CHAPTER IV

RESULTS AND DISCUSSION

The 2.2 kg of dried , ground roots bark of Clausena cambodiana Guill. were refluxed with hexane to remove fat from the material and follow by refluxed with 5 liters of chloroform three times to obtain 156 gm. brown, oily residue. 40.0 gm. of former extract were chromatographed over Silica gel G 60 by Quick column chromatography technique. Eluting with the series of Hexane - chloroform - methanol mixture.

Each individual fraction (F1 - F20) that was eluted from the Quick column chromatography was monitored by TLC. The appropriate fraction were combined and rechromatographed over silica by suitable solvent system. Five compounds were isolated and purified. Compound I was oxidised with m - chloroperbenzoic acid in chloroform and was separated by chromatography over silica followed by recrytallization in diethylether and ethanol to obtained Compound VI. The identification of six compound were performed by physical and chemical method as following.

1.Compound I : 2.12 gm. (5.3 % yeild) of crystal was obtained from F3-F6 . This compound was identified with the following data.

Color and form of crystal : This compound was crystallized by ethanol as a pale yellow elongated prism.

Rf value :

0.32 on TLC solvent system 1

0.42 on TLC solvent system 2

0.39 on TLC solvent system 4

0.43 on TLC solvent system 6

0.65 on TLC solvent system 7

Molecular weight : 328 (EIMS)

Melting point : 132°-134° C (uncorrected)

TLC : The compound was spotted on silica gel GF 254 plate and developed in solvent system 1,2,4,6,7 . After the plate was dried in open air the detection was performed under UV and was sprayed with benzidine reagent.

UV : This compound gave only one spot on TLC in five solvent system and fluoresced either 254 nm (short wavelength) or 365 nm (long wavelength).

Benzidine reagent : This compound gave positive color with benzidine reagent. It is indicated that there has a phenolic group in the structure.

Spectral data

Infrared spectrum (potassium bromide disc) (see Figure 20 appendix)

ν_{max}^{KBr} = 3500 cm^{-1} (OH streching)
1720 cm^{-1} (C=O streching)
1650 cm^{-1} (C=O streching)
1600 cm^{-1} (conjugated double bond at C-3, C-4)
1450 cm^{-1} (aromatic)

Mass spectrum (see Figure 21 appendix)

m/e (%) = 328 (59.8),313 (65.3),285 (15.2)
257 (100.0),245 (15.2),244 (20.5)
229 (17.3)

Nuclear magnetic resonance spectrum

^{1}H-NMR spectrum : 10 mg. of sample was dissolved in dueteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz and analysed in δ value (PPM) (see Figure 22 appendix)

Proton	Chemical shift (PPM)	Multiplicity
OH	12.98	singlet
1H (CH at C-4)	8.04	doublet
1H (CH at C-3)	6.15	doublet
1H (CH at C-16)	6.24	doublet,doublet

Proton	Chemical shift (PPM)	Multiplicity
1H (CH at C-17, trans)	4.91	doublet
1H (CH at C-17, cis)	4.88	doublet
2H (CH_2 at C-11)	2.75	singlet
6H ($2CH_3$ at C-15)	1.64	singlet
6H ($2CH_3$ at C-10)	1.50	singlet

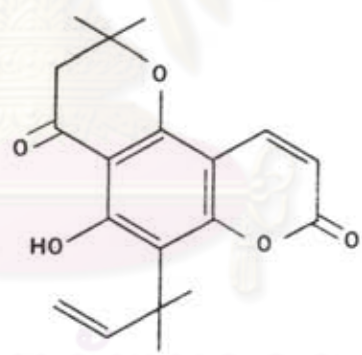
^{13}C NMR spectrum : 50 mg. of sample was dissolved in dueteriochloroform using TMS as reference compound.The spectrum was obtained from 22.5 MHz and analysed in δ value (PPM) (see Figure 23 appendix)

Carbon	Chemical shift (PPM)	Multiplicity
2	160.03	singlet
3	110.78	doublet
4	138.52	doublet
4a	104.02	singlet
5	159.97*	singlet
6	114.47	singlet
7	159.00	singlet
8	103.20	singlet
8a	159.87*	singlet
10	80.02	singlet
11	47.67	triplet

Carbon	Chemical shift (PPM)	Multiplicity
12	198.17	singlet
15	40.95	singlet
16	149.52	doublet
17	108.35	triplet
$2CH_3$ at C-10	26.49	quartet
$2CH_3$ at C-15	29.47	quartet

* The chemical shift may be reversed

All these information , suggest that compound I is Clausenidin



Clausenidin

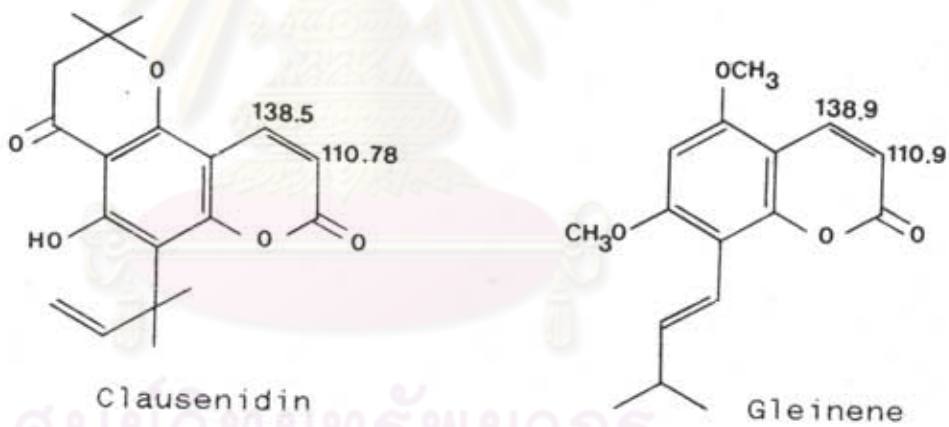
From ^{13}C NMR spectrum, signal from quarternary carbon could be identified by the proton noise decoupling experiment and by the absence of a large one bond C-H coupling when proton information is retain through Gated decoupling experiment and Off resonance experiment.

The carbonyl carbon could be identified by

its chemical shift characteristic.The ketone carbonyl carbon at C-12 downfield to 198.17 ppm. while the lactone carbonyl carbon appeared at 160.03 ppm.

There are three methines carbon in Clausenidin two of them are at C-3 and C-4 and were assinged by compare with Gleinene (42) as in Fig.24

C-3 carbon appeared at 110.78 PPM. and C-4 at 138.52 ppm. The last methine in the structure, which was clearly discriminated from the former, would be C-16 by the result of long - range coupling with proton on methyl group at C-15



Clausenidin Gleinene

Figure 24 Structure of Clausenidin and Gleinene

The remain quarternary carbons in the benzene ring could be separated into two groups. The quarternary carbons that attached to the oxygen atoms (C-8a, C-5, C-7) were downfield more than the other group which have no oxygen (C-4a, C-6, C-8)

The assignment of C-8a, C-5 and C-7 could be made by observing the enviroment of C-8a and C-5 which

were nearly the same while C-7 was different . So, the chemical shift at 159.97 ppm. and 159.87 ppm. belong to C-8a and C-5 respectively (the chemical shift may be reverse). The more intent signal at 159.00 ppm. was C-7. Because C-7 bearing OH functional group that could formed a strong intramolecular interaction with carbonyl group which limited the mobility. So, T_1 were decreased and the peak intensity increased.

The second group (C-4a, C-6, C-8) which had no oxygen attached. C-6 (114.47 ppm.) was easily distinguished from other due to it attachment to the carbonyl group. This functional group caused the C-6 more downfield than other because of the inductive effect .

In Off - resonance spectrum , C-8 could be separated from C-4a by observing the long range coupling. On this basis, the signal at 103.20 ppm. were multiplet because C-8 couples with proton of dimethyl group at C-15

The remaining assignment of the quarternary carbon were C-10 and C-15 . The upfield signal was assigned to be C-15 (40.95 ppm.) and the downfield signal to be C-10 because of the inductive effect from attachment to the oxygen atom.

The methylene carbon of the olefin part (C-17) appeared at 108.35 ppm. while the methylene

carbon of the aliphatic part (C-11) showed at 47.67 ppm. Both signal were triplet in Off - resonance spectrum.

The methyl groups that substituted on C-10 and C-15 were position at 26.49 ppm. and 29.47 ppm. respectively. This assignment were assigned by relative to the signal in proton NMR spectrum of Clausenidin and Clausenidin epoxide (compound IV, Figure 43 appendix).

In proton NMR spectrum of Clausenidin , the signals at 1.64 ppm. and 1.50 ppm. may be the signal of methyl protons of C-15 or C-10 . When we oxidised Clausenidin by m - chloroperbenzoic acid to yeild Clausenidin epoxide. The proton NMR of these compound showed two small singlets at 1.67 ppm. and 1.29 ppm , each signal had the integration equaled to 3 proton while the strong singlet at 1.64 ppm that ever seen in Clausenidin was diappeared. This meaned , the down field signal at 1.64 ppm of Clausenidin must be the signal of methyl proton at C-15 because the epoxide ring made the two methyl groups at C-15 non - equivalence. From this result, the remain signal at 1.50 ppm in Clausenidin must be the signal of methyl proton at C-10. We used these information to assigned the carbon's chemical shifts of C-10 and C-15 by relative to proton NMR because allmost of proton and carbon chemical shifts were correlated.

Compound II : 120 mg. (0.3 % yeild) of crystal was obtained from F8 - F9 . This compound was identified with the following data.

Color and form of crystal : This compound was crystallized by chloroform and methanol as a colorless rod crystal.

Rf value :

0.74 on TLC solvent system 1
0.21 on TLC solvent system 2
0.82 on TLC solvent system 3
0.61 on TLC solvent system 4
0.75 on TLC solvent system 5

Molecular weigth : 380 (EIMS)

Melting point : 198 °C (uncorrected)

TLC : This sample was spotted on silica gel GF 254 plate and developed in solvent system 1,2,3,4,5 . After plate was dried in open air, the detection was performed under UV and was sprayed with benzidined reagent.

UV : This compound gave only one spot in all solvent systems and fluoresced at 254 nm (short wavelength) but did not fluoresced at 365 nm (long wavelength).

Benzidine reagent : Only one spot gaved red

color with benzidine reagent. It was indicated that there was phenolic group in the structure.

Spectral data

Infrared spectrum (potassium bromide disc) (see Figure 25 appendix)

ν_{max}^{KBr} = 3170 cm^{-1} (OH stretching)

1675 cm^{-1} (C=O stretching)

1645 cm^{-1} (conjugated double bond at C-3, C-4)

1610 cm^{-1} (CH=C- stretching)

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

Mass spectrum (see Figure 26 appendix)

m/e (%) :380 (33.44),366 (26.24),365 (100.0)
337 (7.97), 312 (2.35), 311 (3.88)
309 (5.61), 297 (8.10).

Nuclear magnetic resonance spectrum

^{1}H NMR spectrum : 10 mg. of sample was dissolved in dueteriochloroform. Using TMS as reference compound, the spectrum was obtained from 90 MHz and analysed in δ value (PPM). (see Figure 27 appendix)

Proton	Chemical shift (PPM)	Multiplicity
1H (CH at C-4)	7.89	singlet
1H (CH at C-12)	6.55	doublet
1H (CH at C-11)	5.66	doublet
2H (methine, of - vinyl group attached to quarternary carbon)	6.0 - 6.45	multiplet
4H (methylene,of - vinyl group attached to quarternary carbon)	4.7 - 5.2	multiplet
6H (CH_3, at - 2,2 - dimethylchromene ring)	1.47	singlet
6H (CH_3, at C-15)	1.63	singlet
6H (CH_3, at C-18)	1.42	singlet
OH	7.26	singlet

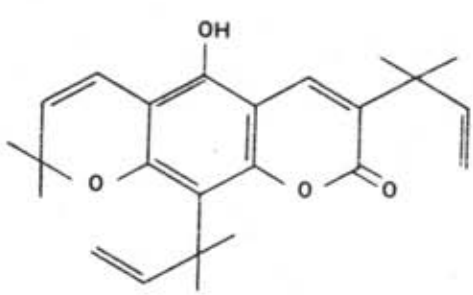
^{13}C NMR spectrum :50 mg. of sample was dissolved in deuteriochloroform. Using TMS as reference compound, the spectrum was obtained from 22.5 MHz and analysed in δ value (PPM). (see Figure 28 - 29 appendix)

Carbon	Chemical shift (PPM)	Multiplicity
2	161.3	singlet
3	128.0	doublet
4	135.0	doublet

Carbon	Chemical shift (PPM)	Multiplicity
4a	106.72	singlet
5	147.5	singlet
6	104.72	singlet
7	155.32*	singlet
8	114.9	singlet
8a	153.04*	singlet
10	76.9	singlet
11	128.94	doublet
12	116.25	doublet
15	40.8	singlet
16	150.12	doublet
17	111.87	triplet
18	40.0	singlet
19	145.0	doublet
20	107.9	triplet
$2CH_3$ at C-10	27.30	quartet
$2CH_3$ at C-15	29.53*	quartet
$2CH_3$ at C-18	26.22*	quartet

* : The chemical shift may be reversed

All these information suggested that compound II is Clausarin



Clausarin

From ^{1}H NMR spectrum of Clausarin, the two sets of overlapping signals (at 4.7 - 5.2 ppm and 6.0 - 6.45 ppm.) were the ABX - type arising from vinyl groups attached to quaternary carbons as show in Figure.30. Two sharp singlets at 1.42 ppm. and 1.63 ppm. (6H each) due to two pairs of dimethyl groups permitted the assignment of $2C_5H_9$ fragment as 1,1 - dimethylallyl group in Clausarin. The signal at 1.42 ppm was assigned as methyl protons at C-18 and signal at 1.63 was assigned as methyl at C-15 by the result of assignment in Clausenidin and Clausenidin epoxide. A singlet at 1.47 ppm.(for 2 equivalent methyl group) together with two olefinic protons forming an AB doublet - doublet at 5.66 ppm. and 6.55 ppm. (J = 9.9 Hz) indicated the presence of a dimethylchromene nucleus. One proton appeared at 7.89 ppm. was identified as C4-H. The last one, the hydroxy peak may be overlap in chloroform peak.

From EI mass spectral fragmentation pattern which showed the expected molecular ion peak at m/e 380 . The cracking pattern was characterized by the loss of a methyl radical (m/e 365) and the subsequent loss of other functional groups that showing fragment ions at m/e 337 [M+ - (Me + CO)], 312 [M+ - C_5H_8], 311 [M+ - C_5H_9], 309 [M+ - (Me + 2CO)], 297 [M+ - (Me+C_5H_8)], 283 [M+ - (C_5H_9 + CO)]

In the ^{13}C NMR spectrum , the signals at

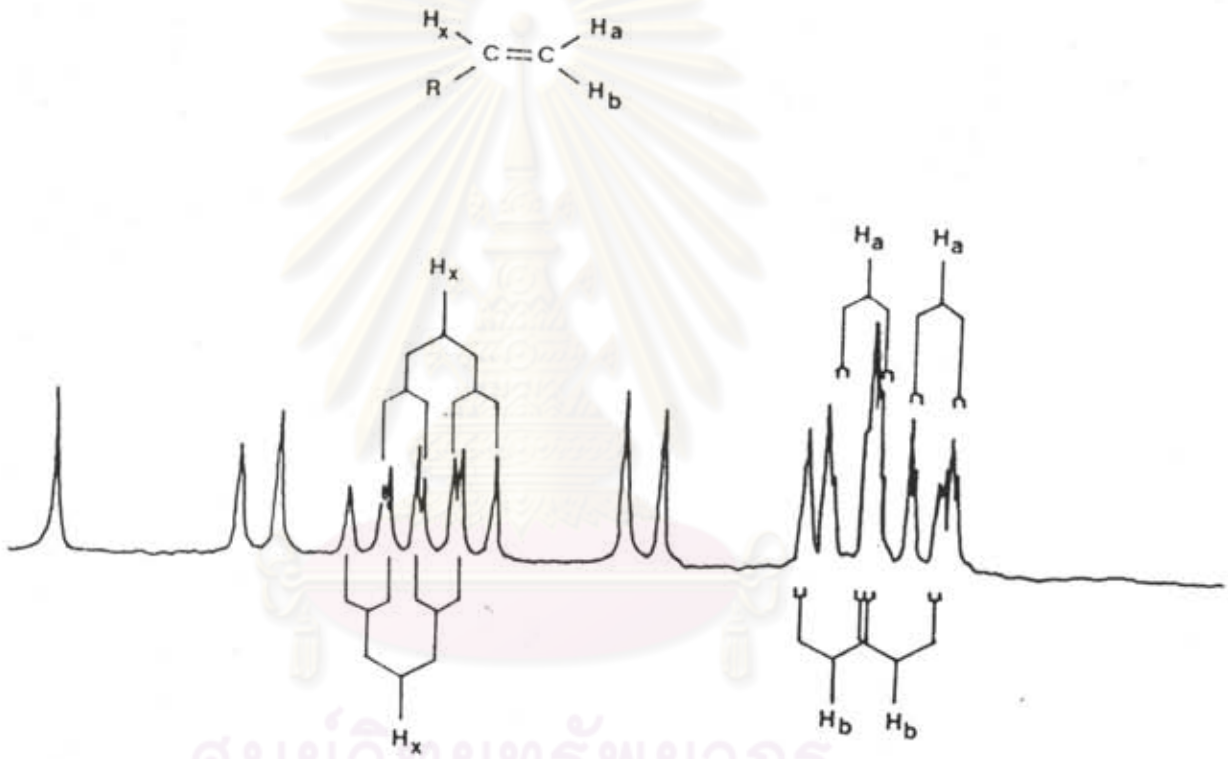


Figure 30. ^{1}H NMR spectrum of Clausarin, showed two set of overlapping signal (at 4.7 - 5.2 ppm. and 6.0 - 6.45 ppm.) which were ABX type.

155.32 ppm., 153.0 ppm. and 147.52 ppm. due to quarternary carbon that attached to oxygen, but the intensity of signal at 147.52 ppm. stronger than other signals . So this signal was assigned to be C-5 because the hydroxy group which attached on C-5 could form intermolecular hydrogen bonding . Resulting in shorten relaxation time.

The remain quarternary benzenoid carbons were C-4a, C-6 and C-8. C-8 could be separated from the other in the group and is assigned at 114.90 ppm. because of the different in environment when compared with C-4a and C-6.

C-6 was assigned at 104.72 ppm. by the multiplet of three bonds and two bonds long - range coupling in Gated decoupling spectra while C-4a showed a doublet because it had only two bonds long range coupling interaction.

The methylene carbon at 1,1 - dimethylallyl at C-3 and C-8 appeared as doublet of doublet instead of triplet in Gated decoupling spectra. Since the methylene proton were non - equivalent and base on the ABX system , so the one bond coupling constant were unequal as showed in Figure 31. The signal of each carbon on 1,1 - dimethylallyl group which substituded at C-3 would appeared upfield than the other one at C-8 because an anisotropic effect from carbonyl functional group of coumarin nucleus.

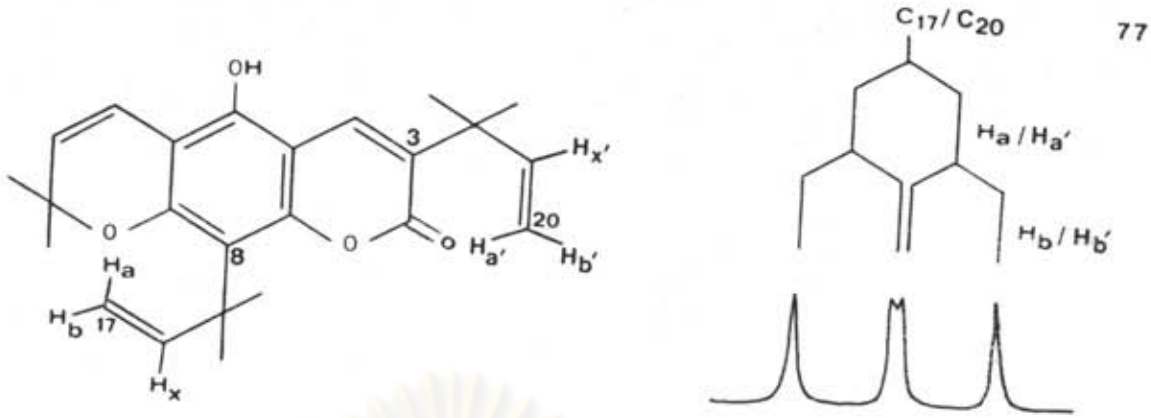


Figure 31. Coupling pattern between carbon and protons of 1,1 - dimethylallyl side chain of Clausarin.

Compound III : 1.2 gm. (3 % yeild) of crystal was obtained from F3 - F6. This compound was identified with the following data.

Color and form of crystal : This compound was crystallized by ethanol as a colorless elongated prism.

Rf value :

0.65 on TLC solvent system 1
0.88 on TLC solvent system 3
0.85 on TLC solvent system 5
0.33 on TLC solvent system 6
0.67 on TLC solvent system 8

Molecular weight : 326 (EIMS)

Melting point : 96° C (uncorrected)

TLC : The compound was spotted on silica gel GF 254 plate and developed in solvent system 1,3,5,6,8 . After the plate was dried in open air ,the detection was performed under UV and was

spray with benzidine.

UV : This compound gave only one spot on TLC in five solvent system and fluoresced either 254 nm (short wavelength) or 365 nm (long wavelength)

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

Spectral data

Infrared spectrum (potassium bromide disc) (see Figure 32 appendix)

ν_{max}^{KBr} = 1725 cm^{-1} (C=O stretching)
1630 cm^{-1} (conjugate double bond at C-3 , C-4)
1610 cm^{-1} (CH=C- stretching)
1450 - 1600 cm^{-1} (aromatic)

Mass spectrum (see Figure 33 appendix)

m/e (%) = 326 (22.1), 312 (21.5), 311 (100.0)
281 (14.0)

Nuclear magnetic resonance spectrum

^{1}H NMR spectrum : 10 mg. of sample was dissolved in dueteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz and analysed in δ value (PPM). (see Figure 34 appendix)

Proton	Chemical shift (PPM)	Multiplicity
1H (CH at C-14)	7.87	doublet
1H (CH at C-12)	6.57	doublet
1H (CH at C-3)	6.19	doublet
1H (CH at C-11)	5.68	doublet
1H (CH at C-16)	6.31	doublet , doublet
1H (CH at C-17 cis)	4.88	doublet
1H (CH at C-17 trans)	5.00	doublet
OCH_3	3.83	singlet
$2CH_3$ (6H at C-10)	1.45	singlet
$2CH_3$ (6H at C-15)	1.66	singlet

^{13}C NMR spectrum :50 mg. of sample was dissolved in dueteriochloroform. Using TMS as reference compound. The spectrum was obtained from 22.5 MHz and analsed in δ value (PPM). (see Figure 35 - 36 appendix)

Carbon	Chemical shift (PPM)	Multiplicity
2	160.68	singlet
3	111.65	doublet
4	138.90	doublet
4a	107.54*	singlet
5	154.31	singlet
6	110.00*	singlet
7	156.00	singlet
8	111.65 @	singlet

Carbon	Chemical shift (PPM)	Multiplicity
8a	151.31	singlet
10	77.42	singlet
11	130.34	doublet
12	116.37	doublet
15	41.23	singlet
16	149.87	doublet
17	108.19	triplet
OCH_3	63.38	quartet
$2CH_3$ (6H at C-10)	26.49	quartet
$2CH_3$ (6H at C-15)	27.57	quartet

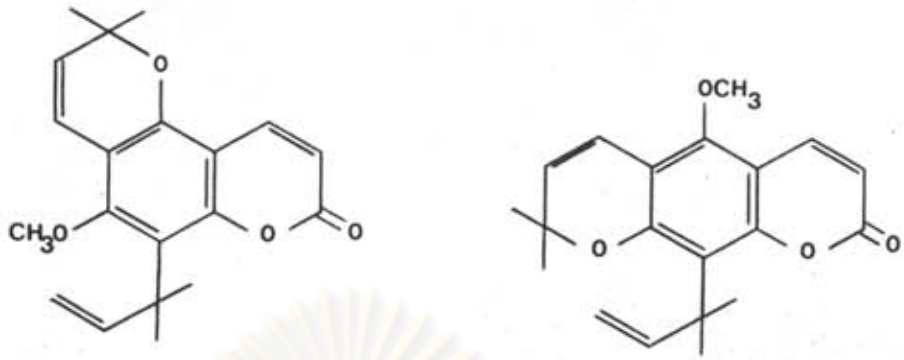
* The chemical shift may be reverse

@ This signal hidden in the C-3 signal

All these information suggested that compound III is Dentatin. Which its structure have been reported by ^1H NMR as angular pyranocoumarin (36) that show in Figure.37 a .But the other structure which could matched to the same spectrum has linear angular pyranocoumarin nucleus as show in Figure 37 b.

The correct structure for this compound was established by use of NOE experiment.

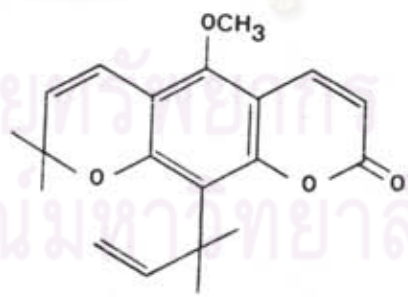
From the experiment, the signals of the OMe in the proton NMR was successively saturated by double irradiation and changes in the integrated intensities of the other proton signals were observed. On saturation of the OMe signal , the



a b

Figure 37 (a) Angular pyranocoumarin nucleus
(b) Linear pyranocoumarin nucleus

intensities of both olefinic 4-H and 12-H in the spectrum of Dentatin were increased about 14 % (see Figure 38 b.). From these result, it was concluded that the OMe group in Dentatin must be proximate to both 4-H and 12-H . So the correct structure is structure b which is linear pyranocoumarin.



Dentatin

For ^{13}C NMR experiment , selective decoupling techinque was used to confirm the chemical shift of C-3 , C-4 , C-11 and C-12 . By irradiated proton H-3 , H-4 , H-11 and H-12 at the center of the

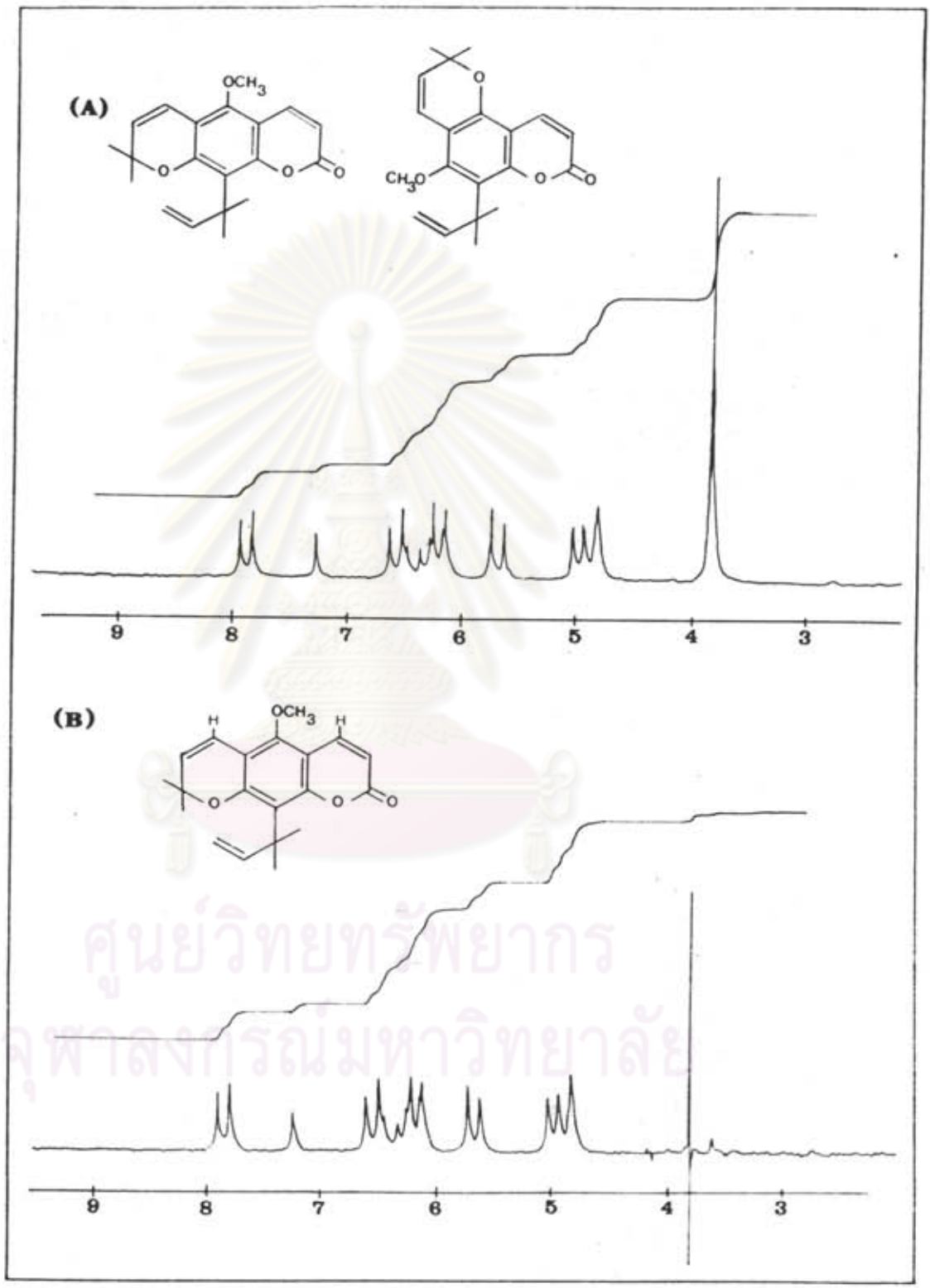


Figure.38 ^{1}H NMR spectrum of dentatin with NOE experiment
(a) Normal spectra (b) decoupling at OCH_3

signals that previously assigned in proton spectrum (see Figure 39) and collected the carbon spectrum as showed in Figure 40 . From this experiment , when irradiate proton at H-4 , the carbon signal at 138.90 ppm. was collaped to singlet and had a highest intensity. So C-4 was assigned at 138.90 ppm. For C-3 , C-11 and C-12 were assigned at 111.65 ppm.,130.34 ppm. and 116.37 ppm. respectively by the same procedure. The chemical shift of carbon which were assigned by selective decoupling technique were correlated to the previously assignment.

Compound IV : 100 mg. (35.7 % yeild) of crystal was obtained from epoxidation of Clausenidin. This compound was identified with the following data.

Color and form of crystal : This compound was crystallized by diethyl ether and ethanol as a yellowish elongated prism.

Rf value :

0.18 on TLC solvent system 1

0.31 on TLC solvent system 2

0.21 on TLC solvent system 4

0.34 on TLC solvent system 6

0.49 on TLC solvent system 7

Molecular weight : 344 (EIMS)

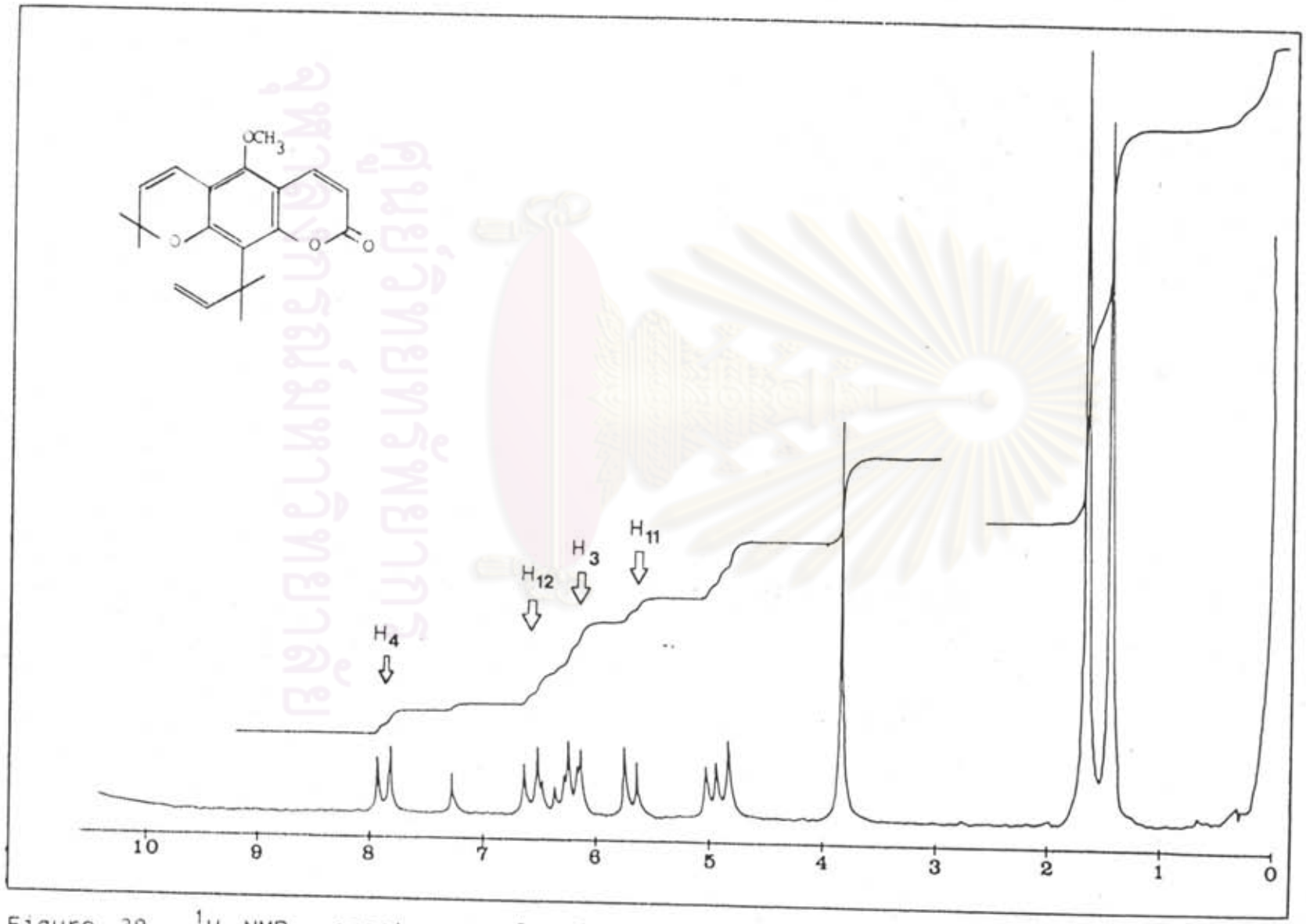


Figure 39 ^{1}H NMR spectrum of dentatin which show the position of irradiation

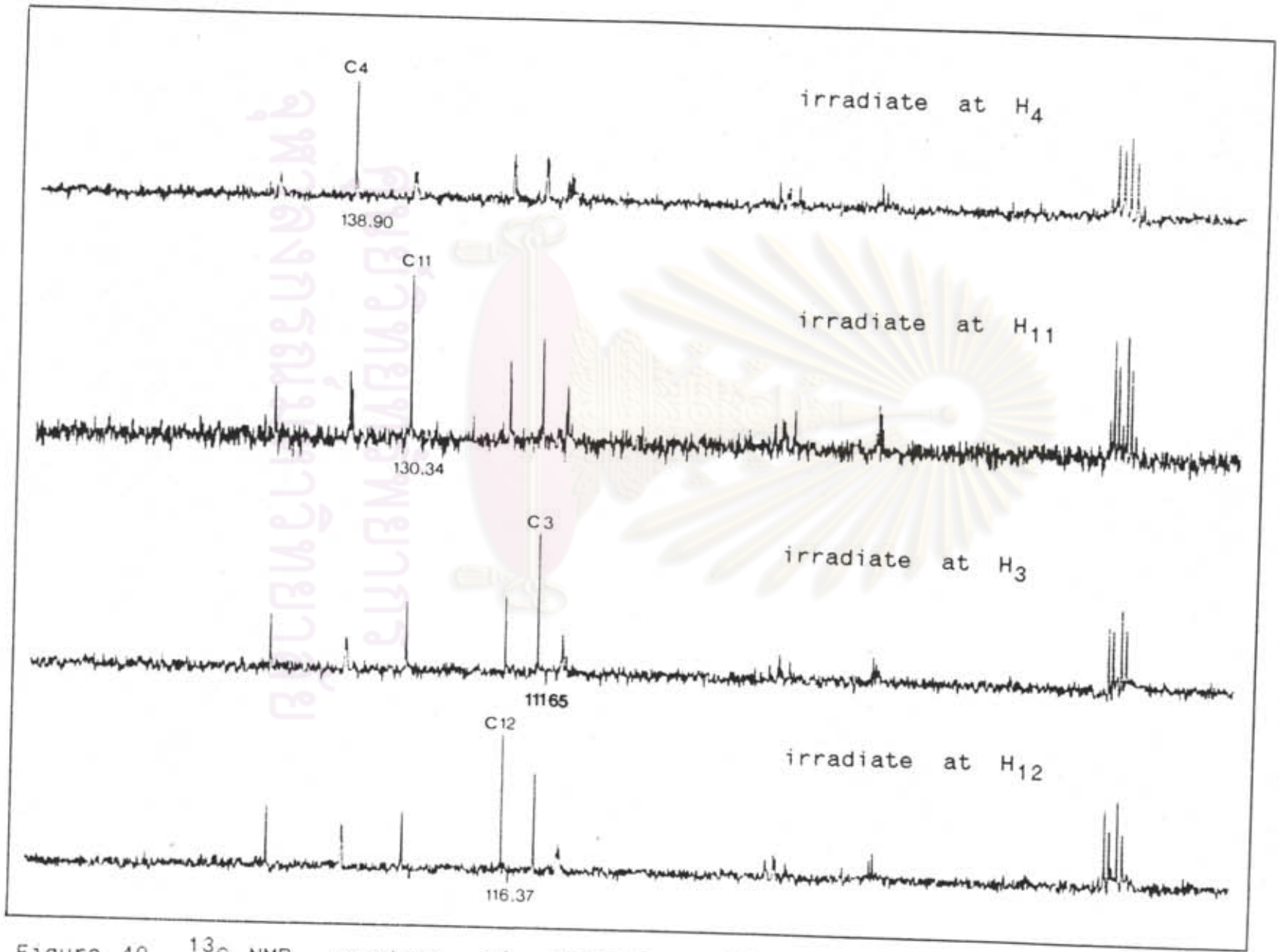


Figure 40 ^{13}C NMR spectrum of dentatin with selective decoupling experiment

Melting point : 146 ° C

TLC : The compound was spotted on silica gel G 254 plate and developed in solvent system 1,2,4,6,7 . After the plate was dried in open air , the detection was performed under UV and sprayed with benzidine reagent.

UV : This compound gave only one spot on TLC in five solvent system and fluoresed either 254 nm (short wavelength) or 365 nm (long wavelength)

Benzidine reagent : This compound gave positive color with benzidine reagent. It is indicated that there has phenolic group in the structure.

Spectral data

Infrared spectrum (Potassium bromide disc) (see Figure 41 appendix)

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

1740 cm^{-1} (C=O strectching)

1640 cm^{-1} (C=O strectching)

1600 cm^{-1} (conjugated double bond at C-3 , C-4)

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

Mass spectrum (see Figure 42 appendix)

m/e (%) = 344 (53.68), 329 (16.22), 314 (18.44)

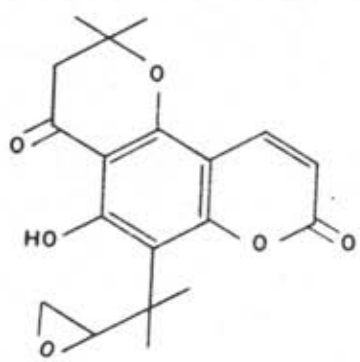
301 (95.89), 245 (100.0)

Nuclear magnetic resonance spectrum

^{1}H NMR spectrum : 10 mg. of sample was dissolved in dueteriochloroform, using TMS as reference compound . The spectrum was obtained from 90 MHz and analysed in δ value (PPM). (see Figure 43 appendix)

Proton	Chemical shift (PPM)	Multiplicity
OH	13.06	singlet
1H (CH at C-4)	8.05	doublet
1H (CH at C-3)	6.18	doublet
1H (CH at C-16)	3.37	triplet
2H (CH_2 at C-17)	2.97	doublet
2H (CH_2 at C-11)	2.79	singlet
3H (CH_3 at C-15)	1.67	singlet
6H ($2CH_3$ at C-10)	1.53	singlet
3H (CH_3 at C-15)	1.29	singlet

All these information , suggested that compound V is Clausenidin epoxide.



Clausenidin epoxide

This reaction selective for the olefinic functional group, that will prove the olefin in the structure of Clausenidin and the result from epoxide formatiom can seperate the methyl groups in the structure of Clausenidin and Clausarin.

From ^{1}H NMR , signal of olefin at 6.24 ppm., 4.91 ppm. and 4.88 ppm. were disappeared when compared with spectrum of Clausenidin. The new upfeild signals in the spectrum would due to methine proton (3.37 ppm.) and methylene protons (2.97 ppm.) and from mass spectrum information showed that m/e increased 16 unit. This meaned , there was oxygen atom added to the structure of Clausenidin

The m - chloroperbenzoic acid is an excellent reagent for epoxidation of olefinic double bonds. It is more stable than other reagent and have been used at an elevated temperature. Reaction is believed to take placed by electrophilic attack of peroxy - acid on the double bond , as illustrated in Figure.44

According to this mechanism, the rate of epoxidation is increased by electron - withdrawing groups in the peroxy acid or electron - donating substituted on the double bond. So , the terminal mono - olefins react only slowly with most peroxy acid but the rate of reaction increases with the degree of alkyl substitution. The α,β - unsaturated acid

Figure.44 Mechanism of epoxidation of m - chloroperbenzoic acid.

or ester react more slowly because conjugation of the olefin with other unsaturated group reduced the rate of epoxidation.

Compound V : 40 mg. (0.1 % yeild) of crystal was obtained from F_{15} - F_{17}. This compound was identified with the following data

Color and form of crystal : This compound was crystallized by methanol as a yellow prism.

Rf value :

0.22 on TLC solvent system 1
0.05 on TLC solvent system 3
0.20 on TLC solvent system 4
0.87 on TLC solvent system 5
0.43 on TLC solvent system 7

Molecular weigth : 312 (EIMS)

Melting point : 183° - 186° C

TLC : The sample was spotted on silica

gel GF 254 plate and developed in solvent system 1 , 2 , 4, 6, 7 . After the plate was dried in open air , the detection was performed under UV and was sprayed with benzidine reagent.

UV : This compound gave only one spot on TLC plate in all solvent system and Fluoresced at 254 nm (short wavelength) but it did not fluoreced at 365 nm (long wavelength)

Benzidine reagent : The spot gave red color with benzidine reagent. It is indicated that there is phenolic group in the structure.

Spectral data

Infrared spectrum (Potussium bromide disc) (see Figure.45 appendix)

ν_{max}^{KBr} = 3240 cm^{-1} (OH strectching)
1680 cm^{-1} (C=O strectching)
1640 cm^{-1} (conjugated double bond at C-3 , C-4)
1600 cm^{-1} (C=CH- strectching)
1450 - 1600 cm^{-1} (aromatic)

Mass spectrum (see Figure.46 appendix)

m/e (%) : 312 (27.28), 298 (20.2)
297 (100.0), 269 (14.0)
241 (17.53)

Nuclear magnetic resonance spectrum

^{1}H NMR spectrum : 10 mg of sample was dissolved in dueteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz NMR and analysed in δ value (PPM).(see Figure 47 appendix)

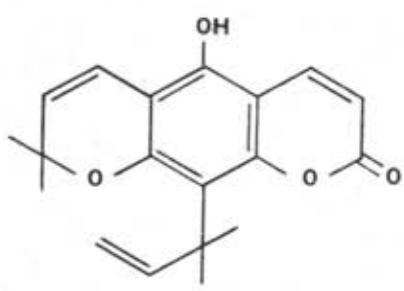
Proton	Chemical shift (PPM)	Multiplicity
OH	5.55	singlet
1H (CH at C-4)	8.02	doublet
1H (CH at C-3)	6.13	doublet
1H (CH at C-12)	6.53	doublet
1H (CH at C-11)	5.67	doublet
1H (CH at C-16)	6.19	doublet - doublet
1H (CH at C-17 trans)	4.90	doublet
1H (CH at C-17 cis)	4.85	doublet
6H (CH_3 at 1,1 - dimethy allyl)	1.64	singlet
6H (CH_3 at 2,2 - dimethychromene ring)	1.44	singlet

^{13}C NMR spectrum : 40 mg of sample was dissolved in dueteriochloroform, using TMS as reference standard. The spectrum was obtained from 22.5 MHz and analysed in δ value (PPM).(see Figure 48 - 49 appendix)

Carbon	Chemical shift (PPM)	Multiplicity
2	162.69	singlet
3	109.26	doublet
4	140.85	doublet
4a	107.43*	singlet
5	148.49	singlet
6	104.94*	singlet
7	156.62 $	singlet
8	115.50	singlet
8a	154.26 $	singlet
10	77.30	singlet
11	128.94	doublet
12	116.58	doublet
15	41.17	singlet
16	150.49	doublet
17	108.02	triplet
$2CH_3$ (at C-10)	27.52	quartet
$2CH_3$ (at C-15)	29.74	quartet

$,* Chemical shift may be reversed.

All these information suggested that coumpound V is Nordentatin



Nordentatin

Compound VI : 60 mg (0.15 % yeild) of crystal was obtained from F_8 - F_9 . This compound was identified with the following data.

Color and form of crystal : This compound was crystallized by ethanol as colorless prism.

Rf value :

0.54 on TLC solvent system 1

0.10 on TLC solvent system 3

0.76 on TLC solvent system 4

0.21 on TLC solvent system 6

0.62 on TLC solvent system 8

Molecular weigth : 258 (EIMS)

Melting point : 132° - 133° C

TLC : The sample was spot on silica gel GF 254 plate and developed in solvent system 1, 3, 4, 6, and 8 After the plate was dried in open air , the detection was performed under UV and was sprayed with benzidine reagent.

UV : This compound gave only one spot on TLC plate in all solvent system and fluoresced either 254 nm (short wavelength) or 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.

Infrared spectrum (Potussium bromide disc) (see Figure.50 appendix)

ν_{max}^{KBr} = 1752 cm^{-1} (C=O stretching)

1635 cm^{-1} (conjugated double bond at C-3 , C-4)

1610 cm^{-1} (CH=C stretching)

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

Mass spectrum (see Figure.51 appendix)

m/e (%) :258 (21.1), 243 (100.0), 227 (30.5) 200 (19.4)

Nuclear magnetic resonance spectrum

^{1}H NMR spectrum : 10 mg. of sample was dissolved in dueteriochloroform , using TMS as reference compound. The spectrum was obtained from 90 MHz and analysed in δ value (PPM). (see Figure.52 appendix)

Proton	Chemical shift (PPM)	Multiplicity
1H (CH at C-3)	6.21	doublet
1H (CH at C-4)	7.83	doublet
1H (CH at C-8)	6.56	singlet
1H (CH at C-11)	5.71	doublet
1H (CH at C-12)	6.56	doublet

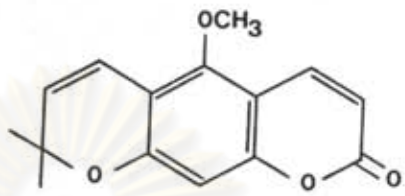
Proton	Chemical shift (PPM)	Multiplicity
OCH_3	3.86	singlet
6H (CH_3 at 2,2 - dimethylchromene ring)	1.46	singlet

^{13}C NMR spectrum : 50 mg. of sample was dissolved in dueteriochloroform , using TMS as reference compound. The spectrum was obtained from 22.5 MHz and analysed in δ value (PPM).(see Figure.53 - 54 appendix)

Carbon	Chemical shift (PPM)	Multiplicity
2	160.95	singlet
3	112.36	doublet
4	138.52	doublet
4a	107.42	singlet
5	152.92	singlet
6	109.59	singlet
7	157.64*	singlet
8	100.82	doublet
8a	155.69*	singlet
10	77.58	singlet
11	130.61	doublet
12	115.88	doublet
OCH_3	63.66	quartet
$2CH_3$ (at C-10)	28.17	quartet

* Chemical shift may be reversed

All these information suggested that compound VI is Xanthoxyletin.



Xanthoxyletin

From ^{13}C NMR spectrum , a joint utilization of coupling pattern and chemical shift criteria led to the assignment of the majority of resonance. The methine carbons C-3 and C-4 centred at 112.36 ppm and 138.52 ppm reflected the splitting due to $^{1}J_{CH}$ but $^{2}J_{(C-C-H)}$ splitting dose not appearantly seem to be present at measurable magnitude.

The another methine that resonated high field at 100.82 ppm due to ortho effect of the oxygen neighbours exhibit no further splitting in gated decoupling , which fitted only C-8 because lack of vicinal proton.

The multiplet that centred at 130.61 ppm reflected the splitting due to $^{3}J_{CH}$ long range coupling with two methyl groups at C-10 . So this carbon must be C-11. The signal at 115.88 ppm was C-12 because it showed small splitting due to $^{2}J_{C11H12}$.

When compare the ^{13}C chemical shift of xanthoxyletin which were assigned here with Bergapten (43) a linear furanocoumarin that isolated from Angelica officinalis. The majority of the chemical shift of this compound were so close to xanthoxyletin because the structure was nearly about between two compounds.(see Figure 55)

Xanthoxyletin Bergapten

Figure 55 Structure of xanthoxyletin and Bergapten