CHAPTER IV

RESULTS AND DISCUSSION

The 2.8 kg of dried powdered root bark of *Clausena harmandiana* Pierre. was extracted with 20 litres of hexane for 17 hours and 56 g of gummy residue was yielded. 5 g of gummy residue was separated by means of column chromatography on 60 g of silica gel G 60 (230-400 mesh ASTM) and eluted with solvent A. A 25 ml of each fraction was collected as F_1, F_2, F_3,..., then solvent B was added and the same amount of each fraction was collected. A total 54 fractions of 25 ml each were obtained. Each individual fraction was monitored by TLC in solvent system 3 and identical fractions were combined. The crystals were obtained in ether-methanol. Total six compounds were isolated. The number of fractions, purification TLC solvent system, the isolated compounds and the identification of these compounds were showed as the following :-

1. Compound I : 39.6 mg (0.79% yield) of crystal was obtained from F_{12}-F_{17}. This compound was identified with the following data :-

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

R_f value : 0.88 on TLC solvent system 1

0.79 on TLC solvent system 2

0.51 on TLC solvent system 3

0.72 on TLC solvent system 6

0.37 on TLC solvent system 7

Molecular weight : 279 (mass spectrometry, EIMS)

Melting point : 171-172℃

TLC : The sample was spotted an silica gal GF 254 plate and developed in solvent system 1,2,3,6,7. After the plate was dried in open air, the detection was performed under UV and was sprayed with benzidine reagent.

UV : This compound gave one spot on five solvent systems and fluoresced at 254 nm (short wavelength) but it did not fluoresce at 365 nm (long wavelength).

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

Spectral data :-

Ultraviolet absorption spectrum (methanol)

λ_{max}^{MeOH} = 236 nm (log ε 4.42)

278 nm (log ε 4.57)

299 nm (log ε 4.60)

342 nm (log ε 4.60)

Infrared absorption spectrum (Potassium Bromide disc)

λ_{max}^{KBr} = 3300 cm^{-1} (-OH or -NH stretching)

2740-3125 cm^{-1} (-CH stretching)

1612 cm^{-1} (aldehyde, -C=C stretching)

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

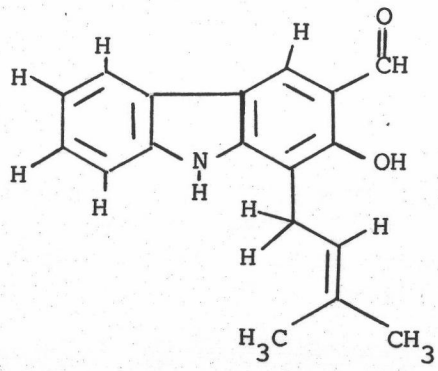
Mass spectrum (EIMS was performed at 195℃)

m/e (%) - 279(20.21), 264(6.68), 262(2.77), 236(5.48), 225(3.66), 224(22.61), 223(3.89), 222(2.53), 195(2.64), 180(2.23), 167(4.53).

Nuclear magnetic resonance spectrum (10 mg of sample was dissloved in deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz and analysed in δ value (PPM).

protons	chemical shift (δ)	multiplicity
-COH	9.9	singlet
-OH	11.63	singlet
$-CH_3$ (6H, at 1,1-dimethylallyl)	1.56, 1.90	singlet
$-CH_2-$ (at 1,1-dimethylallyl)	3.64	doublet
-CH= (methine, 1H, at 1,1-dimethylallyl)	5.32	broad triplet
aromatic (5H)	7.37	triplet
	7.96	doublet
	8.20 or 8.04?	singlet
-NH	8.04 or 8.20?	singlet

All These informations, suggested that compound I is Heptaphylline. It is identical in R_f value, melting point(170-171℃), ultraviolet absorption spectrum, infrared absorption spectrum, mass spectrum, nuclear magnetic resonance spectrum, molecular weight and color reaction with identified Heptaphylline data.[21,25] The spectroscopic spectra were shown in Fig. 9 to 12.



Heptaphylline

2. Compound II : 71.7 mg (1.43% yield) of crystal was obtained form F_{18}-F_{20}. This compound was identified with the following data :-

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

R_f value : 0.77 on TLC solvent system 1
0.37 on TLC solvent system 2
0.22 on TLC solvent system 3
0.51 on TLC solvent system 4
0.62 on TLC solvent system 5
0.82 on TLC solvent system 8

Molecular weight : 380 (mass spectrometry, EIMS)

Melting point : 198-202℃

TLC : The sample was spotted on silica gel GF 254 plate and developed in solvent system 1,2,3,4,5,8. 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 in all solvent systems and fluoresced at 254 nm (short wavelength) but it did not fluoresce at 365 nm (long wavelength).

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

Spectral data :-

Ultraviolet absorption spectrum (methanol)

λ_{max}^{MeOH} = 211 nm (log ε 4.27)
229 nm (log ε 4.31)
280 nm (log ε 4.40)
335 nm (log ε 4.16)

Infrared absorption spectrum (Potassium Bromide disc)

ν_{max}^{KBr} = 3170 cm^{-1} (-OH stretching)
2880-3010 cm^{-1} (-CH stretching)
1672 cm^{-1} (coumarin carbonyl)
1645 cm^{-1} (conjugated double bond at C-3, C-4)

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

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

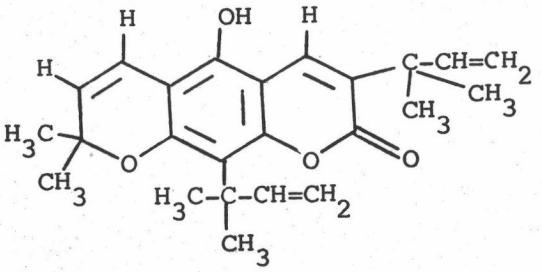
Mass spectrum (EIMS was performed at 230℃)

m/e (%) - 380(11.19), 366(9.66), 365(36.43), 337(2.68), 297(5.80), 69(7.47), 54(2.21), 52(2.66).

Nuclear magnetic resonance spectrum (10 mg of sample was dissolved in deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz NMR and analysed in δ value (PPM.)

protons	chemical shift (δ)	multiplicity
$-CH_3$ (12H, at 3,3-dimethylallyl or prenyl)	1.42, 1.62	singlet
$-CH_3$ (6H, at 2,2-dimethylchromene ring)	1.48	singlet
-OH at C-5	7.27	singlet
-H at C-4	8.07	singlet
-H at C-3'	5.65	doublet
-H at C-4'	6.72	doublet
-CH= (methine, of prenyl, 2H)	6.0-6.5	multiplet
$=CH_2$ (methylene of prenyl, 4H)	4.8-5.2	multiplet

All these informations, suggested that compound II is Clausarin. It is identical in R_f value, melting point (208℃),ultraviolet absorption spectrum, infrared absorption spectrum, mass spectrum, nuclear magnetic resonance spectrum, molecular weight and color reaction with identified clausarin data.[18] The spectroscopic spectra were shown in Fig. 13-16.



Clausarin

To analyse the coupling pattern of proton at δ 6.0-6.5 and δ 4.8-5.2, the method of irradiation was used. Irradiation of proton about δ 6.0-6.5 gave separately a signal at δ 4.8-5.2 into 2 set. Each set of signal at δ 5.11 or at δ 4.94 refered to two protons of methylene group. The signal of each set still showed the multiplicity, residual coupling, due to the protons at δ 6.0-6.5 were incompletly decoupled. In reversely, the irradiation of proton at about δ 4.8-5.2 also gave two set of signal, one proton in each set, at δ 6.3 and δ 6.19. Each proton still showed a residual coupling due to incomplete decoupling.

On the basic knowledge of coupling constant of olefinic compounds, J_{trans} is larger than J_{cis}. Both J_{trans} and J_{cis} are larger than J_{gem}. The coupling pattern of two prenyl groups could be analysed as shown in Fig. 8. One proton of methine (H_3) in one prenyl group showed a *cis* and *trans* coupling with two protons of methylene (H_1, H_2), therefore signal of H_3 splitted into doublet-doublet (dd). The signal of proton H_1 also gave a doublet-doublet due to a *cis* coupling with H_3 and *geminal* coupling with H_2. The coupling pattern of H_2 could be analysed with the same procedure. Our experiment did not indicated which proton of methine in two

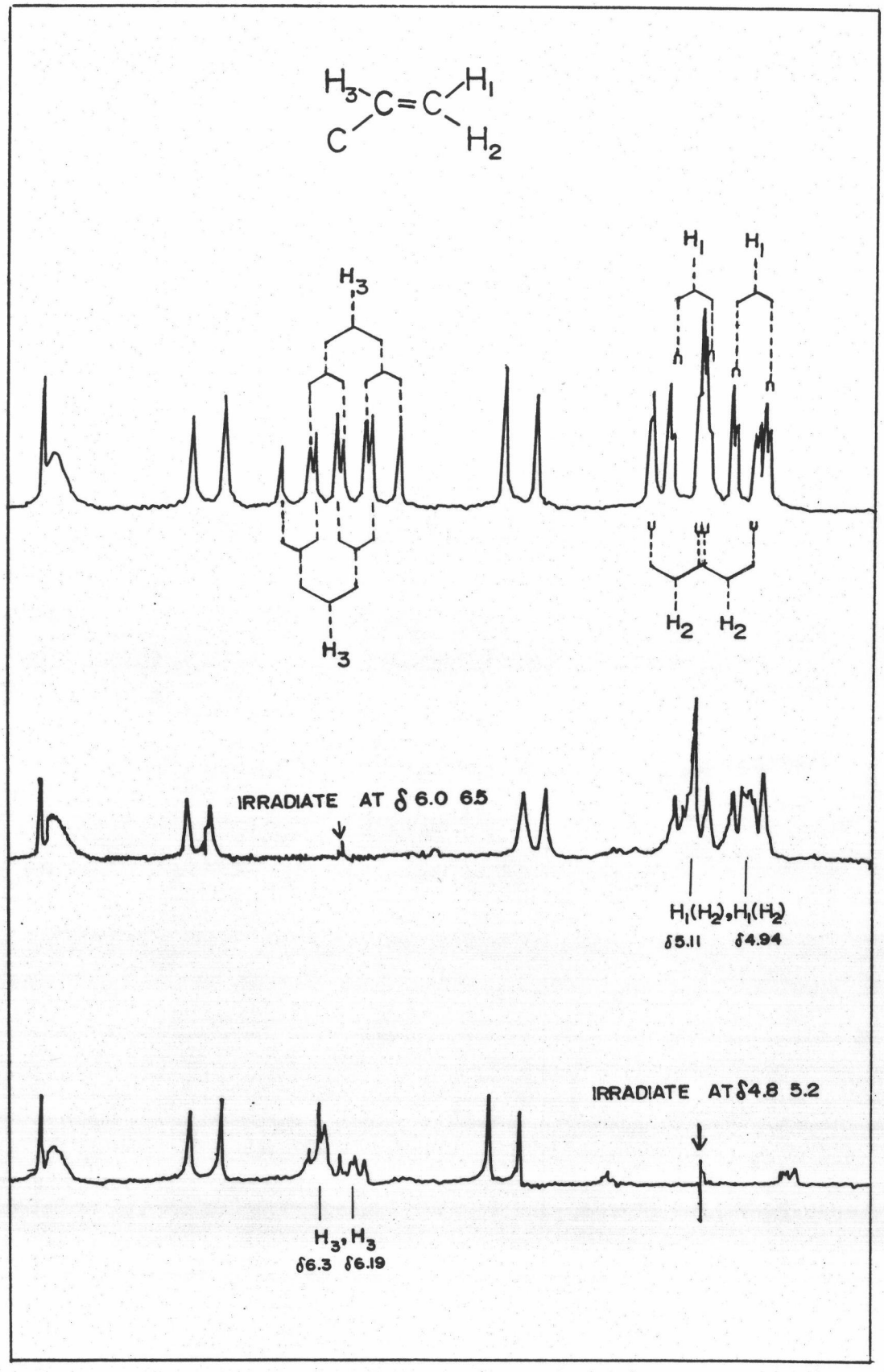


Fig. 8 Normal and irradiation ^{1}H-NMR spectra of compound II (clausarin) in $CDCl_3$.

prenyl groups showed a higher chemical shift. In our knowledge we proposed that the proton of methine in prenyl group, which aparted from oxygen of lactone ring or prenyl group at C-3 position of coumarin nucleus, should show a high chemical shift. This was due to the shielding effect of electron of oxygen at position 1 of coumarin nucleus.

3. Compound III : 600 mg (12% yield) of crystal was obtained from F_{22}-F_{29}. This compound was identified with the following data :-

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

R_f value : 0.65 on TLC solvent system 1
0.34 on TLC solvent system 2
0.20 on TLC solvent system 3
0.75 on TLC solvent system 4
0.67 on TLC solvent system 5

Molecular weight : 326 (mass spectrometry, EIMS)

Melting point : 95°C

TLC : The compound was spotted on silica gel GF 254 plate and developed in solvent system 1,2,3,4,5. 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 systems 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.

Spectral data :-

Ultraviolet absorption spectrum (methanol)

λ_{max}^{MeOH} = 206 nm (log ε 4.14)
231 nm (log ε 4.25)
272 nm (log ε 4.41)
346 nm (log ε 4.01)

Infrared absorption spectrum (Potassium Bromide disc)

ν_{max}^{KBr} = 2870-3080 cm^{-1} (-CH stretching)
1725 cm^{-1} (coumarin carbonyl)
1630 cm^{-1} (conjugated double bond at C-3, C-4)
1610 cm^{-1} (-CH=C- stretching)
1450-1600 cm^{-1} (aromatic)

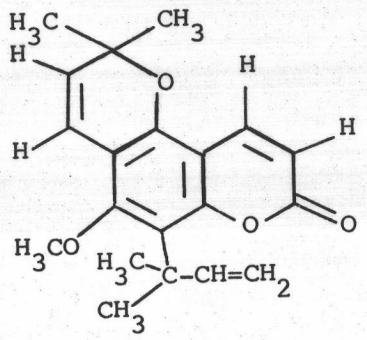
Mass spectrum

m/e (%) - 326(18.9), 312(21.6), 311(100.0), 281(16.2).

Nuclear magnetic resonance spectrum (30 mg of sample was dissolved in 2 ml deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz and analysed in δ value (PPM).

proton	chemical shift (δ)	multiplicity
$=CH_2$ (methylene of prenyl)	4.94	multiplet
-CH= (methine of prenyl)	6.21	doublet
$-CH_3$ (6H, at 3,3-dimethylallyl or prenyl)	1.67	singlet
$-OCH_3$	3.83	singlet
-H at C-3	6.19	doublet
-H at C-4	7.88	doublet
-H at C-3'	5.7	doublet
-H at C-4'	6.57	doublet
$-CH_3$ (6H, at 2,2-dimethylchromene ring)	1.46	singlet

All these informations, suggested that compound III is Dentatin. It is identical in R_f value, melting point(95℃), ultraviolet absorption spectrum, infrared absorption spectrum, mass spectrum, nuclear magnetic resonance spectrum, molecular weight and color reaction with identified dentatin data.[20] The spectroscopic spectra were shown in Fig. 17-20.



Dentatin

4. Compound IV : 150 mg (3.0% yield) of crystal was obtained from F_{39}-F_{41}. This compound was identified with the following data:-

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

R_f value : 0.62 on TLC solvent system 1
0.34 on TLC solvent system 2
0.15 on TLC solvent system 3
0.63 on TLC solvent system 4
0.48 on TLC solvent system 5

Molecular weight : 244 (mass spectrometry, EIMS)

Melting point : 78-81℃

TLC : The compound was spotted on silica gel GF 254 plate and developed in solvent system 1,2,3,4,5. 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.

Spectral data :-

Ultraviolet absorption spectrum (methanol)

λ_{max}^{MeOH} = 206 nm (log ε 4.51)

259 nm (log ε 4.04)

268 nm (log ε 4.04)

321 nm (log ε 4.14)

Infrared absorption spectrum (Potassium Bromide disc)

ν_{max}^{KBr} = 2840-3010 cm^{-1} (-CH stretching)

1728 cm^{-1} (coumarin carbonyl)

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

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

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

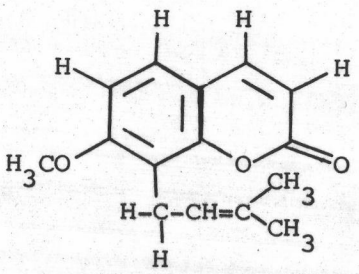
Mass spectrum (EIMS was performed at 100°C).

m/e (%) - 244(2.66), 243(15.31), 242(2.33), 229(2.02), 228(12.14), 212(4.76), 200(7.02), 188(11.22), 186(2.74), 185(2.38), 174(2.50), 158(2.43), 131(3.42), 77(2.07).

Nuclear magnetic resonance 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.)

protons	chemical shift (δ)	multiplicity
$-CH_3$ (6H, at 1,1-dimethylallyl)	1.67, 1.84	singlet
-CH= (methine of 1,1-dimethylallyl)	5.23	triplet, quartet
$-CH_2-$ (at 1,1-dimethylallyl)	3.52	doublet
$-OCH_3$	3.92	singlet
-H at C-3	6.22	doublet
-H at C-4	7.61	doublet
-H at C-5	7.29	doublet
-H at C-6	6.83	doublet

All these informations, suggested that compound IV is Osthol. It is identical in R_f value, melting point, ultraviolet absorption spectrum, infrared absorption spectrum, mass spectrum, nuclear magnetic resonance spectrum, molecular weight and color reaction with identified osthol data.[2] The spectroscopic spectra were shown in Fig. 21-24.



Osthol

5. Compound V : 300 mg (6.0% yield) of crystal was obtained from F_{46}. This compound was identified with the following data :-

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

R_f value : 0.56 on TLC solvent system 1

0.23 on TLC solvent system 2

0.11 on TLC solvent system 3

0.71 on TLC solvent system 4

0.47 on TLC solvent system 5

Molecular weight : 258 (mass spectrometry, EIMS)

Melting point : 132-134℃

TLC : The sample was spotted on silica gel GF 254 plate and developed in solvent system 1,2,3,4,5. 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 systems 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.

Spectral data :-

Ultraviolet absorption spectrum (methanol)

λ_{max}^{MeOH} = 227 nm (log ε 4.36)

268 nm (log ε 4.42)

346 nm (log ε 4.09)

Infrared absorption spectrum (Potassium Bromide disc)

ν_{max}^{KBr} = 2840-3060 cm^{-1} (-CH stretching)

1725 cm^{-1} (coumarin carbonyl)

1638 cm^{-1} (conjugated double bond at C-3, C-4)

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

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

Mass spectrum

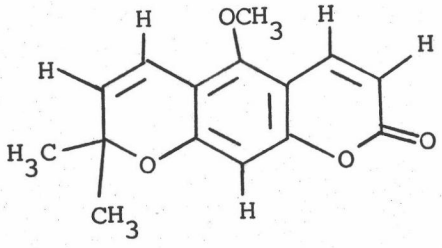
m/e (%) - 258(9.30), 244(7.42), 243(over),228 (over) 200(6.73), 172(1.49), 144(1.49), 129(1.38), 115(1.85), 107(2.02), 100(2.12), 69(2.28).

Nuclear magnetic resonance spectrum (30 mg of sample was dissolved in 2 ml deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz and analysed in δ value (PPM.)

protons	chemical shift (δ)	multiplicity
-H at C-3	6.21	doublet
-H at C-4	7.83	doublet
-H at C-8	6.56	singlet
-H at C-3'	5.71	doublet
-H at C-4'	6.56	doublet
$-OCH_3$	3.86	singlet
$-CH_3$ (6H, at 2,2-dimethyl-chromene ring)	1.46	singlet

All these informations, suggested that compound V is xanthoxyletin. It is identical in R_f value, melting point (132-134°C), ultraviolet absorption spectrum, infrared absorption spectrum, mass

spectrum,nuclear magnetic resonance spectrum, molecular weight and color reaction with identified xanthoxyletin data.[13] The spectroscopic spectra were shown in Fig. 25-28.



Xanthoxyletin

6. Compound VI : 56 mg (1.12% yield) of crystal was obtained from F_{47}-F_{48}. This compound was identified with the following data :-

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

R_f value : 0.20 on TLC solvent system 1
0.07 on TLC solvent system 2
0.02 on TLC solvent system 3
0.20 on TLC solvent system 4
0.18 on TLC solvent system 5
0.40 on TLC solvent system 8
0.57 on TLC solvent system 10

Molecular weight : 312 (mass spectrometry, EIMS)

Melting point : 183-186℃

TLC : The sample was spotted on silica gel GF 254 plate and developed in solvent system 1,2,3,4,5,8,10. 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 systems and fluoresced at 254 nm (short wavelength) but it did not fluoresce 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 :-

Ultraviolet absorption spectrum (methanol)

λ_{max}^{MeOH} = 208 nm (log ε 4.24)
227 nm (log ε 4.25)
278 nm (log ε 4.43)
337 nm (log ε 4.14)

Infrared absorption spectrum (Potassium Bromide disc)

ν_{max}^{KBr} = 3240 cm^{-1} (-OH stretching)
2860-2980 cm^{-1} (-CH stretching)
1685 cm^{-1} (coumarin carbonyl)
1640 cm^{-1} (conjugated double bond at C-3, C-4)
1605 cm^{-1} (-CH=C- stretching)
1450-1600 cm^{-1} (aromatic)

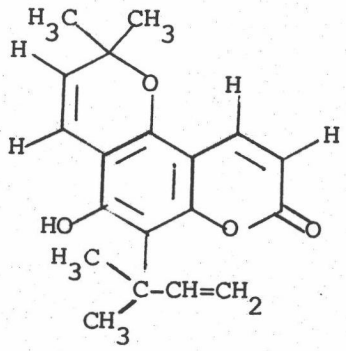
Mass spectrum (EIMS was performed at 200°C)

m/e (%) - 312(9.56), 298(6.44), 297(30.58), 269(4.58), 241(4.49), 77(3.48), 52(3.60).

Nuclear magnetic resonance spectrum (10 mg of sample was dissolved in deuteriochloroform, using TMS as reference compound. The spectrum was obtained from 90 MHz NMR and analysed in δ value (PPM).

protons	chemical shift (δ)	multiplicity
$=CH_2$ (methylene of prenyl)	4.9	triplet, quartet
-CH= (methine of prenyl)	6.19	doublet
$-CH_3$ (6H, at 3,3-dimethylallyl or prenyl)	1.64	singlet
$-CH_3$ (6H, at 2,2-dimethyl chromene ring)	1.44	singlet
-OH	6.44	singlet
-H at C-3	6.13	doublet
-H at C-4	8.02	doublet
-H at C-3'	5.67	doublet
-H at C-4'	6.53	doublet

All these informations, suggested that compound VI is nordentatin. It is identical in R_f value, melting point (182°C), ultraviolet absorption spectrum, infrared absorption spectrum, mass spectrum, nuclear magnetic resonance spectrum, molecular weight and color reaction with identified nordentatin data.[20] The spectroscopic spectra were shown in Fig. 29-32.



Nordentatin

Clausena harmandiana was the plant growth in the northeast of Thailand. This plant have been told to be used as a folkloric medicine as a stomachica. In the identification of plant indicated that it was a member of family Rutaceae. The root bark gave pungent odor, and was tasted bitter and acrid. Therefore the methods of extraction of coumarins were selected to isolate and separate the compound from this plant.

Two methods of extraction were used, an extraction with hexane by refluxing and an extraction with petroleum ether using soxhlet apparatus. In the analysis of the extracts, from both methods, by TLC showed that the hexane extract countained more compounds, in large amount, than the petroleum ether extract. Also the hexane extract gave more clearly crude extract than petroleum ether extract which usually contained fatty and resinous compounds. These may be concerned about the polarity of both solvents and also the solubility of the compound in solvent (petroleum ether is less polar than hexane). The method using hexane as solvent, was selected to extract in large amount of plant.

In order to obtained a large amount of compounds, column chromatography was used to purify the crude extract. Gradient elution was performed by using solvent A (petroleum ether : diethyl ether = 5:1) and solvent B (diethyl ether). Respectively, the fractions were collected, each individual fraction was monitored by TLC and the identical one were conbined. Each combined fraction were tried to crystallize, and in order to obtain the pure compound recrystalli zation may be used. Because of large amount to compound contained in this plant, only such purification could obtain a pure crystal. There were only two compounds heptaphylline and clausarin, which needed recrystallization to obtain a more pure one.

In the isolation of *Clausena harmandiana* Pierre. root bark, yield heptaphylline (0.79%), clausarin (1.43%), dentatin (12.00%), osthol (3.00%), xanthoxyletin (6.00%), and nordentatin (1.12%). There were no reported about these compounds in this plant before. This was the reported, at least five coumarins, two of them contained in large amount (dentatin and xathoxyletin). Heptaphylline, an only one alkaloid which was isolated, was an exceptional due to the solubility. This compound gave a positive alkaloidal test which was clearly shown the difference from isolated compound. It was surprisingly that this compound came out in non polar fraction, but, was in small amount. Therefore the small amount of heptaphylline would not indicate the actual yield of this compound in plant. By using the alkaloidal method of extraction, a large amount of this compound may be obtained.

According to the classification of coumarin, the compounds

found in this plant could be classified into two main types. Clausarin and xanthoxyletin were pyranocoumarin type (linear xanthyletin), dentatin and nordentatin were also be classified into pyranocoumarin type but in angular subtype. The only one simple coumarin type was osthol.

The analysis of all these compounds were based on the informations of spectral data of ultraviolet spectrophotometry, infrared spectrophotometry, nuclear magnetic resonance spectroscopy and mass spectrometry. The molecular weight of each one was first obtained from electron impact mass spectrometry. This was done by using the calculation of M+1 or M+2 and also the fragmentation pattern of some coumarins. This analysis of other functional groups in the structure were completed by UV, IR, NMR spectra. Some special techniques such irradiation of proton in NMR also was performed to confirm such a functional group. Another informations could be obtained by TLC, melting point and also from chemical test. All the informations obtained, were used to identify the compound by comparing with the reported data of known one.

UV absorption spectrophotometry

- The UV spectrum of heptaphylline showed a maximum absorption at 299 nm. The phenolic group was characterized by using the appearance of bathochromic shift on addition a few drops of sodium hydroxide solution. This was confirmed with the chemical test with benzidine reagent.

The pyranocoumarin which contained hydroxy group showed a little longer wavelength than the one which contained methoxy group.

Clausarin (λ_{max} at 280 nm) and nordentatin (λ_{max} at 278 nm), a phenolic pyranocoumarin, showed this property in some wavelength over dentatin (λ_{max} at 272 nm) and xanthoxyletin (λ_{max} at 268 nm). An only simple coumarin, osthol showed a λ_{max} at 206 nm.

IR spectrophotometry

- The IR spectrum of heptaphylline in KBr showed a -OH and -NH stretching at 3300 cm^{-1}. The aldehyde C=O stretching was at 1612 cm^{-1}, low frequency than normal aldehyde due to the intramolecular hydrogen bond.

The hydroxy pyranocoumarins showed the -OH streching at normal region (3704-3125 cm^{-1}). The carbonyl streching of hydroxy pyranocoumarins gave normally at lower frequency than the carbonyl streching of methoxy pyranocoumarins. This may be due the inductive effect of methoxy and hydroxy group on carbonyl absorption. Clausarin and nordentatin showed a carbonyl stretching at 1672 cm^{-1} and 1685 cm^{-1} which both dentatin and xanthoxyletin showed at 1725 cm^{-1}. The aromatic absorption band of all pyranocoumarins were formed at usual region of 1450-1600 cm^{-1}. This was similar to aromatic absorption in simple coumarin. The α-β unsaturated lactone ring. a structure in coumarin also has been formed at frequency of 1625-1650 cm^{-1}. Osthol, a simple coumarin, showed a carbonyl stretching at 1728 cm^{-1} similar to dentatin and xanthoxyletin. Other absorption peaks were similar to all common peak found in coumarin.

Electron impact mass spectrometry (EIMS)

- Heptaphylline, only one compound showed a odd number of molecular weight, indicated that this compound contained the odd

number of nitrogen. Clausarin and nordentatin showed a molecular weight at 380 and 312 respectively, the difference of 68 unit of molecular weight, was due to one additional prenyl group in clausarin. Therefore EIMS of both compound were similar. The loss of methyl group in 2,2-dimethylchromenopyrone and CO gave the peak at m/e 365, 337 for clausarin and m/e 297, 269 for nordentatin.

Dentatin showed a molecular ion peak at m/e 326. On the analysis of the EIMS, indicated that the loss of methyl group in 2.2-dimethylchromenopyrone, and a carbonyl group gave the peak at m/e 311 and 283 respectively.

On EIMS, osthol showed the intense peak at m/e 244, 229, 201 and 186, indicated that the molecular weight was 244 and the loss of CH_3 in prenyl group, CO of the pyrone ring, and CH_3 of methoxy group.

Xanthoxyletin showed a molecular weight of 258. The fragmentations also were occured in similar as osthol. The loss of methyl in 2,2-dimethylchromenopyrone gave a peak at m/e 243. The loss of methyl of methoxy gave a peak at m/e 228.

NMR spectrometry

- Heptaphylline showed peak aldehyde at δ 9.9 (s). The two methyl group at 1,1-dimethylallyl group were showed at δ 1.56, 1.90 (s), the methine was showed at δ 5.32 (broad triplet) and methylene group was showed at δ 3.64 (d). The signals of aromatic ring were showed at δ 7.37 (t), 7.96 (d).

The most common NMR spectra of coumarins were the absorption peaks at δ 6.2 and δ 7.6 due to the proton at C-3 and C-4 of coumarin nucleus. Each peak formed a doublet with J about 9.5 Hz. The analysis of the chemical shift and coupling pattern was showed before, base on the basic knowledge and known data previously report.