# CHAPTER III

## RESULTS AND DISCUSSIONS

Dried, powdered root bark of *Clausena cambodiana* , Guill 850 grams was extracted by refluxing with n-hexane. The hexane extract was concentrated on rotary evaporator to yield a gummy residue. Total amount of 35 gm of gummy residue was left over night for crystallization of compounds. Crystal have been washed with cool methanol and dissolved the crystal with hexane and recrystallization to get compound-1 Combine all filtrate fractions and concentrate to yield about 40 ml which have then been separated by column chromatography to get compound-2 and compound-3. Total weight 100 gm of compound-2 was brought to react with potassium carbonate 5 gm. in acetone and reflux for 9 hours and treated product with hydrochloric acid, white precipitate of compound-4 was obtained. (29)

The identification of four compounds were performed by physical and chemical method. The result were summarized as following:-

Compound-1 A rhombic crystal 2.7 gm of compound-1 (0.23%), was obtained from recrystalization filtrate was dissolved in acetone and spotted on TLC plate, developed in five solvent systems(1,2,3,4,5). After developed

the plate was left to dry at room temperature and detected spot by using iodine vapor and ultraviolet light. Only one spot showed on TLC plate indicated pure compound obtained.

Color and form: Colorless, rhombic

Rf-value(Siliga gel 60 GF 254 E.Merk in five solvent systems)

| | | | |
|---|---|---|---|
| Rf-value | = | 0.71 | Solvent system 1 |
| | | 0.38 | Solvent system 2 |
| | | 0.26 | Solvent system 3 |
| | | 0.82 | Solvent system 4 |
| | | 0.73 | Solvent system 5 |

Melting point: 96.5°C (Uncorrected)

IR Spectrum (Potassium bromide disc)(Figure 21 appendix)

$\nu_{Max}^{KBr}$ = 2990 $cm^{-1}$ (-CH stretching)
1725 $cm^{-1}$ ( C=O carbonyl, coumarin)
1618 $cm^{-1}$ ( conjugated double bond)
1590 $cm^{-1}$ (-CH=CH- stretching)
1460 $cm^{-1}$ (aromatic)

Mass spectrum (EIMS): (Figure 22 appendix)

m/e(%relative intensity) = 326(26.6), 312(21.4), 311(100)
218(13.0)

Molecular weight 326 (EIMS)

H-NMR Spectrum of compound 1 in $CDCl_3$ (TMS as reference), was reported as follow:(Figure 23 appendix)

| proton | chemical shift (ppm) | multiplicity |
|---|---|---|
| 1H(C4) | 7.86 | Doublet(J=9.9Hz) |
| 1H(C3) | 6.18 | Doublet(J=9.9Hz) |
| 1H(C11) | 5.69 | Doublet (J=10.8Hz) |
| 1H(C12) | 6.57 | Doublet(J=10.8) |
| 3H($-OCH_3$) | 3.83 | Singlet |
| 6H($2CH_3$ on C10) | 1.45 | Singlet |
| 6H($2CH_3$ on C15) | 1.66 | Singlet |
| 1H(C16) | 6.31 | Doublet doublet (J cis=9.9Hz) (J trans=18.9Hz) |
| 1H(C17 cis) | 4.88 | Doublet(J=9.9Hz) |
| 1H(C17 trans) | 5.00 | Doublet(J=18.9Hz) |

From data obtained indicated that this compound should be dentatin(Figure 24)

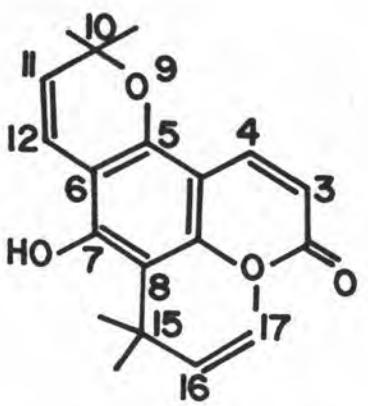


Figure 24 Structure of dentatin (30)

By data from TLC experiment, the compound showed only one spot on TLC plates which were developed in 5 difference polarity of 5 solvent systems, which indicated that this compound was more purely. In IR spectrum showed a strongly absorption peak of C=O stretching, carbonyl group of lactone, at 1725 cm . The peak at 1618 cm indicated a conjugated double bond. (31)

From data obtained from this experiment we can concluded that this compound should be dentatin. The molecular weight 326 which correspond to $C_{20}H_{22}O_4$ . The loss of mass 15 (m/e 311, 100%) indicated that one methyl group will be cleavage. The loss of mass 14 (m/e 312, 21.4%)indicated one methylene group will be cleavage by hemihexterolysis process (Figure 24): (32)

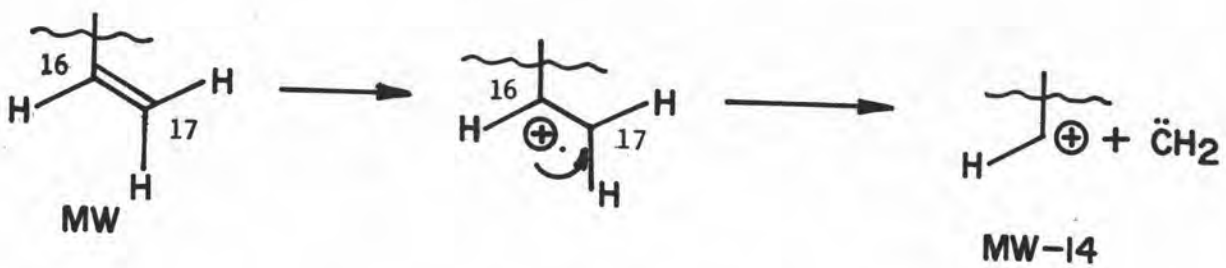


Figure 24 The mechanism of hemihexterolysis process.

From NMR spectrum a doublet at 7.86 ppm and 6.18 ppm (H4 and H3) which J-value is 9.9 Hz indicated that this structure might contain coumarin nucleus. The doublet at 5.69 ppm and 6.57 ppm (H11 and H12) which J-value is 10.8 Hz indicated that a olefinic coupling proton exists. The methoxy group showed a singlet at 3.83 ppm. Four methyl group are showed at 1.45,1.66 ppm. A pattern of proton at C16 and two protons at C17 were analysed to be at 6.3 and 4.83-5.04 respectively. The multiplicity of proton at C16 showed a doublet-doublet which correspond to cis and trans coupling (J cis=9.9, J trans =18.9). The proton at C16 (H16) will be coupling with protons at C17(H-cis) and at C17(H-trans) to form a doublet doublet. The multiplicity at region 5.01ppm-4.81ppm were assigned for proton at C17(cis-trans) coupling with proton at C16. The coupling pattern of H16(cis) and two protons at H17(cis-trans) have been shown as in the diagram below (Figure 26).

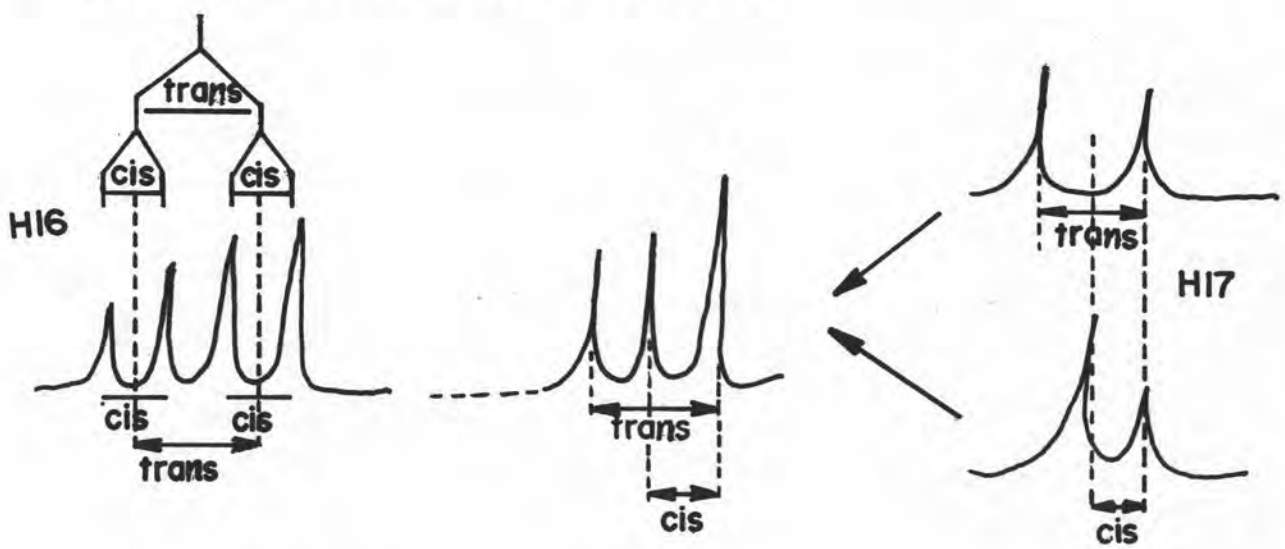


Figure 26 Pattern of spectrum coupling between one proton at C16 and two protons at C17.

There were no geminal coupling of H17 cis and H17-trans due to the J-value varied from 0-32 Hz( 26 ).

All of the data obtained were compared with the data obtained reference compound dentatin, and the result showed that this compound must be dentatin.

Compound-2 Total weight 3.1 gm (0.36%) was obtained from extracted root bark of Clausena cambodiana and separated by column chromatography process. This compound was recrystallized from ethanol and tested purities by TLC. The identification and structure elucidation of compound was based on the following data:

Color and form: Yellowish, needle prism.

Rf-value (Siliga gel 60 GF 254 E.Merk in five solvent systems.)

| | | | |
|---|---|---|---|
| Rf-value | = | 0.34 | Solvent system 1 |
| | | 0.45 | Solvent system 2 |
| | | 0.43 | Solvent system 3 |
| | | 0.28 | Solvent system 4 |
| | | 0.56 | Solvent system 9 |

Melting point: 134°C (uncorrected)

IR Spectrum (Potassium bromide disc) (Figure 27 appendix)

$\nu_{Max}^{KBr}$ = 3500 $cm^{-1}$(OH stretching)

1718 $cm^{-1}$(C=O stretching)

1650 $cm^{-1}$(C=O stretching)

1600 $cm^{-1}$(conjugated double bond)

Mass spectrum (EIMS): (Figure 28 appendix)

m/e(%relative intensity) = 328(75.9), 313(100.0), 285(15.6), 257(100.0), 245(15.3), 244(21.2)

Molecular weight: 328 (EIMS)

H-NMR spectrum (Figure 29 appendix)

The H-NMR spectrum of this compound in $CDCl_3$ (TMS as reference), was summarized as follows:

| proton | chemical shift (ppm) | multiplicity |
|---|---|---|
| 1H(OH) | 12.98 | Singlet |
| 1H(C4) | 8.04 | Doublet(J=9.9Hz) |
| 1H(C3) | 6.15 | Doublet(J=9.9Hz) |
| 2H(C11) | 2.75 | Singlet |
| 6H($2CH_3$) | 1.64 | Singlet |
| 6H($2CH_3$) | 1.5 | Singlet |
| 1H(C16) | 6.24 | Doublet,Doublet (J=18,J=10.2) |
| 1H(C17 cis) | 4.88 | Doublet(J=10.2) |
| 1H(C17 trans) | 4.91 | Doublet(J=18Hz) |

From data obtained indicated that this compound was Clausanidin(Figure 30)

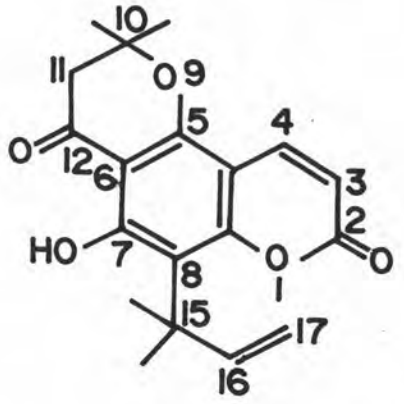


Figure 30 Structure of clausanidin. (33)

In IR spectrum showed a strongly absorption peak of OH stretching at 3500 $cm^{-1}$ and C=O stretching, carbonyl group of lactone at 1718 cm and at 1650 cm . The peak at 1600 cm indicated a conjugated double bond.

The molecular weight 328 correspond to $C_{19}H_{20}O_5$. The loss of mass 15 (m/e 313, 100%) indicated that one methyl group will be cloven, same as compound-1. (34)

From NMR spectrum a doublet at 8.04ppm and 6.15ppm which J-value is 9.9 Hz indicated that this structure contains a coumarin nucleus. The absence of doublet at about 5.69ppm and 6.57ppm showed no olefinic coupling proton compared to compound-1,dentatin. No singlet peak of methoxy group showed in the spectrum but a singlet peak at 12.98 ppm which no assigned to be the proton of hydroxy group was shown. Twelve protons of four methyl groups showed singlet peak at 1.64ppm and

1.5ppm Singlet peak at 2.75 ppm was assigned to be two protons of methylene group at C11. A pattern of coupling proton at C16 and C17 were analysed same as compound-1(dentatin). Therefor the conclusion can be made that compound was clausanidin structure showed above.

Compound-3 Total weight 0.1 gm (0.01%) was obtained from extracted root bark of Clausena cambodiana and separated by fractional column chromatography process. This compound was recrystallized in acetone and tested purities by TLC. The identification and structure elucidation of compound was based on the following data:

Color and form: Colorless needle prism.

Rf-value (Siliga gel 60 GF 254 E.Merk in seven solvent systems.)

| | | | |
|---|---|---|---|
| Rf-value | = | 0.71 | Solvent system 1 |
| | | 0.47 | Solvent system 2 |
| | | 0.13 | Solvent system 3 |
| | | 0.57 | Solvent system 4 |
| | | 0.43 | Solvent system 5 |
| | | 0.31 | Solvent system 8 |
| | | 0.65 | Solvent system 9 |

Melting point: 132 C-132.5 $^{\circ}$C

IR Spectrum (Potassium bromide disc) (Figure 31 appendix)

$\nu_{Max}^{KBr}$ = 2990 $cm^{-1}$(-CH stretching)

1724 $cm^{-1}$(C=0 Carbonyl, coumarin)

1620 $cm^{-1}$(conjugated double bond)

1600 $cm^{-1}$(-CH=C- stretching)

1465 $cm^{-1}$(aromatic)

Mass spectrum (EIMS): (Figure 32 appendix)

m/e(%relative intensity) = 258(21.1), 243(100.0), 227(30.5), 200(19.4)

Molecular weight: 258 (EIMS)

H-NMR spectrum (Figure 33 appendix)

The H-NMR spectrum of this compound in $CDCl_3$ (TMS as reference), was summarized as follows:

| proton | chemical shift (ppm) | multiplicity |
|---|---|---|
| 1H(C3) | 6.2 | Doublet(J=9.0Hz) |
| 1H(C4) | 7.85 | Doublet(J=9.0Hz) |
| 1H(C8) | 6.54 | Singlet |
| 1H(C11) | 6.57 | Doublet(J=10.8) |
| 1H(C12) | 5.69 | Doublet(J=10.8) |
| 3H($OCH_3$ on C5) | 3.86 | Singlet |
| 6H($2CH_3$ on C10) | 1.64 | Singlet |

From data obtained indicated that this compound was xanthoxyletin(Figure 34).

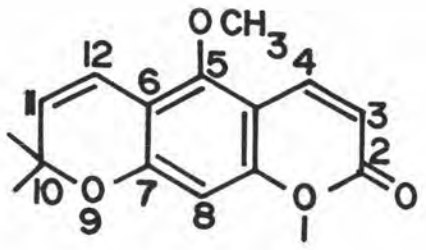


Figure 34 Structure of xanthoxyletin. (35)

In IR spectrum showed a strongly absorption peak CH stretching at 2990 cm, and C=O stretching, carbonyl group of lactone, at 1724 $cm^{-1}$. The peak at 1620 cm indicated a conjugated double bond and peak at 1600 indicated -CH=CH- stretching.

The molecular weight 258 which correspond to $C_{15}H_{14}O_4$.The loss of mass number 15 (m/e 243, 100%) indicated that one methyl group will be cleavages the same as compound-1. The loss of mass 16 (m/e 227, 30.5%)indicated that one atom of oxygen will loss. The proposed mechanism of of methyl group and oxygen atom will be show in Figure 35 (26,31)

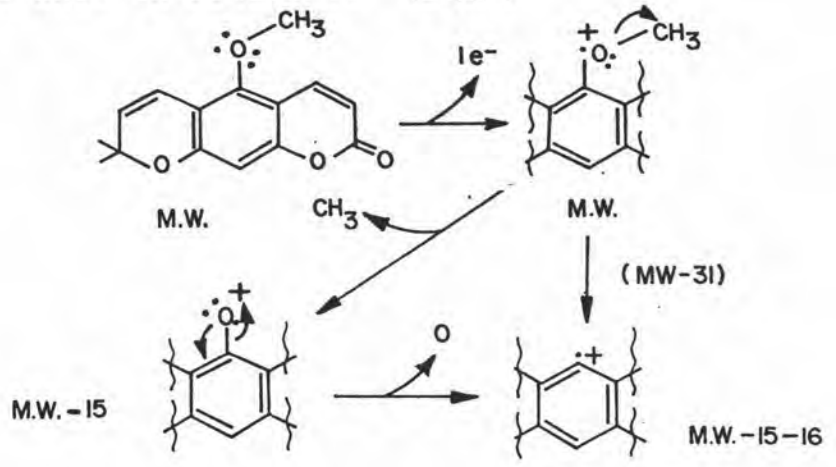


Figure 35 The mechanism of methyl group and oxygen atom cleavage from xanthoxyletin molecule.

From NMR spectrum a doublet at 8.04 and 6.15 which J-value is 9.9 Hz indicated that this structure contains comma nucleus. By compare this spectrum with dentatin, the present of doublet at 5.69ppm and 6.57ppm (J-value 10.8 Hz) show no olefinic coupling proton at C11 and C12. The peak of one methoxy group showed a singlet at 3.86ppm. Six protons from two methyl groups showed singlet peak at 1.64ppm. A single peak at 6.54ppm was assigned to an aromatic protons (H8).

The confirmation of this structure was performed by the irradiation technique. The methyl group at 3.68ppm was irradiated in order to determined the nucleus over hauser effect (NOE effect). The spectra of compound-3 before and after irradiated were compared and showed on Figure 36

Effect of irradiation of methyl group will cause the row intensity of peak of proton at 5.69ppm (H12) and 7.85ppm (H4) but would not be effect the intensity peak of proton at 6.54ppm (H8). From data of this experiment the intensity peak of proton at 5.69ppm (H12) and 7.85ppm (H4) were decrease but the intensity of peak of proton at peak 6.54ppm (H8) was also decreased. These can be explained that before irradiation intensity peak of proton (H8) was added up by intensity of proton (H12), after irradiation the chemical shift of H12 was changed. Therefore the isolated peak was showed with out the additional of intensity of H12. This will cause the

low intensity peak of H8 after irradiation

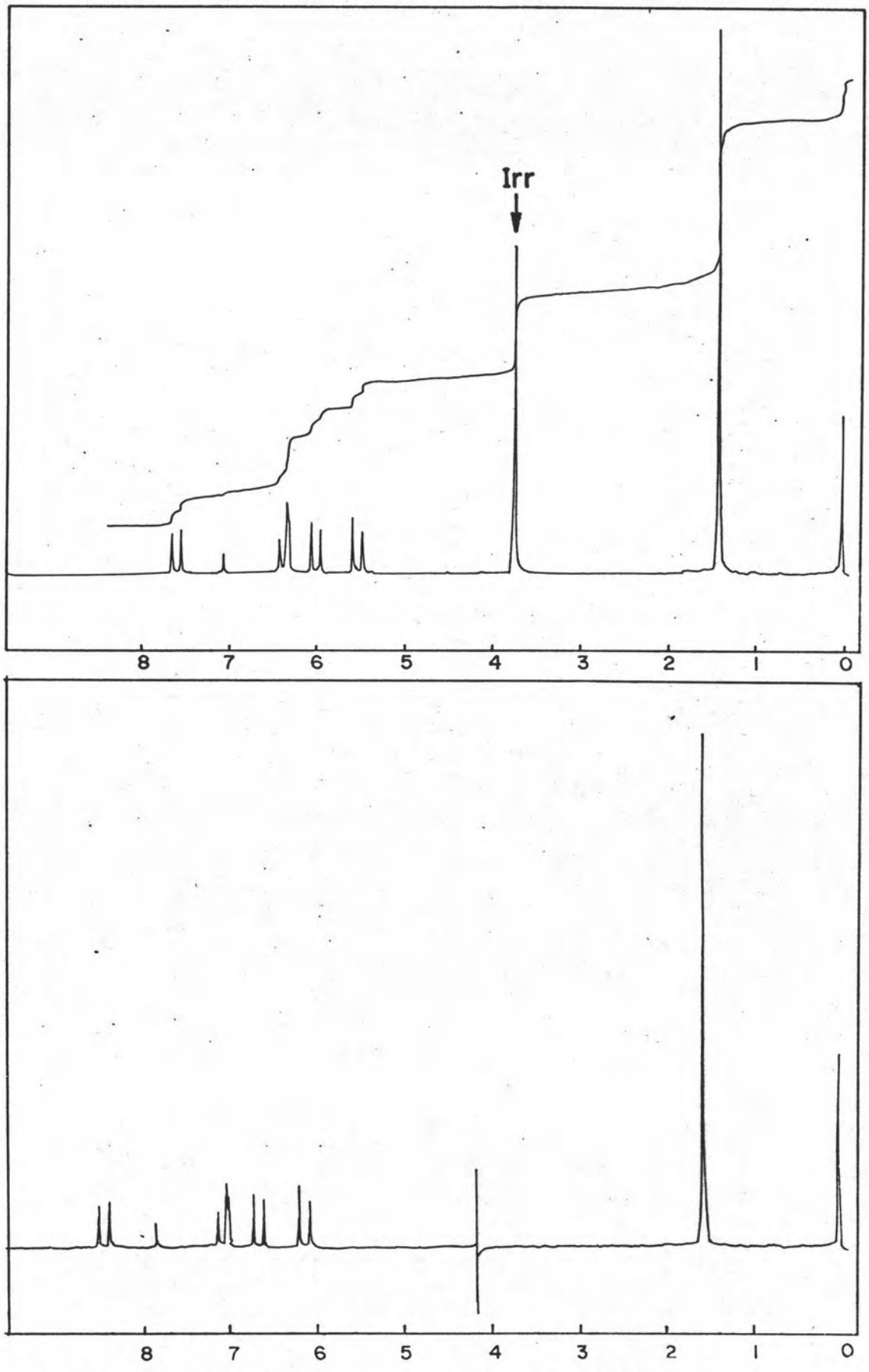


Figure 36. Compare spectrum of compound-3 and compound-3 when irradiate at methyl group at 3.68ppm.

low intensity peak of H8 after irradiation.

So the conclusion of this structure was xanthoxyletin.

Compound-4 Total weight 0.03 gm was obtained from reaction of clausenidine 100 mg., potassium carbonate 5 gm. in acetone reflux for 9 hours and treated product with hydrochloric acid. This product was checked purities by TLC technique.

Color and form: Colorless needle prism.

Rf-value (Siliga gel 60 GF 254 E.Merk in five solvent systems.)

| | | |
|---|---|---|
| Rf-value = | 0.02 | Solvent system 1 |
| | 0.02 | Solvent system 2 |
| | 0.13 | Solvent system 3 |
| | 0.01 | Solvent system 4 |
| | 0.03 | Solvent system 5 |
| | 0.01 | Solvent system 8 |
| | 0.21 | Solvent system 9 |

Melting point: 210°C

IR Spectrum (Potassium bromide disc) (Figure 37 appendix)

$\gamma_{Max}^{KBr}$ = 3250 $cm^{-1}$ (-OH stretching)

1620 $cm^{-1}$ (C=O Stretching Carbonyl of acid)

1540 $cm^{-1}$ (C=O Stretching Carbonyl of keto)

1400 $cm^{-1}$ (aromatic)

Mass spectrum (EIMS): (Figure 38 appendix)

m/e(%relative intensity) = 346(65.3),332(24.7),331(100.(
313(16.1),301(16.9),258(11.2)
257(67.7),231(17.6)

Molecular weight: 346 (EIMS)

H-NMR spectrum (Figure 39 appendix)

The H-NMR spectrum of this compound in pyridine-d5 was summarized as follows:

| proton | chemical shift (ppm) | multiplicity |
|---|---|---|
| 1H(OH) | 13.87 | Singlet |
| 1Ha | 8.58 | Doublet (J=18.9Hz)trar |
| 1Hb | 7.45 | Doublet (J=18.9Hz)trar |
| 2Hc(CH ) | 2.74 | Singlet |
| 1Hd(CH) | 4.46 | Quartet(J=6.5) |
| 15H(5CH ) | 1.35-1.13 | Multiplex |
| H(COOH) | 6.19 | Singlet broad |

From data obtained indicated that this compound possibility will be structure like this:(Figure 40)

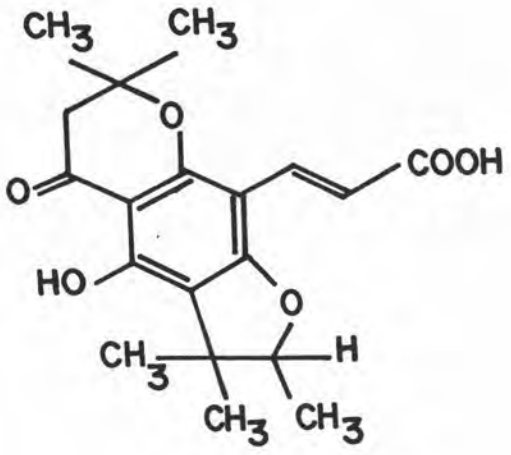


Figure 40 The purpose structure of product from cyclization of clausenidin in alkaline hydrolysis. (36,37)

From IR spectrum showed a strong absorption peak of OH group at 3250 cm indicated that the carboxylic acid was formed. There was also a strong absorption peak at 1620 $cm^{-1}$ which corresponded to C=O stretching of carboxylic acid, at 1540 $cm^{-1}$ which showed of C=O stretching of keto group.

A molecular weight of 346 which corresponded to a formulas $C_{19}H_{22}O_6$. Loss of one methyl group which mostly occurred in clausenidin was showed at m/e 331 (100% intensity).

A proton NMR in pyridine-d5 showed and olefinic coupling of two proton ( 8.58,7.45) J-value equal to 18.9 Hz, is strong evidence to supported that lactone ring was opened. The assignment of methylene proton ($-CH_2-$) at 2.74ppm due to a singlet peak. Methine proton (>CH-) showed chemical shift at 4.46ppm. This proton was

coupling with three protons of methyl group, resulted in a quartet with J-value was 6.5 Hz. It is very difficult to assign chemical shift of each proton in methyl groups, but all five methyl groups will showed in a region of 1.35ppm-1.13ppm. Two protons of hydroxy groups were showed at 13.87 ppm (Phenolic OH which form intramolecular hydrogen bonding) and 6.19ppm (Carboxylic acid which peak was boarded). Therefore these conclusion can be made a suitable structure must be as show above.(38)

The mechanism of all reaction can be purposed for the opening of lactone ring and cyclization to form five member ring as showed on Figure 41 (39)

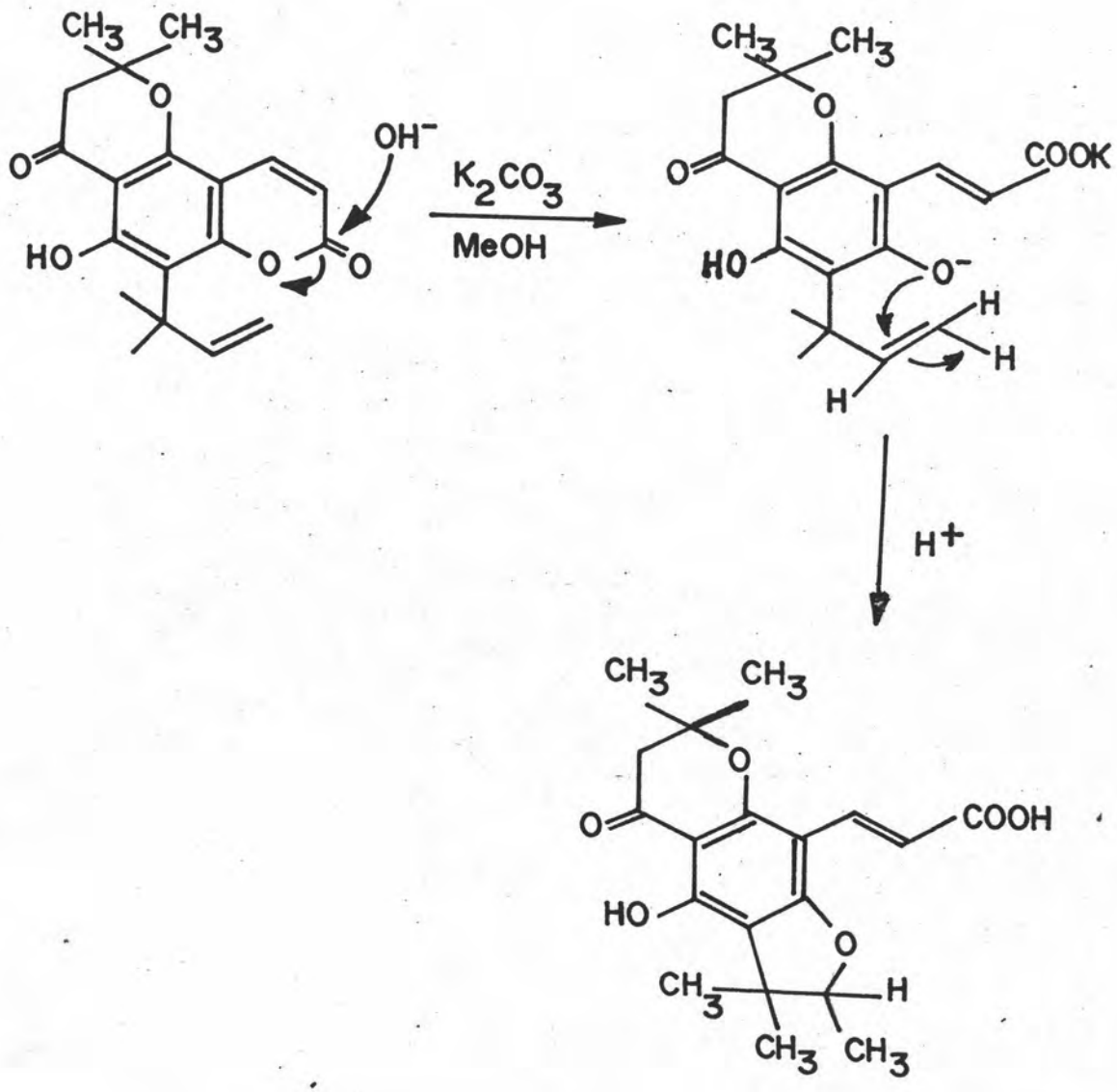


Figure 41 The purpose mechanism for cyclization of clausenidin in alkali hydrolysis.