CHAPTER IV

RESULTS AND DISCUSSION

The 1.2 kg of dried, ground roots bark of *Micromelum minutum* Wight & Arn. were refluxed with 4 liters of hexane twice to obtain 18 g of gummy residue. A 10.0 g of gummy reside was chromatographed on silica gel by fractional column chromatography, then eluting with hexane : ethyl acetate (6 : 1). Each individual fraction that was eluted from the column chromatography was monitored by TLC. Compound I was isolated and purified.

The chloroform filtrate that were refluxed with 4 liters of chloroform three times obtained a brown oily residue (31 g). Ten grams of the residue were chromatographed over column chromatography. Compound II was isolated and recrystallized.

The combined methanol that were refluxed with 6 liters of methanol three times obtained 95 g gummy residue. 50 g of gummy residue were chromatographed by column chromatography, then eluting with hexane : ethyl acetate (1 : 10). Each individual fraction that was eluted from the column chromatography was monitored by TLC. The appropriate fraction were combined and rechromatographed over silica by suitable solvent system. Compound III was isolated and purified. The identification

of three compounds were performed by physical and chemical method as following.

1. Compound I : 324 mg (3.24% yield) of crystal was obtained from F_{22} - F_{30}. This compound was identified on the basis of the following data.

Color and form of crystal : This compound was crystallized from diethyl ether as a colorless prism.

Rf value :

0.64 on TLC solvent system 1

0.57 on TLC solvent system 2

0.90 on TLC solvent system 3

0.83 on TLC solvent system 4

0.77 on TLC solvent system 5

Molecular weight : 244 (EIMs)

Melting point : 80 - 81^{o} (uncorrected)

TLC TECHNIQUE : The compound was spotted on silica gel GF 254 plate and developed in solvent system 1, 2, 3, 4, 5. After drying the plate, the detection was performed under UV and sprayed with benzidine reagent.

UV DETECTION : This compound gave only one spot on TLC in five solvent systems and fluoresced under 254 nm (short wavelength) and 365 nm (long wavelength)

Benzidine reagent : This compound gave negative color with benzidine reagent. It is indicated that there is no phenolic group in the structure.

SPECTRAL DATA :

Ultraviolet absorption spectrum (in Methanol) (see figure 38 appendix)

λ_{max}^{MeOH} = 321 nm (log ε 4.21)

= 258 nm (log ε 3.77)

= 207 nm (log ε 4.65)

Infrared Spectrum (potassium bromide disc) (see Figure 39 Appendix)

ν_{max}^{KBr} = 2880-3010 cm^{-1} (-CH streching)

= 1730 cm^{-1} (C=O streching)

= 1610 cm^{-1} (conjugated double bond at C-3, C-4)

= 1450-1600 cm^{-1} (aromatic)

Mass spectrum (see Figure 40 Appendix)

m/e(%) = 244(100.0), 229(76.8), 213(35.8), 201(49.9), 189(52.1), 175(15.3), 159(17.3), 131(28.5), 115(15.8), 77(15.3)

NUCLEAR MAGNETIC RESONANCE SPECTRUM

^{1}H-NMR SPECTRUM : 25% w/v of sample of deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 500 MHz and analysed in δ value (ppm) (see Figure 41 Appendix).

Proton	Chemical Shift (ppm)	Multiplicity
Coumarin H_4	7.59	Doublet (1H) J = 9.4 Hz
Coumarin H_5	7.26	Doublet (1H) J = 8.6 Hz
Coumarin H_6	6.82	Doublet (1H) J = 8.6 Hz
Coumarin H_3	6.18	Doublet (1H) J = 9.4 Hz
Butene 2 CH	5.23	Broad triplet (1H) J = 7.3 Hz
7-OCH_3	3.90	Singlet (3H)
Butene 1 CH_2	3.51	Broad double (2H) J = 7.3 Hz
Butene E CH_3	1.84	Singlet (3H)
Butene Z CH_3	1.68	Singlet (3H)

^{13}C NMR SPECTRUM : 25% w/v of sample in deuteriochloroform using deuteriochloform as reference compound at 76.9 ppm. The spectrum was analysed in δ value (ppm) (see Figure 42 appendix).

Carbon	Chemical Shift (ppm)	Multiplicity
Coumarin C_2	160.9	Singlet
Coumarin C_7	159.8	Singlet
Coumarin $C8_a$	152.4	Singlet
Coumarin C_4	143.5	Doublet
Butene C_3	132.1	Singlet
Coumarin C_5	126.0	Doublet
Butene 2 CH	120.9	Doublet
Coumarin C8	117.3	Singlet
Coumarin $C4_a$	112.5	Singlet
Coumarin C 3	112.4	Doublet
Coumarin C 6	107.0	Doublet
$-OCH_3$	55.6	Quartet
Butene Z CH_3	25.4	Quartet
Butene 1 CH_2	21.5	Triplet
Butene E CH_3	17.5	Quartet

DEPT-90 SPECTRUM : (CH only) same as ^{13}C NMR except there is a ^{13}C 90° pulse and a ^{1}H 90° pulse. (see Figure 42 appendix).

Carbon	Position
Coumarin C_4	Negative (δ 143.5)
Coumarin C_5	Negative (δ 126.0)
Butene 2 CH	Negative (δ 120.9)
Coumarin C_3	Negative (δ 112.4)
Coumarin C_6	Negative (δ 107.0)

DEPT-135 SPECTRUM : Same as Dept-90 except there is a ^{1}H 135° pulse. (see Figure 42 appendix).

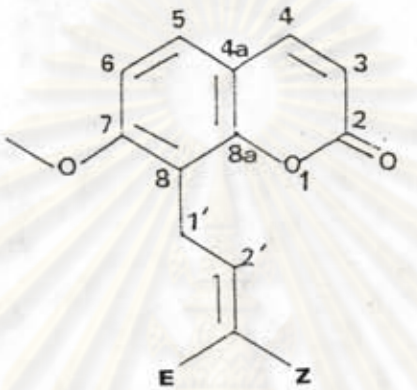
Carbon	Position
Coumarin C_4	Positive (δ 143.5)
Coumarin C_5	Positive (δ 126.0)
Butene 2 CH	Positive (δ 120.9)
Coumarin C_3	Positive (δ 112.4)
Coumarin C_6	Positive (δ 107.0)
$-OCH_3$	Positive (δ 55.6)
Butene Z CH_3	Positive (δ 25.4)
Butene 1 CH_2	Negative (δ 21.5)
Butene E CH_3	Positive (δ 17.5)

H-H COSY SPECTRUM : 25% w/v of sample in deuteriochloroform. The spectrum was obtained from H-H COSY pulse sequence and analysed in row and column projections plotted. (see Figure 43 appendix)

NOESY SPECTRUM : Same as H-H COSY. The spectrum was obtained from NOESY pulse sequence. (see Figure 44 appendix)

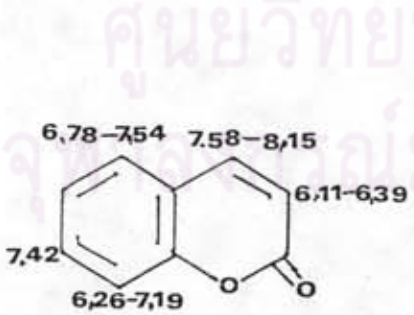
C-H COSY SPECTRUM : The spectrum was obtained from C-H COSY pulse sequence and analysed in term of the correlation between carbon and proton. (see Figure 45 appendix)

LONG RANGE C-H COSY SPECTRUM : same as one bond C-H COSY. But the spectrum was analysed in term of the two or three bond (long range coupling) correlation between carbon and proton. (see Figure 46, 47 appendix) All these information, suggest that compound I is osthol [7-methoxy-8-(3-metheylbut-2-enyl) coumarin].

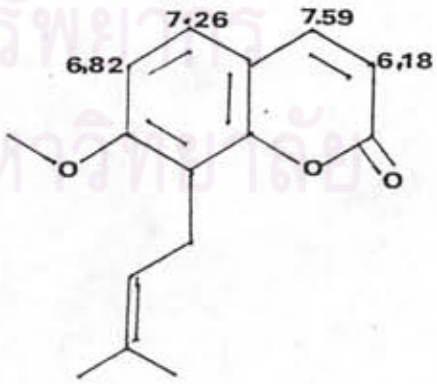


osthol

From ^{1}H-NMR spectrum of osthol, the protons of coumarin nucleus (H-3, H-4, H-5, H-6) were assign by comparing with the chemical shift of coumarin nucleus, that was reported by T.R. Seshadri (8) as in Figure 48.



coumarin nucleus



osthol

Figure 48 structure of coumarin nucleus and osthol.

The coupling constant ($J_{3,4}$ = 9.4 Hz) confirms that the protons at the 3 and 4 positions were cis to each other as at the 5 and 6 positions. It indicated that there were two substituted group at the 7 and 8 positions. The assignments are confirmed by H-H cosy that displays the correlation between H-4 and H-3 and between H-5 and H-6. For the assignment of the methine of butene side chain is confirmed by the correlation with the methylene and two methyl group of butene from H-H COSY spectrum.

The signal at 1.84 and 1.68 were assigned as methyl group at E-butene and Z-butene position respectively by the interpretation from NOESY spectrum. The position of the substituents was most easily demonstrated by the NOESY spectrum. This showed the close approach of proton pair H_4, H_5 and H_6 to the methoxy group. This confirmed the two substiuents must be on carbon 7 and 8 with the methoxy group on carbon 7.

From ^{13}C-spectrum, the multipicity of signal could be identified by the off resonance experiment that was possible to discriminate between methyl, methylene, methine, and quarternary carbons. These were actually determined from DEPT-90 and DEPT-135 spectra. The DEPT-90 spectrum showed only the methine carbon signal at the negative position. The DEPT-135 spectrum displayed the methine and methyl carbon signal at the positive position and the methylene carbon signal at the negative position.

The carbonyl carbon could be identified by its chemical shift charteristic. The lactone carbonyl carbon appeared at 160.9 ppm.

The quarternary carbons in the benzene ring could be separated into two groups. The quarternary carbons that attached to the oxygen atoms (C-8a, C-7) were downfield more than the other group which have no oxygen (C-4a, C-8).

The assignment of C-4a and C-8 could be separated by observing the environment of C-8 which was nearly the oxygen atom. So the chemical shift of C-8 was down field to 117.3 ppm while C-4a signal appeared at 112.5 ppm. These confirmed by the long range C-H COSY spectra (Figure 46, 47). The C-8 correlated to H-6 with three bond coupling and correlated to methylene of butene with two bond coupling. The C-4a correlated to H-3 and H-6 with long range coupling.

The second group (C-7, C-8a) which had oxygen attached. The C-7 (159.8 ppm) which was identified by the long range C-H COSY spectra correlated to methyl proton of 7-methoxy and H-5 with three bond coupling. The upfield signal was assigned to be C-8a (152.4 ppm) because there are the three bond correlation between C-8a and H-4 & H-5. The last quarternary carbon signal which appeared at 132.1 ppm was the C-3 butene which was confirmed by the long range C-H COSY.

The assignment of the methine carbons in coumarin nucleus could be separated by observing the environment of C-3 and C-6 which had the ortho effect of the oxygen neighbours. C-3 and C-6 resonated high field at 112.4 ppm and 107.0 ppm. These were confirmed by the one bond C-H COSY spectrum. C_4 (143.5 ppm) and C_5 (126.0 ppm) which were identified by the one bond C-H COSY and the long range C-H COSY spectra correlated to H-5 and to H-4 with three bond coupling. The methine carbon at the side chain which appeared at 120.9 ppm was confirmed by the long range C-H COSY. The C-2 butene correlated to methylene with two bond and to two methyl groups with three bond coupling.

The signal at 25.4 and 17.5 were assigned as methyl carbon at Z-butene and E-butene respectively by the interpretation from the one bond C-H COSY and the long range C-H COSY spectra.

From EI mass spectral fragmentation pattern which showed the expected molecular ion peak at m/e 244. The carcking pattern was characterized by the loss of methyl radical (m/e 229). Seshadri and Vishwapaul (8) have demonstrated that C-prenylated coumarins lose methyl radical from the prenyl substituent rather than from the methoxy group. So osthol eliminated methyl radical from prenyl substituent to highly conjugated ion (II) (Figure 49).

The data obtained indicated that this compound was osthol agreeing with the data from literature (44).

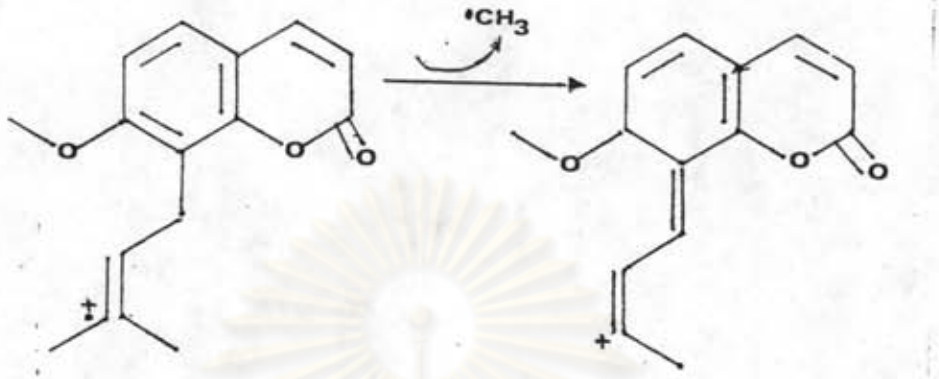


Figure 49 The cracking pattern of osthol.

2. Compound II : 183 mg (1.83% yield) of crystal was obtained from F_{41} - F_{50}. This compound was identified with the following data.

Color and form of crystal : This compound was crystallized by diethy ether and methanol as colorless needle crystals.

Rf value :

0.36 on TLC solvent system 1

0.28 on TLC solvent system 2

0.74 on TLC solvent system 3

0.60 on TLC solvent system 5

0.41 on TLC solvent system 6

Molecular weight : 288 (EIMs)

Melting point : 212 - 214°C (uncorrected)

TLC TECHNIQUE : The compound was spotted on silica gel GF 254 plate and developed in solvent system 1, 2, 3, 5, 6. After drying the plate, the detections were performed under UV, I_2 vapor and sprayed with benzidine reagent.

UV DETECTION : This compound gave only one spot on TLC in five solvents system and fluoresced under 254 nm (short wavelength) and 365 nm (long wavelength) and gave a brown spot in I_2 vapor.

Benzidine reagent : This compound gave negative color with benzidine reagent. It is indicated that there is no phenolic group in the structure.

SPECTRAL DATA :

Ultraviolet absorption spectrum (in Methanol) (see Figure 50 appendix)

λ_{max}^{MeOH} = 320 nm (log ε 4.22)

= 207 nm (log ε 4.58)

Infrared spectrum (Potassium bromide disc) (see Figure 51 appendix)

ν_{max}^{KBr} = 2800-3010 (-CH streching)

1770 (C=O streching, -lactone ring)

1735 (C=O streching, coumarin lactone)

1630 (conjugated double bond at C-3, C-4)

1450-1600 (aromatic)

Mass spectrum (see Figure 52 appendix)

m/e (%) = 288(100.0), 229(54.6), 214(16.0), 213(38.8), 203(11.4), 186(16.2), 158(12.4), 43(15.3)

NUCLEAR MAGNETIC RESONANCE SPECTRUM

^{1}H-NMR SPECTRUM : This sample was run as a saturated solution in 0.7 ml of deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 900 MHz and analysed in δ value (ppm). (see Figure 53 appendix)

Proton	Chemical Shift (ppm)	Multiplicity
Coumarin H_4	7.67	Doublet (1H) J = 9.4 Hz
Coumarin H_5	7.38	Singlet (1H)
Coumarin H_8	6.88	Singlet (1H)
Coumarin H_3	6.32	Doublet (1H) J = 9.4 Hz
Furanyl H_2	5.56	Singlet (1H)
Furanyl H_3	4.05	Singlet (1H)
Coumarin 7-OCH_3	3.96	Singlet (3H)
Furanyl 4-CH_3	1.68	Singlet (3H)

^{13}C-NMR SPECTRUM : This sample was run as a saturated solution in 0.7 ml of deuteriochloform, using deuteriochloroform as reference compound at 77.0 ppm. The spectrum was analysed in δ value (ppm) (see Figure 54 appendix).

Carbon	Chemical Shift (ppm)	Multiplicity
Coumarin C5	172.3	Singlet
Coumarin C2	160.4	Singlet
Coumarin C7	159.9	Singlet
Coumarin C8a	156.6	singlet
Coumarin C4	142.9	Doublet
Coumarin C5	127.6	Doublet
Coumarin C4a	120.2	Singlet
Coumarin C3	114.2	Doublet
Coumarin C6	112.4	Singlet
Coumarin C8	99.9	Doublet
Furanyl C2	77.4	Doublet
Furanyl C3	63.5	Doublet
Furanyl C4	57.3	Singlet
Coumarin 7-OCH_3	56.5	Quartet
Furanyl 4-CH_3	11.3	Quartet

DEPT-90 SPECTRUM : (CH-only) same as ^{13}C-NMR except there is a ^{13}C 90^{o} pulse and a ^{1}H 90^{o} pulse. (see Figure 54 appendix)

Carbon	Position
Coumarin C4	Negative (δ 142.9)
Coumarin C5	Negative (δ 127.6)
Coumarin C3	Negative (δ 114.2)
Coumarin C8	Negative (δ 99.9)
Furanyl C2	Negative (δ 77.4)
Furanyl C3	Negative (δ 63.5)

DEPT-135 SPECTRUM : Same as Dept-90 except there is a ^{1}H 135° pulse. (see Figure 54 appendix)

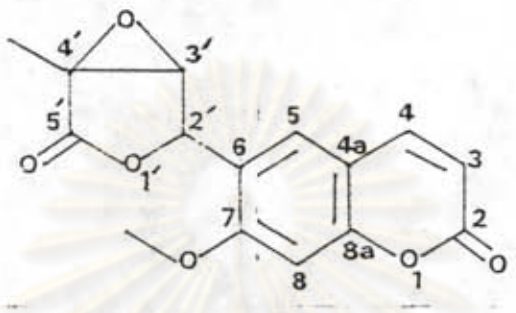
Carbon	Position
Coumarin C4	Positive (δ 142.9)
Coumarin C5	Positive (δ 127.6)
Coumarin C3	Positive (δ 114.2)
Coumarin C8	Positive (δ 99.9)
Furanyl C2	Positive (δ 77.4)
Furanyl C3	Positive (δ 63.5)
Coumarin 7-OCH_3	Positive (δ 56.5)
Furanyl 4-CH_3	Positive (δ 11.3)

H-H COSY SPECTRUM : This sample was run as a saturated solution in 0.7 ml of deuteriochloroform. The spectrum was obtained from H-H cosy pulse sequence and analysed in row and column projections plotted. (see Figure 55 appendix)

NOESY SPECTRUM : Same as H-H COSY the spectrum was obtained from NOESY pulse sequence. (see Figure 56, appendix)

C-H COSY SPECTRUM : The spectrum was obtained from C-H COSY pulse sequence and analysed in term of the correlation between carbon and proton. (see Figure 57 appendix)

All these information, suggest that compound II is micromelin [7-methoxy-6-(3,4-epoxy-4-methyl-5-oxotetrahydrofuran-2-yl) coumarin]



micromelin

From IR spectrum of micromelin, a band at 173.5 cm^{-1} was showed the typical of coumarin lactone and a second strong carbonyl band at 1770 cm^{-1} was attributed to the γ -lactone system (44).

From ^{1}H-NMR, the aromatic ^{1}H-NMR resonance patterns (7.38, ppm, s, 1H; 6.88 ppm, s, 1H; 7.67 ppm, d, 9.4 Hz; and 6.32 ppm, d, 9.4 Hz) (4) indicated that the coumarin was disubstituted. This was confirmed by H-H COSY, NOESY and C-H one bond COSY spectrum. The methoxy group (3.96 ppm) correlated to H-8 (6.88 ppm) which was displayed in H-H COSY and NOESY spectrum. It indicated that the methoxy group substituted at C-7. From H-H COSY and NOESY spectrum, the furanyl H2 correlated to H5 (7.38 ppm). This indicated that there was a γ -lactone ring substituted at C-6. The chemical shift of methyl group

(1.68 ppm) was closed to the range observed (1.3-1.5 ppm) for a methyl group attached to a carbon bearing an epoxide ring (44), the additional downfield shift being consistent with the proximity of the C-methyl group to the lactone carbonyl group. This was confirmed by H-H COSY spectrum, the methyl group correlated to furanyl H-2 and furanyl H-3. It indicated that the methyl group substituted at the γ-lactone ring. The lack of coupling between two aliphatic methine proton (furanyl H-2, furanyl H-3) was consistent with a trans relationship between these protons which resulted in an approximate 90^{o} dihedral angle (4).

From ^{13}C-NMR spectrum, the carbonyl carbon could be identified by its chemical shift characteristic. The γ-lactone carbonyl at furanyl C-5 downfield to 172.3 ppm. while the coumarin lactone carbonyl carbon appeared at 160.4 ppm.

The quarternary carbons in the coumarin nucleus could be separated into two groups. The quarternary carbon that attached to the oxygen atoms (C-7, C-8a) were downfield more than the other group which had no oxygen (C-4a, C-6). The last quarternary carbon signal which appeared at 57.3 ppm. was the C-4 furanyl.

The assignment of the methine carbons in coumarin nucleus which was identified by Dept-90, DEPT-135 could be discriminated by observing the environment of C-3

and C-8 which had the ortho effect of the oxygen neighbours. C-3 and C-8 resonated high field at 114.2 ppm and 99.9 ppm respectively. These were confirmed by the one bond C-H COSY spectrum. C-4 (142.9 ppm) and C-5 (127.6 ppm) were identified by the one bond C-H COSY spectrum.

The assignment of the methine carbons in γ-lactone side chain were identified by the one bond C-H cosy spectrum. The C-2 furanyl peak which was overlapped in chloroform peak appeared at 77.4 ppm. The other methine (C-3 furanyl) appeared highfield at 63.5 ppm.

The signal at 56.5 and 11.3 were assigned as methoxy carbon at C-7 and as methyl carbon at C-4 furanyl respectively by the interpretation from the one bond C-H COSY.

For the stereochemistry of the epoxide group, the methyl group and the neighbouring proton in both isomers were more or less in the same place with respect to the rest of the molecule. So the reliable evidence in any of the spectra to show the stereochemistry of the epoxide group was slightly clear.

The data obtained indicated that this compound was micromelin and were agree with the data from literature (4,44,45).

3. Compound III : 12.1 mg (0.02% yield) as a gum was obtained from methanol fraction which were chromatographed by column chromatography eluting with chloroform : methanol (98 : 1). This compound was identified with the following data.

Rf value :

0.31 on TLC solvent system 3

0.21 on TLC solvent system 4

0.21 on TLC solvent system 5

0.45 on TLC solvent system 7

0.64 on TLC solvent system 8

Molecular weight : 276 (EIMs)

TLC TECHNIQUE : The compound was spotted on silica gel GF 254 plate and developed in solvent system 3, 4, 5, 7, 8. After drying the plate, the detection was performed under UV, I_2 vapor and sprayed with benzidine and ferric chloride reagent.

UV DETECTION : This compound gave only one spot on TLC in five solvent systems and fluoresced under 254 nm (short wavelength) and 365 nm (long wavelength) and gave a brown spot in I_2 vapor.

Benzidine reagent : This compound gave negative color with benzidine reagent. It is indicated that there is no phenolic group in the structure.

Ferric chloride reagent : This compound gave red color. It is indicated that there was hydroxamic acid in the structure.

SPECTRAL DATA

INFRARED SPECTRUM (potassium bromide demountable cell) (see Figure 58 appendix)

ν_{max}^{KBr} = 3450 cm^{-1} (OH streching)

2800-3010 cm^{-1} (CH streching)

1720 cm^{-1} (C=O strching)

1610 cm^{-1} (conjugated double bond at C-3, C-4)

1450-1600 cm^{-1} (aromatic)

1115 cm^{-1} (C-O streching)

840 cm^{-1} ($=CH_2$ wag of methylene)

Mass spectrum : (see Figure 59 appendix)

m/e (%) = 258(0.68), 205(100.0), 206(24.8)
175(15.8), 71(1.1)

NUCLEAR MAGNETIC RESONANCE SPECTRUM

^{1}H-NMR SPECTRUM : 10 mg of sample was dissolved in deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz and analysed in δ value (ppm). (see Figure 60 appendix)

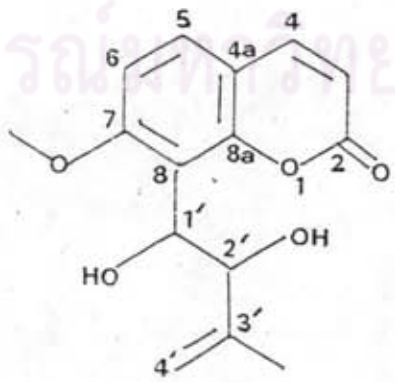
Proton	Chemical Shift (ppm)	Multiplicity
Coumarin H_4	7.65	Doublet (1H) J = 10.6 Hz
Coumarin H_5	7.40	Doublet (1H) J = 9.5 Hz
Coumarin H_6	6.87	Doublet (1H) J = 9.5 Hz
Coumarin H_3	6.20	Doublet (1H) J = 10.6 Hz
CH at C-1′	5.33	Broad doublet (1H)
CH at C-2′	4.64	Broad doublet (1H)
CH_2 at C-4′	4.60	Broad (2H)
Coumarin 7-OCH_3	3.94	Singlet (5H)
CH_3 at C-3′	1.73	Singlet (3H)

^{1}H-NMR DEUTERATED SPECTRUM : same as ^{1}H-spectrum. The spectrum was obtained deuterium oxide as solvent. (see Figure 61 appendix)

^{13}C NMR SPECTRUM : The sample was dissolved in deuteriochloroform using TMS as reference compond. The spectrum was obtained from 22.5 MHz and analysed in value (ppm).(see Figure 62 appendix)

Carbon	Chemical Shift (ppm)	Multiplicity
Coumarin C2	160.5	Singlet
Coumarin C7	159.9	Singlet
Coumarin C8a	152.4	Singlet
Coumarin C4	143.9	Doublet
C3′	143.7	Singlet
Coumarin C5	128.4	Doublet
Coumarin C8	115.8	Singlet
Coumarin C3	113.1	Doublet
Coumarin C4a	112.5	Singlet
Coumarin C6	112.3	Doublet
C4′	107.7	Singlet
C1′	75.6	Doublet
C2′	68.9	Doublet
Coumarin 7-OCH_3	55.9	Quartet
3′-CH_3	17.0	Quartet

All these information, suggest that compound III may be murrangatin.



murrangatin

From IR spectrum of compound 3, a band at 1720 cm^{-1} was showed the typical of coumarin lactone, a broad band at 3450 cm^{-1} was displayed two hydroxy groups and 1610 cm^{-1} was attributed to conjugated double bond at C-3, C-4.

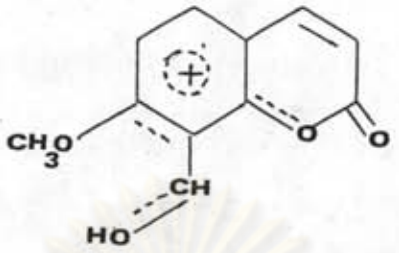
From ^{1}H-NMR, the aromatic ^{1}H-NMR resonance patterns [6.20 ppm (d, J = 10.6 Hz, H-3), 7.65 ppm (d, J = 10.6 Hz, H-4), 7.40 ppm (d, J = 9.5 Hz, H-5), 6.87 ppm (d, J = 9.5 Hz, H-6)] indicated that the coumarin was disubstituted at C-7, C-8 (46). This was comfirmed by ^{13}C-NMR. Two substituted groups, the one was methoxy group (a sharp 3H singlet at 3.94 ppm) which could be subsituted at C-7. The other group that could be substituted at C-8 may be butene side chain which had one methyl group and two hydroxyl groups substituted. Because there were a methyl signal at 1.76 ppm, a broad 1H signal (5.32 ppm) of H-1′ which was sharpened in D_2O to a clear doublet and a doublet signal (4.64 ppm) of H-2′ which partially hidden under a broad 2H singlet at 4.60 ppm (CH_2-4′). The signal of hydroxylic proton could be hidden under a singlet of OCH_3 at 3.94. That was approved by obtaining a spectrum in deuterium oxide (Figure 61). The integrated area of signal at 3.94 decreased from 5H to 3H. It indicated that the hydroxyl peak disappeared on deuteration (46).

From ^{13}C-spectrum, the multiplicity of signal could be identified by the off resonance experiment.

The lactone carbonyl carbon appeared at 160.5 ppm. The assignment of quarternary carbons in the bezene ring was same as in the assignment of osthol (C-7, 159.9; C-8a, 152.4; C-8, 115.8; C-4a, 112.5). The last quarternary carbon signal which appeared at 143.7 was the C-3′ butene.

The assignment of the methine carbons in coumarin nucleus was same as in the assignment of osthol (C-4, 143.9; C-5, 128.4; C-3, 113.1; C-6; 112.3). The methine carbons at the side chain could be separated by observing the environment. C-1′ resonated down field at 75.6 and C-2′ resonated high field at 68.9. There was a singlet signal at 107.7 which could be methylene carbon of the side chain. The signal at 55.9 and 17.0 were assigned as methoxy carbon and methyl carbon of the side chain.

From EI mass spectrum, fragmentation pattern showed the peak at m/e 258 which could be characterized by the loss of water. The distinct M-18 peak which is given by loss of water has noticed in spectra of alcohols (47). So the expected molecular ion peak could be showed at m/e 276. The fission of the 1′-2′ bond has given rise to a stable ion(a) which appeared as the base peak at m/e 205 (46).



stable ion(a)

From IR, ^{1}-NMR and mass spectra were obtained corresponded to the data in the literature (46, 48). This was confirmed by ^{13}C-NMR and the deuteration of ^{1}-H-NMR. Therefore the compound III may be identified to be murrangatin.