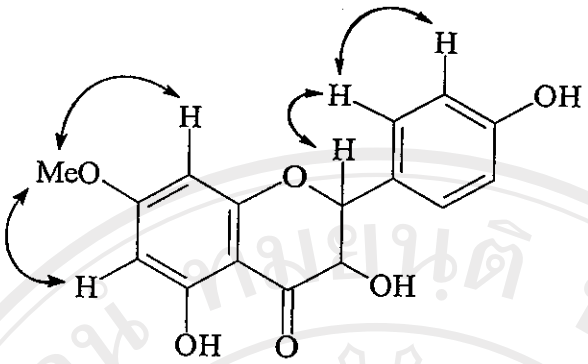


Figure 20. The correlations in the NOEDS of EU9M135

Table 4.24 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M135

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	84.3	*ax* 5.11	d	H-3$_{ax}$ (12.0)
3	73.1	*ax* 4.68	dd	H-2$_{ax}$ (12.0), 3-OH (3.6)
4	198.3	-	-	-
5	164.4	-	-	-
6	95.6	6.08	d	H-8 (2.1)
7	169.0	-	-	-
8	94.5	6.05	d	H-6 (2.1)
9	163.7	-	-	-
10	101.9	-	-	-
1′	128.8	-	-	-
2′	130.1	7.42	d	H-3′ (8.4)
3′	115.7	6.90	d	H-2′ (8.4)
4′	158.6	-	-	-
5′	115.7	6.90	d	H-6′ (8.4)
6′	130.1	7.42	d	H-5′ (8.4)
3-OH	-	4.74	d	H-3 (3.6)
5-OH	-	11.67	br s	-
4′-OH	-	8.53	br s	-
7-OMe	56.3	3.86	s	-

14. Compound EU9M138K

The high resolution chemical ionization mass spectrum exhibited a molecular ion peak at *m/z* 303.0874 which represented MH^+ for $C_{16}H_{15}O_6$ (calculated mass 303.0869). The ^{13}C-NMR spectrum of EU9M135 in acetone-d_6 (Figure 140) showed 16 carbon peaks which represented 16 carbon atoms. These carbons were determined as one methyl, one methylene, six methine and eight quaternary carbons by HSQC (Figure 143) and DEPT (Figure 141) experiments. These data established its tentative molecular formula as $C_{16}H_{14}O_6$.

The ^{1}H-NMR spectrum of EU9M138 in acetone-d_6 (Figure 139) showed the characteristic of 5-OH-flavanone pattern including a singlet signal at δ12.17 ppm (5-OH), and the typical ABX system of three protons at δ5.43 (1H, dd, J_{AX} = 12.6, J_{BX} = 3.0 Hz; H-2$_{ax}$), δ3.15 (1H, dd, J_{AB} = 17.1 Hz, J_{AX} = 12.6; H-3$_{ax}$) and δ2.75 ppm (1H, dd, J_{AB} = 17.1 Hz, J_{BX} = 3.0 Hz; H-3$_{eq}$). Two olefinic proton signals at δ5.97 ppm (1H, d, J = 2.4 Hz; H-8) and δ5.95 ppm (1H, d, J = 2.4 Hz; H-6) indicated 5,7-disustitution on ring A. The remaining three proton signals, appeared around 7.0 ppm supportive of disubstitution on ring B, and without evidence of vicinal coupling among these protons, suggested 3′,5′-disubstitution in ring B.

The ^{1}H-^{1}H COSY spectrum of compound EU9M138 (Figure 142) showed correlations between proton at δ5.43 ppm (H-2$_{ax}$) and δ3.15 ppm (H-3$_{ax}$) and the proton-proton correlations and assignments are summarized in Table 4.25. The assignment of the position of the methoxyl group and finalization of the structure was accomplished by using the HMBC experiment (Figure 144-145). The NOEDS (Figure 146-147) exhibited a correlation between the proton signal at δ3.86 ppm and the proton signal at δ6.97 ppm which then indicated the placement of the methoxyl group in ring B.

Compound EU9M138 was therefore identified as the known 5,7,3′-trihydroxy-5′-methoxyflavanone (alyssifolium) which has been reported on one occasion from the aerial parts of *Teucrium alyssifolium* (155); this is the first time it has been isolated from *C. odorata*.

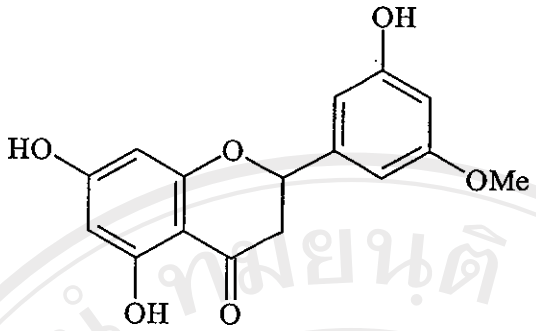


Figure 21. The chemical structure of compound EU9M138

Table 4.25 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M138

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	79.7	*ax* 5.43	dd	H-3$_{ax}$ (12.6), H-3$_{eq}$ (3.0)
3	43.3	*ax* 3.15	dd	H-3$_{eq}$ (17.1), H-2$_{ax}$ (12.6)
		eq 2.75	dd	H-3$_{ax}$ (17.1), H-2$_{ax}$ (3.0)
4	196.8	-	-	-
5	165.0	-	-	-
6	96.7	5.95	d	H-8 (2.4)
7	167.0	-	-	-
8	95.7	6.05	d	H-6 (2.4)
9	164.0	-	-	-
10	103.1	-	-	-
1′	132.6	-	-	-
2′	118.6	6.97	m	-
3′	147.3	-		-
4′	112.1	6.97	m	-
5′	148.4	-	-	-
6′	114.2	7.05	m	-
5-OH	-	11.67	br s	-
7-OH	-	-	-	-
3′-OH	-	-	-	-
5′-OMe	56.2	3.86	s	-

15. Compound EU9M147

The high resolution chemical ionization mass spectrum gave a molecular ion peak at *m/z* 303.0876 which represented MH^+ for $C_{16}H_{15}O_6$ (calculated mass 303.0869). The ^{13}C-NMR spectrum of EU9M147 in acetone-d_6 (Figure 150) showed 14 carbon peaks which were representative of 16 carbon atoms. These carbons were determined as one methyl, eight methine and seven quaternary carbons by HSQC (Figure 153) and DEPT (Figure 151) experiments. These data established its tentative molecular formula as $C_{16}H_{15}O_6$.

The ^{1}H-NMR spectrum of EU9M147 in acetone-d_6 (Figure 149) exhibited resonance signals that were similar to those of compound EU9M135. The typical AX system of H-2$_{ax}$ and H-3$_{ax}$ was indicated by signals at δ5.12 ppm (H-2$_{ax}$; J_{AX} = 11.7 Hz) and δ4.66 ppm (H-3$_{ax}$; J_{AX} = 11.7 Hz). The large coupling constant (J_{AX} = 11.7 Hz) showed a *trans*-diaxial relationship and indicated a dihydroflavonol structure for compound EU9M147. The proton signals at δ7.51 ppm (2H, d, J = 8.7 Hz; H-2′ and H-6′) and δ6.99 ppm (2H, d, J = 8.7 Hz; H-3′ and H-5′) suggested a *para*-monosubstituted ring B.

The ^{1}H-^{1}H COSY spectrum of EU9M147 (Figure 152) showed vicinal couplings as follows : the proton signals at δ7.51 ppm to the protons at δ6.99 ppm and the proton at δ5.12 ppm to the proton at δ4.66 ppm. The proton correlations and assignments are summarized in Table 4.26. The assignment of the position of the methoxyl group (δ3.83) to C-4′ was accomplished by using the HMBC experiment (Figure 154-155) and the NOEDS (Figure 156-157). The correlation of protons in the NOEDS is shown in Figure 22.

Compound EU9M147 was therefore identified as the known 3,5,7-trihydroxy-4′-methoxyflavanone (dihydrokaempferide) which was previously isolated from wood of *Salix caprea* (156), but this is the first report from *C. odorata*.

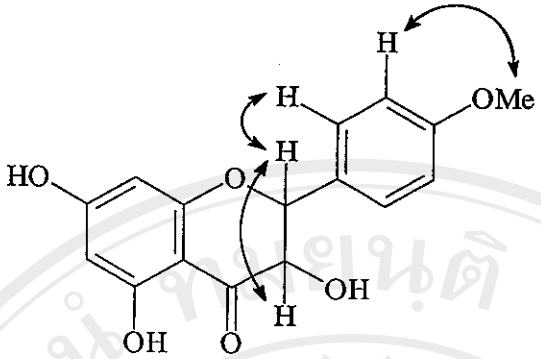
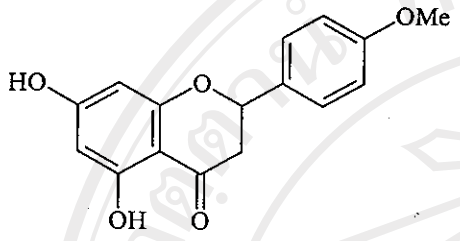


Figure 22. The correlations in the NOEDS of EU9M147

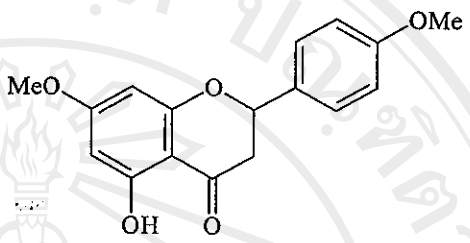
Table 4.26 Carbon and proton chemical shift assignments and proton-proton correlations of compound EU9M147

	δC (ppm)	δH (ppm)	Splitting pattern	Multiplicity (*J* value, Hz)
1	-	-	-	-
2	84.0	*ax* 5.12	d	H-3$_{ax}$ (11.7)
3	73.0	*ax* 4.66	dd	H-2$_{ax}$ (11.7)
4	197.8	-	-	-
5	164.7	-	-	-
6	97.0	6.00	d	H-8 (2.1)
7	167.5	-	-	-
8	95.9	5.96	d	H-6 (2.1)
9	163.8	-	-	-
10	101.4	-	-	-
1′	130.0	-	-	-
2′	129.9	7.51	d	H-3′ (8.7)
3′	114.3	6.99	d	H-2′ (8.7)
4′	160.8	-	-	-
5′	114.3	6.99	d	H-6′ (8.7)
6′	129.9	7.51	d	H-5′ (8.7)
3-OH	-	-	-	
5-OH	-	11.70	br s	-
7-OH	-	-	-	-
4′-OMe	55.5	3.83	s	-

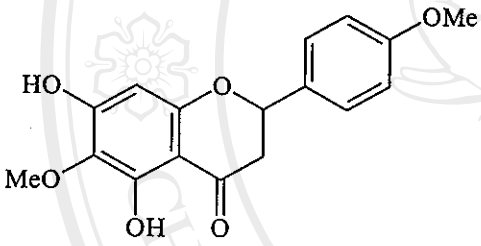
All of the isolated compounds from the aerial parts of *C. odorata* are flavonoids derivatives which could be classified into four subclasses including flavanones (EU9M021, 028, 044, 067, 068, 089, 104, 138), flavanonols (EU9M055, 135, 147), flavones (EU9M058, 112), flavonols (EU9M101) and chalcones (EU9M029) (see the structures below).



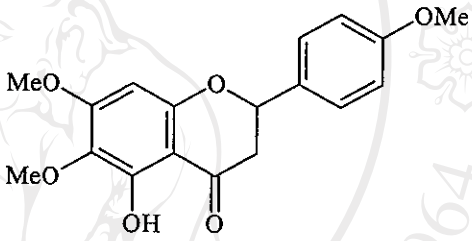
EU9M021



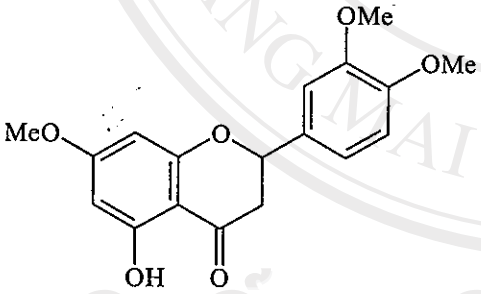
EU9M028



EU9M044



EU9M067



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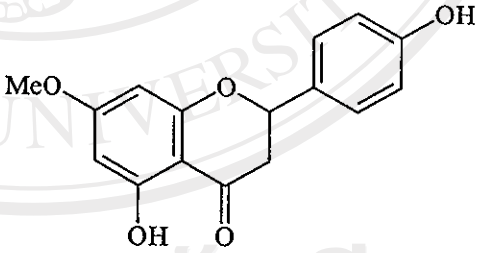

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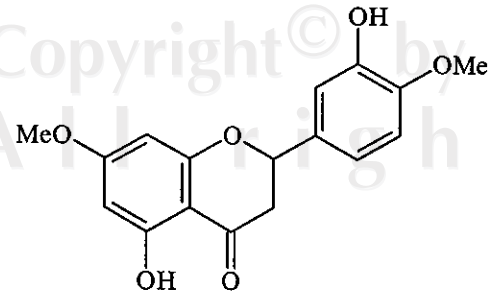
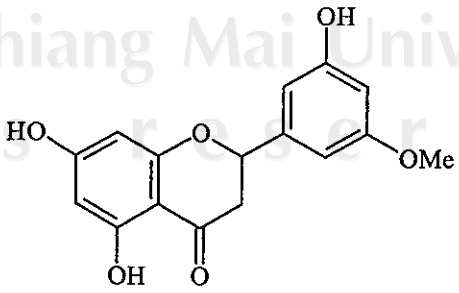
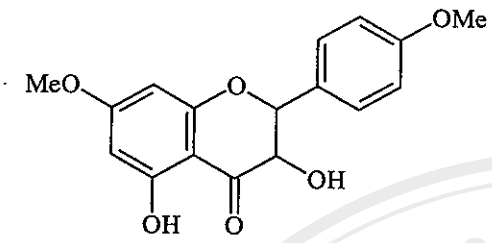


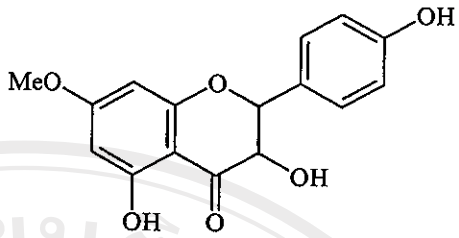
EU9M104



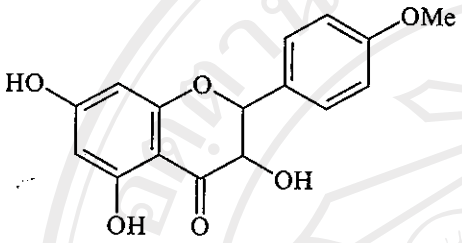
EU9M138



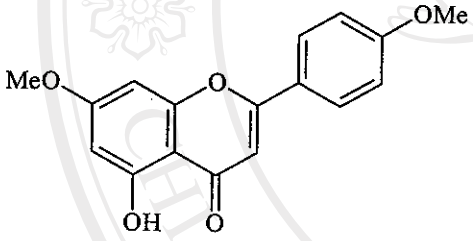
EU9M055



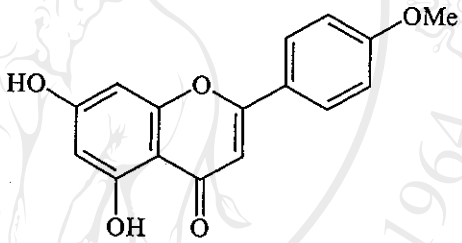
EU9M135



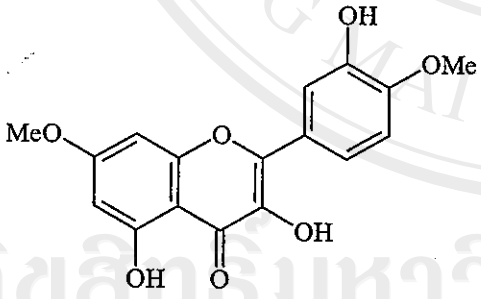
EU9M147



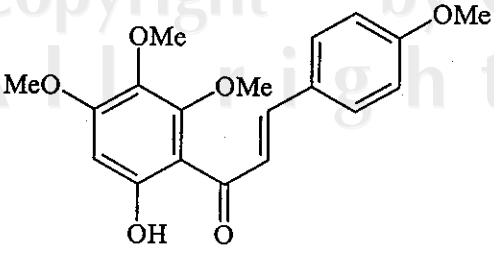
EU9M058



EU9M112

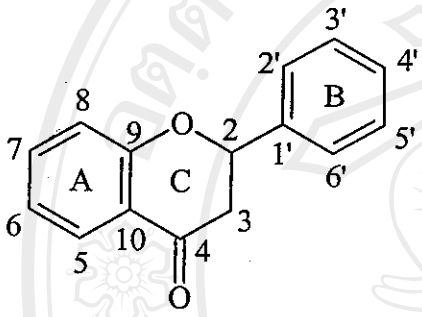


EU9M101

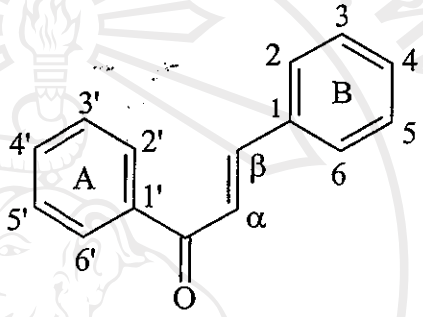


EU9M029

The flavonoids contain fifteen carbon atoms in their basic nucleus and these are arranged in a C_6-C_3-C_6 configuration, that is, two aromatic rings linked by three carbon unit which may or may not form a third ring. For convenience the rings are labeled A, B and C and the individual carbon atoms are referred to by a numbering system which utilizes ordinary numerals for the A- and C-rings and "primed" numerals for the B-ring (see [1] but modified numbering systems used for chalcones, [2]) (157-158).



[1]



[2]

The flavonoids variants are all related by a common biosynthetic pathway which incorporates precursors from both the "shikimate" and "acetate-malonate" pathways, the first flavonoid being produced immediately following confluence of the two pathways (Figure 23). The flavonoid initially formed in the biosynthesis is now though to be the chalcone and all other forms are derived from this by a variety of routes (Figure 23). Further modification of the flavonoid may occur at various stages resulting in : additional (or reduced) hydroxylation; methylation of hydroxyl groups or of the flavonoid nucleus; isoprenylation of hydroxyl groups or of the flavonoid nucleus; methylenation of *ortho*-dihydroxyl groups; dimerization (to produce biflavonoids); and most importantly, glycosylation of hydroxyl groups (to produce flavonoid *O*-glycosides) or of the flavonoid nucleus (to produce flavonoid *C*-glycosides) (157-158). In this study, all of the isolated compounds are flavonoid aglycones.

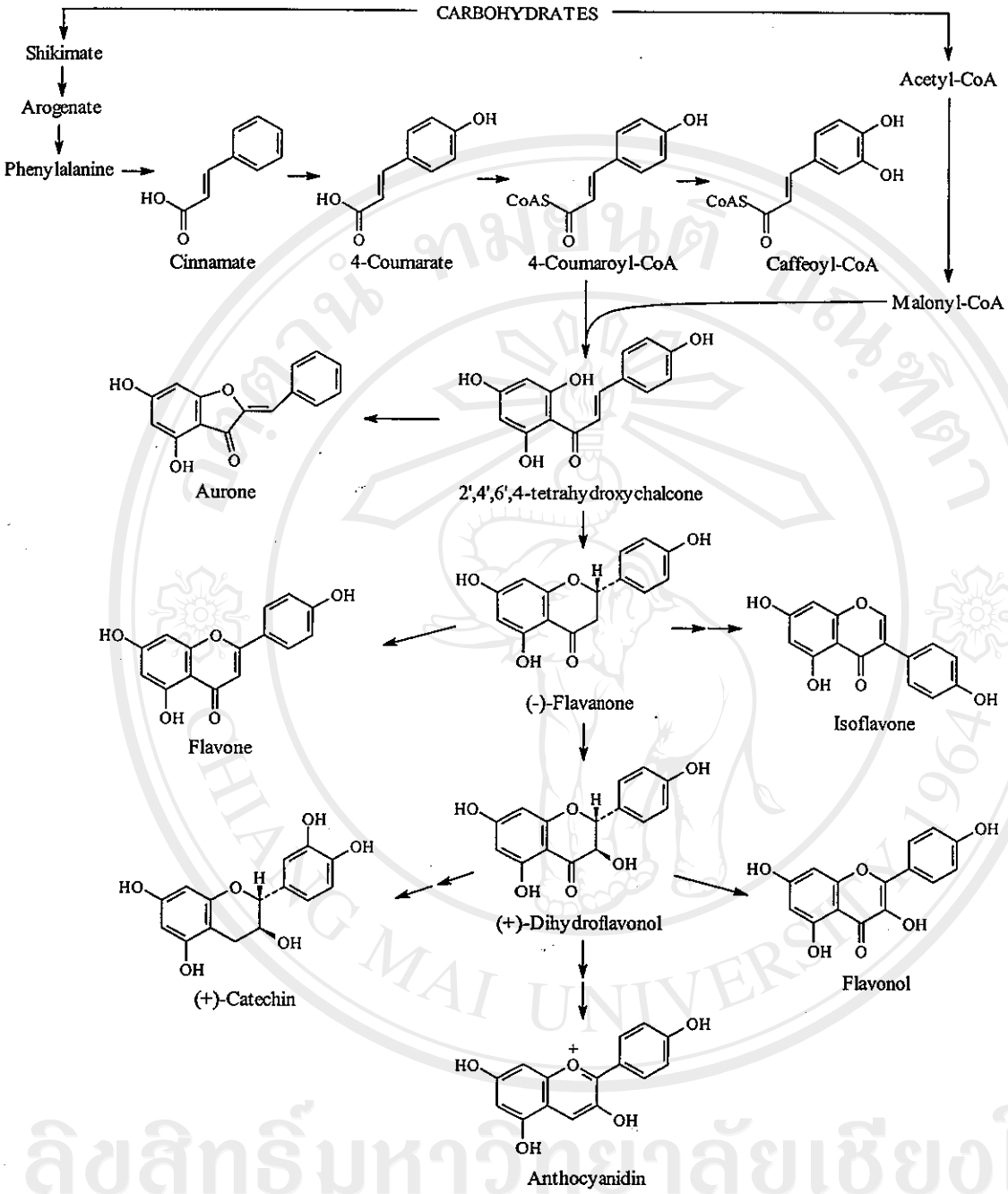


Figure 23. The proposed biosynthesis and interrelationships between flavonoid monomer types