CHAPTER 3

RESULT AND DISCUSSION

Fissistigma lanuginosum and Polyalthia hookerian were two different spesies that have been studied for their chemical content. Alkaloids were found in both species whereas flavonoids were only isolated from *Fissistigma lanuginosum*.

3.1 Flavonoids Extract of *Fissistigma Lanuginosum*

The stem barks of this species were extracted by petroleum - ether (60 - 80 ^{0}C). Petroleum - ether extracts were evaporated to dryness and tested for flavonoids. The general procedure described in Experimental and yielded 1.82 %. The petroleum - ether extract was introduced to column chromatography with dichloromethane and methanol as solvents. A total of fractions were collected and groups into series of fractions after monitoring with TLC. A major part of this crude mixture consisted of orange needle crystals, which was crystallized as labelled FL1 (22.1 %) , FL2 (10.0 %), FL3 (8.3 %) from dichloromethane and it represents 40.5 % of the total flavonoid content. A second fraction consisted of a yellow flavonoid, labelled as FL4 (10.2 %) and FL5 (9.0 %).

3.1.1 Structural Elucidation of Compound labelled FL1

Useful information of the structure of flavonoids can be gained by comparising of their UV spectra in methanol. Flavonoid, FL1 orange crystals which were crystallized from dichloromethane has been the major flavonoid compound of this species. This compound easily identified by their blue fluorescence in UV light.

Flavonoid FL1 was identified as a chalcone because it turn red in alkali. They are often referred to as the ' anthochlor ' pigment. The UV - Vis spectrum of FL1 shows strong absorption at 240 and 315 nm, characterise the chalcone skeleton. In the presence of NaOH, the absorption peaks were detected at 219 and 286 nm. Bathochromic shift was observed due to the presence of hydroxyl group in the system. Bands are also attributed to transitions appear in the spectra of molecules that have conjugated system.

The IR spectrum showed a strong peak at 1635 cm^{-1} typical of conjugated ketone. A sharp peak at 3533 cm^{-1}, typical of an intermolecular hydrogen bonded hydroxyl group. Its mass spectrum showed a molecular ion peak at m / z 330, thus giving the possibility of the molecular formula to be $C_{18}H_{18}O_6$. Other significant fragmentation observed were m / z 226 and 211 (refer to Scheme 8).

The ^1H NMR spectrum showed three distinct methoxyl peaks at 3.75, 4.05 and 3.60 ppm which were probably positioned at C - 3' , 4' and 6'. Multiplets

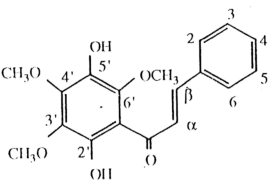
coresponding to five aromatic protons of ring B were revealed at 7.45 - 7.52 ppm and 7.26 - 7.32 ppm which were attributable to H - 2 , 6 and H - 3 , 4 , 5 respectively. H - 2 and H - 6 have higher chemical shift compared to other three aromatic protons due to the dishielding effect.

Two hydroxyl groups were observed at 5.30 ppm and 12.70 ppm . The observed value (12.70 , s , OH - 2 ') were more downfield which may be due to the effect of intermolecular hydrogen bonding between both functional group (OH and CO) . Peak at 5.30 ppm corresponding to OH at C - 5 ' confirmed by literature [34,35] . This proved that ring A was not substituted by any proton.

Furthermore, a doublet peaks at 6.75 ppm and 7.85 ppm which were most probably positioned protons at C - α and C - β[33]. The latter resonated at a lower field due to it being β to the carbonyl group.

The ^{13}C NMR spectrum showed three methoxyl peaks at 61.4, 61.7 and 62.4 ppm which belonged to C - 3' , C - 4' and C - 6'. C - 6' resonates at 62.4 ppm because of being sterically hindered. A very downfield shift was observed at 193.9 ppm attributed to carbonyl group. Furthermore, aromatic carbons are 130.9 ppm (C - 4), 128.9 ppm (C - 3, 5) and 129.4 ppm (C - 2, 6). In the observation, the methylenes shift for C - α and C - β can be observed at 126.6 and 144.3 ppm. The first peak showed slight shielding as compared to the second peak.

Finally, after comparison of spectral data obtained with the literature, one concluded that flavonoid FL1 could be **pedicin** **21** [16, 17].



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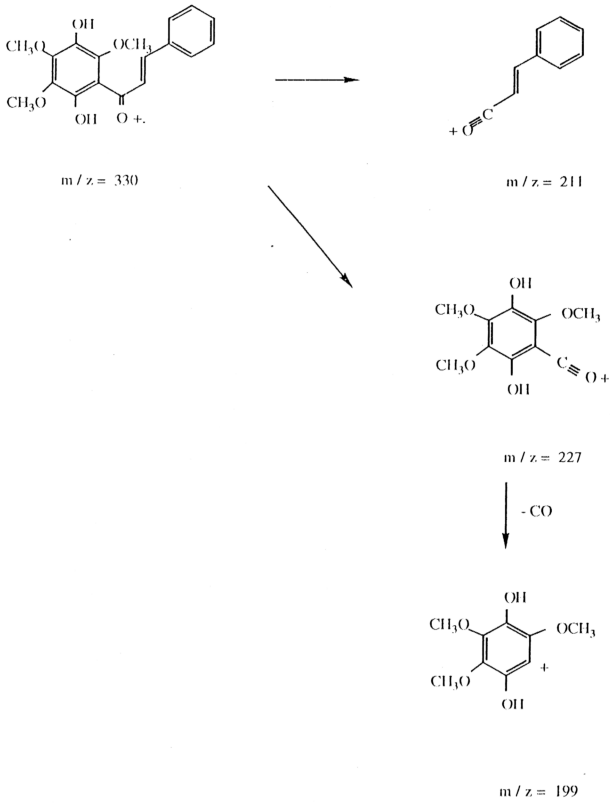
3.1.2 Structural Elucidation of compound labelled FL2

Compound labelled FL2 was identified as one of the flavonoid group, dihydro-chalcone by the UV - Vis spectra in literature [34]. The UV spectra of FL2 in methanol revealed maxima absorption at 281 and 361 nm, typical of a flavonoid. In alkaline medium, it experienced a bathochromic shift suggested that there was a hydroxyl group present.

The IR spectrum showed a strong absorption at the carbonyl group and hydroxyl region. Sharp peaks at 1650 cm^{-1} and 3530 cm^{-1} attributed to carbonyl and hydroxyl groups. In addition, the mass spectra revealed a molecular ion peak at m / z 332 which corresponded to the molecular formula $C_{18}H_{20}O_6$, which has two more



Scheme 8 : The MS Fragmentation Patterns of Flavonoid FL1

(pedicin)

hydrogens in addition to that of the chalcone, pedicin **21**. Moreover, a peak was observed at m / z 227 and a peak at m / z 91 was also visible.

From comparison of the ^{1}H and ^{13}C NMR data of FL1 and FL2 showed that the substitution pattern in ring A and ring B of both compounds was identical. The spectra indicated clearly that the signals of the α and β CH of FL1 were replaced by two methylenes (Table 8) and thus FL2 is dihydropedicin. This was confirmed by conversion of FL1 to FL2 using catalytic hydrogenation.

Finally after comparison of spectral data obtained with the literature, one concluded that flavonoid FL2 could be none other than **2, 5' - dihydroxy - 3', 4', 6' - trimethoxydihydrochalcone 22.**

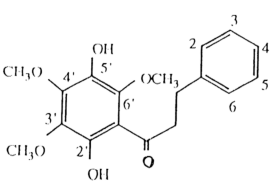


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Table 8 : ^{13}C and ^{1}H MNR Data for compounds FL1 and FL2

Position	FL1		FL2	
	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)
α	126.6	6.75 d (15Hz)	44.7	3.40 t (7Hz)
β	144.3	7.85 d (15Hz)	30.2	3.05 t (7Hz)
CO	193.9		205.4	
1	135.5		141.0	
2,6	129.4		128.1	
3,5	128.9		128.1	
4	130.9		125.7	
1'	111.2		110.0	
2'	152.5		151.2	
3'	136.3		135.7	
4'	147.5		143.0	
5'	134.7		135.5	
6'	143.5		146.5	
3' - OMe	61.4	3.75 s	60.6	3.90 s
4' - OMe	61.7	4.05 s	60.6	4.04 s
6' - OMe	62.4	3.60 s	61.0	3.88 s
2' - OH		12.70 s		12.85 s
5' - OH		5.30 s		5.40 s

3.1.3 Structural Elucidation of Compound labelled FL3

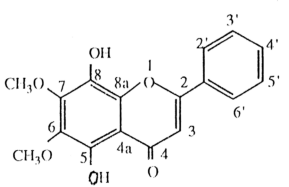
Flavonoid FL3 was obtained as yellow crystals in dichloromethane with melting point 180 - 182 ^{0}C. Compound FL3 was identified as one of the flavonoid groups, furanoflavone by the UV - Vis spectra in literature [34]. It has UV maxima characteristic of a flavone at 284 and 360 nm. From the UV maxima absorption peak in methanol, it indicating a covalently unsaturated group responsible for electronic absorption. In alkaline medium, it experienced a bathochromic shift, suggested that there was a hydroxyl group present.

The IR spectrum showed a strong absorption at a carbonyl and hydroxyl region. A peak at 1658 cm $^{-1}$ was a characteristic of a ketone group while a peak at 1616 cm $^{-1}$ was due to the C = C stretching band. Conjugation with a C = C bond results in the delocalization of the electron in both unsaturated groups. Delocalization of the electron of the C = C group reduces the double - bond character of the C - O bond, causing absorption to occur at a lower wavenumbers. Sharp peak at 3532 cm $^{-1}$ showed intermolecular hydrogen bonding.

The MS spectrum displayed a molecular ion peak at m / z 314 which matched the molecular formula $C_{17}H_{14}O_6$. Other significant fragmentations observed were m / z 299, 240 and 225 (please refer to Scheme 9).

The ^1H NMR spectrum showed multiplets at the region 7.52 - 7.99 ppm corresponding to five protons in the B ring with no substituent. Two peaks for protons methoxyl group at 3.98 and 4.15 ppm substituted at C - 6 and C - 7. Methoxyl group at C - 6 was at higher fields than the signal from methoxyl at C - 8 In this compound, the spectrum showed three singlets (1H) at 12.30, 5.45 and 6.70 ppm assigned to a chelated OH located at C - 5, to a second OH at C - 8 and to H - 3 respectively. The OH group at C - 5 showed HMBC correlations with C - 6 (136.1 ppm) and C - 4a.

In addition from HMBC correlations were observed between one of the OMe and C - 6 and between the second OMe C - 7 (146.4 ppm). The C - 8 with typical upfield shift (129.7 ppm) thus bears the second OH. This was supported by the upfield shift C - 4a (107.3 ppm) indicating the present of an OMe at C - 7 [38]. Thus compound FL3 is **5, 8 - dihydroxy - 6, 7 - dimethoxyflavone 23** which is a previously reported compound [39] and confirmed by comparison with literature (Figure 3 and Table 9).



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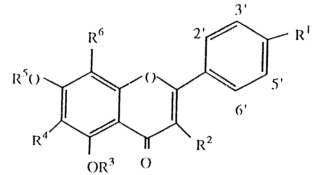


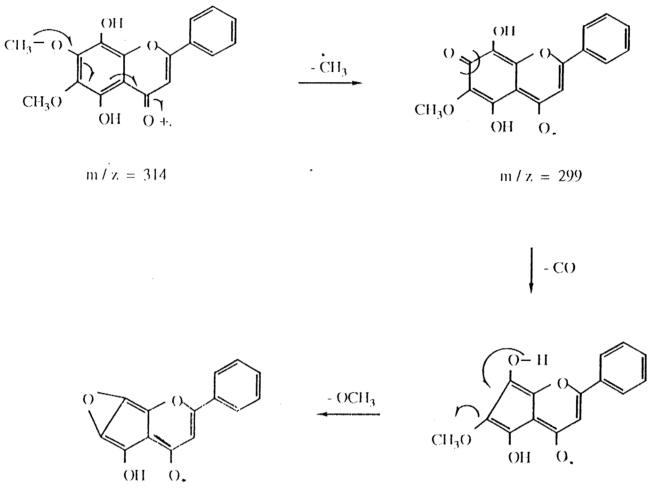
	24	25	26	27	28	29	30	31	32
R^1	H	H	H	H	OMe	H	H	H	H
R^2	H	H	H	H	H	OMe	H	H	OMe
R^3	H	Me	Me	Me	Me	H	Ac	Me	Ac
R^4	OMe	OMe	OMe	H	OMe	OH	OMe	OMe	OAc
R^5	Me	Me	Me	Me	H	Me	Me	Me	Me
R^6	OH	OH	OMe	H	OMe	OMe	H	OAc	H

24 5, 8 - dihydroxy - 6, 7 - dimethoxyflavone
25 8 - hydroxy - 5, 6, 7 - trimethoxyflavone
26 5, 6, 7, 8 - tetramethoxyflavone
27 5, 7 - dimethoxyflavone
28 7 - hydroxy - 4', 5, 6, 8 - tetramethoxyflavone
29 5, 6 - dihydroxy - 3, 7 - dimethoxyflavone
30 3 - acetate - 6, 7 - dimethoxyflavone
31 8 - acetate - 5, 6, 7 - trimethoxyflavone
32 5, 6 - diacetate - 3, 7 - dimethoxyflavone

Figure 3 Substituted Flavones

Table 9 : ^1H NMR Data (270 MHz , $CDCl_3$, TMS as internal Standard)

	3 - H	6 - H	8 - H	2',6' - H	3',4',5' - H	OMe	OH
24	s 6.69	-	-	m 7.94	m 7.59	s 4.05 s 4.03	s 12.71
25	s 6.70	-	-	m 7.93	m 7.52	s 4.07 s 4.03 s 3.96	-
26	s 6.70	-	-	m 7.94	m 7.54	s 4.12 s 4.04 s 3.95 s 3.94	-
27	s 6.70	d 6.39	d 6.59	m 7.88	m 7.60	s 3.97 s 3.93	-
28	s 6.73	-	-	d 7.88	d 7.03	s 4.07 s 4.04 s 3.95 s 3.79	-
29	-	-	s 6.45	m 8.11	m 7.55	s 4.01 s 3.88	s 12.38
30	s 6.66	-	-	m 7.90	m 7.54	s 4.06 s 3.88	-
31	s 6.73	-	-	m 7.93	m 7.54	s 4.06 s 3.96 s 3.94	-
32	-	-	s 6.80	m 8.10	m 7.53	s 4.00 s 3.86	-



Scheme 9 : The MS Fragmentation Patterns of Flavonoid FL3

(5,8 - dihydroxy - 6,7 - dimethoxyflavone)

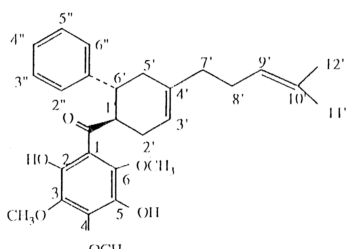
3.1.4 Structural Elucidation of Compound Labelled FL4

This new compound labelled FL4 was afforded as a yellow gum. FL4 was recognized as chalcone from detail spectroscopic criteria[34] and compare with literature[40]. They can also be considered to be biogenetically derived from a Diels-Alder type cyclisation between a chalcone and a monoterpenic diene. The chalcone in the present case is **21** (FL1). The diene is β- myrcene instead of β- ocimene for schefflerin and isoschefflerin[40]. The detail spectrums were described below.

In the ultra - violet region, it absorbed at 288 and 367 nm. The infra - red spectrum showed a peak about 3535 cm^{-1} typical of an intermolecular hydrogen bonded hydroxyl group. Alkali caused a bathochromic shifts in its UV spectrum indicating its phenolic nature. In addition, a peak was observed at 1629 cm^{-1} which is due to carbonyl group. The mass spectrum revealed a M^+ peak at m / z 466 (calc. 466.2355) which gave possible a molecular formula of $C_{28}H_{34}O_6$.

The ^1H and ^{13}C NMR spectra revealed signals of a trimethoxy - dihydroxyl - benzoyl moiety similar to that of **21** (FL1). The HMBC experiment (Figure 4) indicated the same substitution pattern. As for the second moiety, the 1D spectra showed clearly the presence of a γγ - dimethylallyl group and a phenyl ring (Table 10).

The COSY and HMQC experiments showed two spin system as indicated on figure 3. Analysis of the HMBC spectrum confirmed the assignment and the connectively of all the protons and carbons as depicted in FIA. The correlations H - 3' / C - 5', H - 2' / C - 4' and H - 5' / C - 4' proved the closure of the cyclohexene ring. The cross peaks H - 1' / CO and H - 6' / C - 1" and C - 2", 6" supported the positioning of the trimethoxy - dihydroxy - benzoyl moiety and the phenyl ring at C - 1' and C - 6' respectively. Finally, the position of the aliphatic chain at C - 4' was deduced unambiguously from the correlations H - 7' / C - 4', H - 7' / C - 5' and H - 3' / C - 7'. As for the relative stereochemistry, the value of the coupling (J = 10 Hz) between H - 1' (4.30 ppm) and H - 6' (3.25 ppm), confirmed by decoupling experiments, indicated their trans diaxial relationship. Comparison of the observed spectral data confirmed that it is a new 'condensed' chalcone named as **fissistin 33**.



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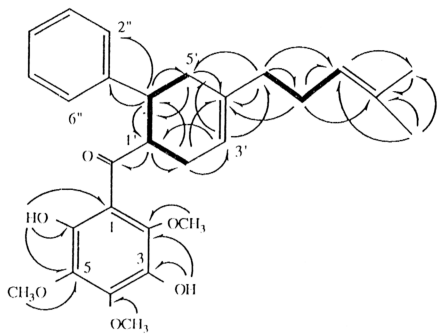
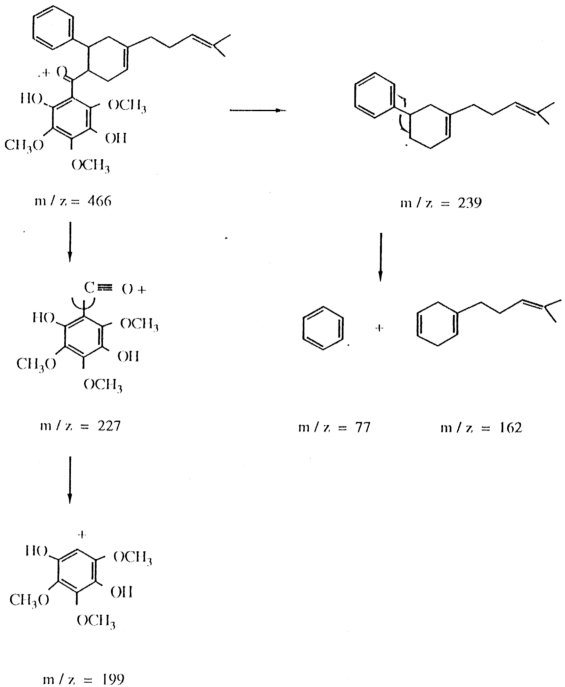


Figure 4 : COSY (▬) and HMBC (——) Correlations for Compound FL4



Scheme 10 : The MS Fragmentation Patterns of Flavonoid FL4

(fissistin)

3.1.5 Structural Elucidation of Compound Labelled FL5

Compound FL5 were purified from dichloromethane as a yellow gum and recognized as chalcone from spectroscopic criteria [34].

Comparison of the spectral data (UV, IR, MS, 1D NMR Table 10) for **fissistin 33** and **isofissistin 34** suggested that FL5 is an isomer of FL4. Since the splitting pattern of H - 1' (4.28 ppm) and H - 6' (3.18 ppm) (Table 10) were respectively similar to that of H - 6' and H - 1' of FL4, hence the two proton were trans diaxial. Therefore, FL5 was a positional isomer of FL4 which could be easily explained by the postulated biogenetical origin of the two compounds through a Diels Alder like coupling. The coupling is apparently not enantioselective, since **33** and **34** are racemates. This was also observed for the schefflerins and other related ' condensed' chalcones[40].

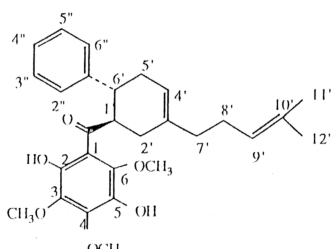


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Table 10 : ^{1}H NMR Data for Compounds FL4 and FL5 ($CDCl_3$)

Position	FL4 ^{1}H ppm (J Hz)	FL5 ^{1}H ppm (J Hz)
1'	4.28 ddd (10, 10, 5)	4.30 ddd (10, 8, 8)
2'	2.50 m	2.36 m
3'	5.62 br s	—
4'	-	5.56 brs
5'	2.38 d (8)	2.36 m
6'	3.25 ddd (10, 8, 8)	3.18 ddd (10, 10, 5)
7'	2.12 m	2.08 m
8'	2.20 m	2.14 m
9'	5.20 t (7)	5.18 t (7)
10'	-	-
11'	1.80 s	1.73 s
12'	1.75 s	1.63 s
1"	-	-
2", 3", 4", 5",6"	7.30 - 7.70 m	7.08 - 7.22 m
3 - OMe	3.83 s	3.73 s
4 - OMe	4.03 s	3.93 s
6 - OMe	4.17 s	4.08 s
2 - OH	11.85 s	11.90 s
5 - OH	5.20 s	5.28 s

3.2 Alkaloids extract of *Fissistigma Lanuginosum*

Stem barks of this species were first defatted with petroleum - ether (60 - 80°C) and followed by dichloromethane. A crude bases (0.80 %) was obtained from dichloromethane extracts by general procedure. Additionally, the crude extracts were chromatographed over silica gel. The column chromatography was successively eluted with dichloromethane and methanol as solvents. The fractions collected were grouped into a series of fraction with the aid of TLC. First part of this crude mixture consisted of a yellow compound, labelled FL6 (0.19 %), which crystallized out from dichloromethane as a fine yellow needles, with melting point 112 - 113° C.

Further separation of the crude alkaloid resulted in the isolation of large quantities of violet amorphus solid, labelled FL7, represents 1.87 % of the total crude extract. Third and fourth fractions also consisted of a violet coloured crystals, labelled as FL8 (1.14 %) and FL9 (0.38 %). Two oxoaporphine alkaloids were isolated from crude extracts, named as liriodenine (0.71 %) and lanuginosine (0.36 %). They were easily recognized from the characteristic red colour observed in acidic medium.

3.2.1 Structure Elucidation of Compound Labelled FL6

It was isolated as a yellow coloured solid. The mass spectrum revealed a molecular ion peak which is also the base peak at m / z 328, hence gave possible the molecular formula of $C_{18}H_{16}O_6$. Peaks were also observed at m / z 269, 257 and 209. The UV spectrum gave maxima at 298 nm and 402 nm, indicating the existence of a

highly unsaturated chromophore and typical of the chalcone skeleton. In addition, the IR spectrum showed strong peaks typical of a conjugated ketone at 1681 cm^{-1}, 1637 cm^{-1} and 1600 cm^{-1}.

The ^{1}H NMR spectrum, showed three distinct methoxyl peaks at 3.95 ppm, 4.00 ppm and 4.08 ppm which were most probably positioned at C - 3', C - 4' and C - 6' respectively. Multiplets corresponding to five aromatic protons of ring B were revealed at 7.38 - 7.42 ppm and 7.54 - 7.60 ppm which were attributable to H - 3', 4', 5' and H - 2', 6'. Furthermore, a doublet peaks at 6.95 ppm and 7.52 ppm which were most probably positioned protons at C - α and C - β.

For further structural study ^{13}C NMR of FL6 was performed (see Table 11) and based on comparison, we suggested that the carbonyl group at positioned C - 2' and C - 5' revealed peaks at 179.4 ppm and 182.4 ppm. All the data obtained suggested that compound FL6 was quinone - chalcone named as **3', 4', 6' - trimethoxy - 2', 5' - quino - chalcone 35**. Obviously, it was proceeded by the intermediacy of the quinone corresponding to **pedicin 21**, the known quinone **35** prepared by treatment of **21** with Ag_2O[16,41].

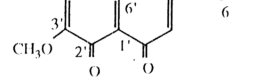

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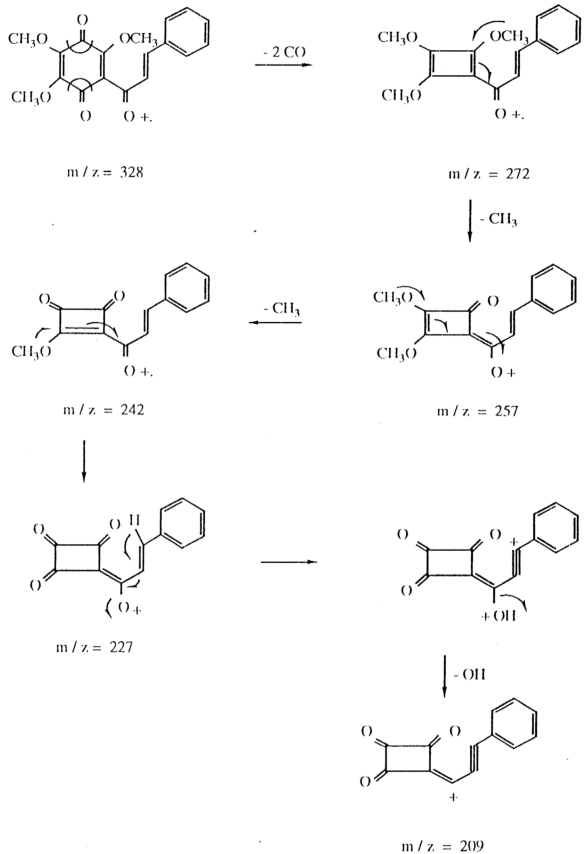
Table 11 : ^{13}C NMR Data for Compound FL6

Position	^{13}C (ppm)
CO	191.3
1	131.4
2,6	129.2
3,5	128.9
4	128.1
1'	121.1
2'	179.2
3'	144.6
4'	143.2
5'	182.4
6'	153.4
α	128.1
β	147.0
3' - OMe	61.0
4' - OMe	61.0
6' - OMe	61.5



Scheme 11 : The MS Fragmentation Patterns of Flavonoid FL6
(3',4',6' - trimethoxy - 2',5' - quinochalcone)

3.2.2 Structural Elucidation of Compound Labelled FL7

This compound was afforded as violet amorphus solid from dichloromethane. A single spot on TLC of this compound were observed under UV light and spray with Dragendroff reagent. The results of the screening are blue flourescence under UV light and a negative spot for alkaloid. Pure compound were then send for spectroscopy.

From the UV spectrum, it showed UV maxima at 318 and 511 nm. The IR revealed bands at 3575 cm^{-1} indicating the presence of the amine group. Other peaks observed were at 1670 and 1570 cm^{-1}. The former was caused by the carbonyl group while the latter was due to the C = C stretching vibrations of the aromatic protons. The mass spectra displayed a molecular ion peak at m / z 434 which corresponded to the molecular formula $C_{26}H_{30}N_2O_4$.

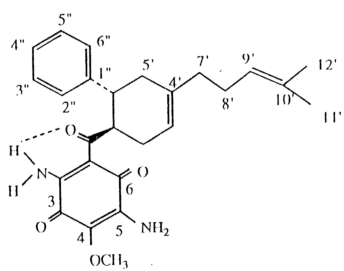
The ^{1}H and ^{13}C NMR spectra showed clearly the presence of a $\gamma\gamma'$-dimethylally group and a phenyl ring same as compound labelled **33** (FL4). The cosy and HMQC experiments showed two spin system as indicated on figure 3 as compound **33**. Analysis of the HMQC spectrum confirmed the assignment and the connectivity of all the protons and carbons (Table 12). The correlations H - 3' / C - 5', H - 2' / C - 4' and H - 5' / C - 4' proved the clossure of the cyclohexene ring. The cross peaks H - 1' / CO and H - 6' / C - 1" and C - 2", 6" supported the positioning of the phenyl ring at C - 1" and C - 6" respectively. Finally, the position of the aliphatic chain at C - 4' was deduced unambigously from the correlations H - 7' / C - 4', H - 7' / C - 5' and H - 3' / C - 7'.

Table 12 : ^{13}C and ^{1}H NMR Data for Compounds FL4 and FL7 ($CDCl_3$)

Position	FL4 ^{13}C (ppm)	FL4 ^{1}H ppm (JHz)	FL7 ^{13}C (ppm)	FL7 ^{1}H ppm (JHz)
CO	210.2		204.6	
1	111.7		104.7	
2	150.5		169.7	
3	135.9		131.7	
4	142.7		156.1	
5	133.7		177.2	
6	146.3		143.0	
1'	51.1	4.28ddd(10,10,5)	49.0	4.28ddd(10,10,5)
2'	30.6	2.50 m	30.6	2.45m(17) 2.12m
3'	119.2	5.62 br s	119.5	5.55 d (1.5)
4'	137.5		137.7	
5'	37.8	2.38 d (8)	38.2	2.02
6'	44.0	3.25 ddd(10,8,8)	43.3	3.20 ddd(10,8,8)
7'	37.4	2.12 m	37.6	2.02 m
8'	26.5	2.20 m	26.6	2.12 m
9'	124.2	5.20 t (7)	124.4	5.25 t (7)
10'	131.7		132.7	
11'	25.8	1.80 s	25.8	1.62 s
12'	17.8	1.75 s	17.8	1.70 s
1"	144.4		145.9	
2", 6"	128.3	7.30 - 7.70 m	128.3	7.08 - 7.22 m
3", 5"	127.6	,,	127.5	,,
4"	126.2	,,	126.0	,,
3 - OMe	60.9	3.83 s		
4 - OMe	61.8	4.03 s	59.7	3.90 s
6 - OMe	61.4	4.17 s		
2 - OH		11.85 s		
5 - OH		5.20 s		
3 - NH_2				11.55 s
6 - NH_2				7.55 s

From ^{13}C NMR (Table 12), only one methoxyl group observed at 59.7 ppm. This data, coupled with the presence of two downfield CO (169.7 ppm and 177.2 ppm). Formation of aminoquinones from methoxyquinones by substitution of one methoxy group to the CO with NH_3 is well known[42]. Furthermore, the ^1H NMR spectrum recorded in $CDCl_3$ showed two singlets at 11.55 ppm and 7.55 ppm assigned to NH. The $^1H-^{15}N$ HMQC spectrum revealed that the two protons correlated with the same nitrogen. The downfield shift of the proton at 11.55 ppm was most probably due to the chelation between the 6 - NH_2 and the non quininic CO group.

Finally after comparison of spectral data obtained with the literature, one concluded that compound FL7 could be as in **36**. Obviously **36** can be prepared by treatment of **33** (FL4) with ammonia. Furthermore, this **36** product was found artefact. It was not obtained unless ammonia was used in the alkaloid extraction and could be prepared by treating with NH_4OH.



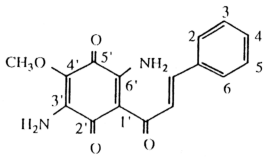
36

3.2.3 Structural Elucidation of Compound Labelled FL8

From an alkaloidal extract of the plant, compound labelled FL8 was afforded in its violet coloured crystals, crystallized from dichloromethane with melting point 226 - 228° C.

Its UV - Vis spectrum showed maxima at 334 and 508 nm. In the IR spectrum, bands at 3375, 1670 (w) and 1543 cm^{-1} were observed. The MS spectrum, showed a molecular ion peak at m / z 298 corresponding to the molecular formula $C_{16}H_{14}N_2O_4$.

The ^1H and ^{13}C NMR exhibit the characteristic signal of the α and β CH and the CO of a chalcone similar to those of **21** and those unsubstituted B ring chalcone (Table 13). The remaining part of the molecule corresponded to the formula $C_7H_{14}N_2O_3$ and contained a methoxy group (59.4 ppm). These data, coupled with the presence of two downfield CO (169.9 ppm and 179.2 ppm) in the ^{13}C NMR spectrum, suggested the diaminoquinone structure is illustrated in **37**. Formation of aminoquinones from methoxyquinones by substitution of one or two methoxy groups to the CO with NH_2 is well known[42]. Finally, compound labelled FL8 was illustrated as **37** and obviously that **37** was also obtained from **21** (FL1) by treatment with NH_4OH supported further the proposed structure. It was also found that **37** is a artefact.



37

3.2.4 Structure Elucidation of Compound Labelled FL9

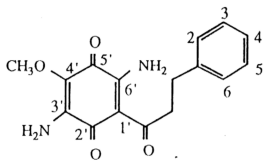
This compound crystallized as violet amorphus solid with melting point 190 - 192 ^{0}C. The mixture of FL8 and FL9 were isolated by coloum chromatography of the alkaloid extract.

Compound FL9 showed UV maxima at 328 and 512 nm. The IR revealed bands at 3375, 1670 (w), 1612 and 1562 cm^{-1}. The MS displayed a molecular ion peak at m / z 300 corresponding to the molecular formula $C_{16}H_{16}N_2O_4$. The NMR spectra were reminiscent to that of **37** (FL8) for the signals at position and which implied a saturated chalcone (Table 13).

For further structural study the ^{1}H and ^{13}C NMR (Table 13) of FL9 was performed as **38** and peaks assignment were based on comparison with **37**. The **38** product was also found artefact and could be prepared by treating **22** (FL2) with NH_4OH.

Table 13 : ^{13}C and ^{1}H NMR Data for Compounds FL8 and FL9

Position	FL8 ^{13}C ppm	FL8 ^{1}H ppm (JHz)	FL9 ^{13}C ppm	FL9 ^{1}H ppm (JHz)
CO	189.5		200.1	
α	129.2	8.10 d (16)	47.7	3.72 t (7)
β	141.2	8.63 d (16)	31.4	3.28 t (7)
1	136.9		145.9	
2,6	129.2	7.70 - 7.77 m	128.9	7.53 - 7.58 m
3,5	129.7	7.20 - 7.35 m	129.2	7.42 - 7.50 m
4	130.5	,,	126.3	,,
1'	105.8		104.8	
2'	169.9		169.8	
3'	133.5		136.5	
4'	159.1		156.5	
5'	179.2		178.6	
6'	146.3		145.8	
4' - OMe	59.7	3.90 s	59.4	4.01 s



38

3.2.5 Structural Elucidation of Compound Labelled FL10

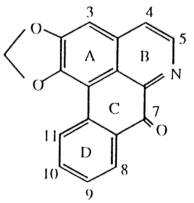
Alkaloid FL10 was isolated as yellow crystals with melting point 268 - 270 ^{0}C. Oxoaphorphinic nature was deduced base on its intense yellow colour and deep red colouration it produced in acid medium (e.g. trifluoroacetic acid). This was also supported by UV - Vis and IR spectroscopic data. The former showed absorption bands at 266, 314 and 412 nm, indicating a highly unsaturated chromophoric system which are typical of an oxoaporphine. A highly conjugated carbonyl functional group was also observed at 1664 cm^{-1} in its IR spectrum.

The mass spectrum of FL10 exhibited a molecular ion peak at m / z 275, suggesting a possible molecular formula $C_{17}H_9O_3N$. Fragmentation peak at m / z 247 $(M - CO)^+$ due to fragmentations involving the ketone group (refer to Scheme 12).

The ^{1}H NMR spectrum of FL10 exhibited the characteristic AB quartet significant of H - 4 and H - 5 at 7.74 and 8.87 ppm respectively. The aromatic region

also showed a series of multiplets integrated thus : two protons between 7.60 - 7.76 ppm and two other protons between 8.54 - 8.62 ppm, indicating on unsubstituted ring D. Proton at C - 3 was appeared at 7.14 ppm as a singlet. A singlet peak at 6.35 ppm corresponds to two protons indicate that a methylenedioxy group was present in ring A located at C - 1, 2.

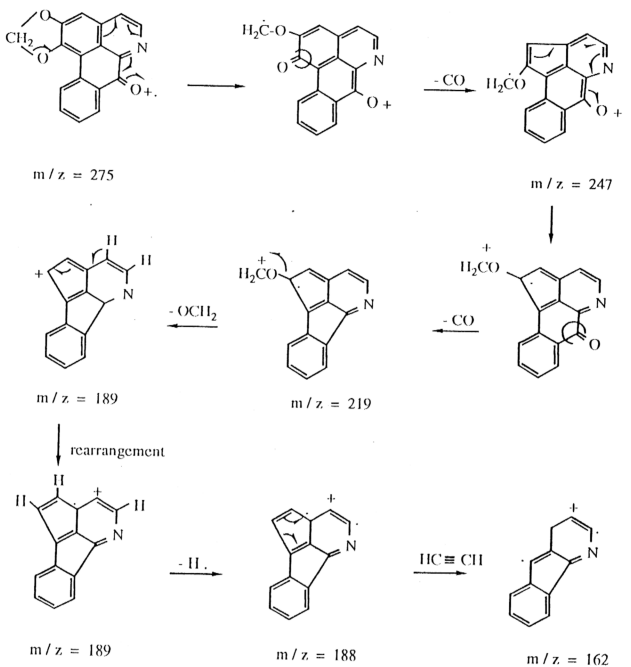
By comparison the spectroscopic data obtained from FL10 and liriodenine[43,44], confirmed that FL10 was **liriodenine 39**.



39

3.2.6 Structural Elucidation of Compound Labelled FL11

Alkaloid FL11 was obtained as a yellow needle crystals with melting point 303 - 306 °C. The physical characteristic as well as the UV - Vis and IR spectral data of FL11 also pointed to an oxoaphorphinic skeleton. The UV - Vis maxima were observed



Scheme 12 : The MS Fragmentation Patterns of Alkaloid FL10

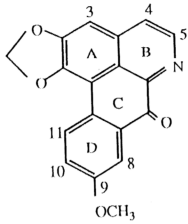
(liriodenine)

at 217, 272, 424 nm indicating the highly conjugated oxoaphorphine system. On acidification, the spectrum is shifted to longer wavelenght. The IR spectrum showed a conjugated ketone peak at 1665 cm^{-1}.

In addition, the Mass spectrum revealed a molecular ion peak at m / z 305, which gave possible molecular formula of $C_{18}H_{11}O_4N$. Other significant peak at m / z 275, ascribed to $M^+ - CH_2O$ fragment. All illustration of the suggested fragmentation patterns is illustrated in Scheme 13.

Furthermore, the ^1H NMR spectrum clearly showed a methylenedioxy group on the planar oxoaphorphine ring system as a two protons singlet at 6.34 ppm, an aromatic methoxyl group as a three protons singlet at 3.99 ppm and six aromatic protons as signals in the 7.14 - 8.88 ppm region. A careful analysis of the aromatic region of the spectrum revealed signals for two highly deshielded protons centered at 8.88 ppm (1H, d, J = 5.8 Hz) and 8.56 ppm (1H, d, J = 9 Hz) located at C -5 and C - 11 respectively. Morever, appearance of a doublet C - 4 proton at 7.75 ppm, a doublet C - 8 proton at 8.02 ppm and a quartet (dd) C - 10 proton at 7.24 ppm necessitate the allocation of the methoxyl group at C - 9. The proton C - 3 signal at 7.14 ppm as a singlet in the same region as those in **39** indicated that C - 3 in ring A is unsubstituted.

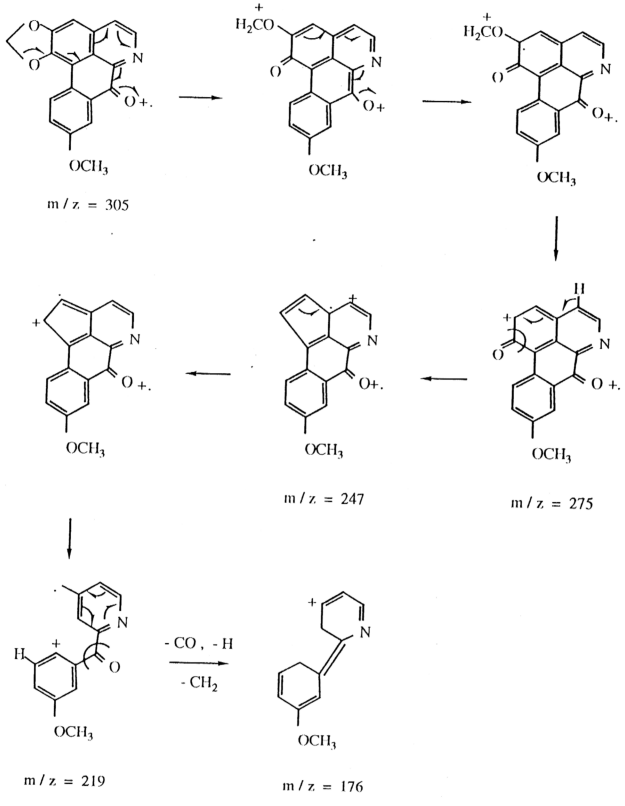
Finally, comparison of the observed data and the literature values [43, 45] of a known compound left no doubt that alkaloid FL11 was **lanuginosine 40**.



40

3.3 Alkaloids extract of *Polyalthia hookerian*

Bark of this species was defatted with petroleum - ether (60 - 80° C) and extracted with dichloromethane for their alkaloid contents using Soxhlet extractor and yield 0.39 % of crude bases. The crude alkaloids were subjected to chromatotron or column chromatography over silica gel using dichloromethane and methanol. The fractions collected were groups into a series of fraction after monitoring with TLC. Alkaloid PH1 was obtained as yellowish amorphus solid crystallized in dichloromethane represents 0.22 % of the total alkaloids content. Further isolation of yellow needle crystals labelled PH2 and this represents 0.54 % of the total alkaloid content. Third compound isolated as deep yellow coloured crystals labelled PH3 and this represents 0.41 %. Other alkaloids were also present but have not been successfully isolated.



Scheme 13 : The MS Fragmentation Pattern of Compound Labelled FL11 (lanuginosine)

3.3.1 Structural Elucidation of the Compound Labelled PH1

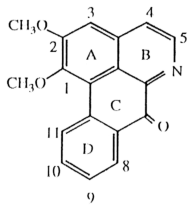
PH1 was isolated as a yellow amorphus solid with melting point 206 - 208° C. PH1 was easily recognized as oxoaporphine from the characteristic red colour observed in acidic medium and bathochromic shift of the UV spectrum due to existence of the conjugated ketone. Additionally, the infra - red spectra revealed an intense peak at 1667 cm^{-1} which indicated the presence of a highly conjugated carbonyl function.

In the Ultraviolet spectrum, maxima were observed at 237, 274, 305 and 402 nm. The Mass spectrum of PH1, exhibited the stable molecular ion peak at m / z 291 as the base peak which corresponded to the molecular formula $C_{19}H_{13}O_5N$. The fragmentation peaks consistent with the losses of a methoxyl substituent in the system.

The ^1H NMR spectrum, showed two distinct methoxyl peaks at 4.03 and 4.11 ppm which were most probably positioned at C - 1 and C - 2. No methylenedioxy peak was observed. Multiplets corresponding to two aromatic protons of ring D were revealed at 7.58 - 7.81 ppm which were attributable to H - 9 and H - 10. A very downfield signal which appeared at 9.18 ppm was assigned to H - 11. This value is typical of this particular proton when C - 1 is substituted with a methoxy group due to extensive hydrogen bonding between the proton and the oxygen of the methoxyl group. Additionally, the spectrum showed a quartet (dd) at 8.61 ppm which

belonged to H - 8 that experienced a shielding effect from the neighbouring C - 7 carbonyl.

Base on the observed data, PH1 was deduced as **lysicamine 41**. The spectral data were full agreement with the reported value [46, 47, 48]. Finally, having knowing the structure of alkaloid PH1, it is possible to suggest the fragmentation patterns of this alkaloid as illustrated in Scheme 14 [50, 51].



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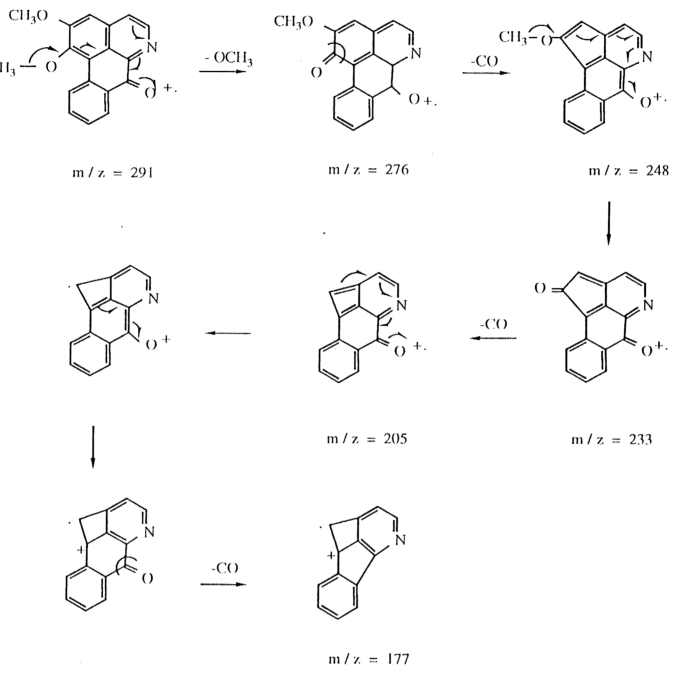
3.3.2 Structural Elucidation of Compound Labelled PH2

Alkaloid PH2 was isolated as yellow needle crystals. In dichloromethane, it revealed an intense yellow - green colour and in acid medium it gave a deep red colour solution. Hence, from these observations an oxoaporphine nature was deduced for this alkaloid.



Scheme 14 : The MS Fragmentation of Compound Labelled PH1

(lysicamine)

The UV spectrum exhibited maxima at 264, 270, 316 and 409 nm which were typical of an oxoaporphine. The IR spectrum showed a strong carbonyl absorption at 1665 cm^{-1}. In addition, its Mass spectrum showed a molecular ion peak which was also the base peak at m / z 275. Other peaks were also observed which were identical with that of alkaloid **39** (FL10) (refer to Scheme 12).

Comparison of its spectral data (IR, ^{1}H and ^{13}C NMR, UV) with that of alkaloid **39** and **liriodenine**[43, 44, 52, 53] proved that they were identical.

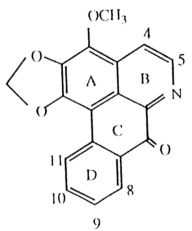
3.3.3 Structural Elucidation of Compound Labelled PH3

The alkaloid PH3 was obtained as a deep yellow crystals with melting point 280 - 282 ^{0}C. The UV spectrum gave maxima at 248, 280, 315 and 435 nm indicating the existence of a highly unsaturated chromophore. The IR spectrum showed a strong peak typical of a conjugated ketone at 1664 cm^{-1}. In addition, the Mass spectrum revealed a molecular ion peak which is also the base peak at m / z 305, hence gave possible the molecular formula of $C_{18}H_{11}O_4N$. Peaks were also observed at m / z 290 and 262 which suggested the loss of a methyl and a carbonyl (Scheme 15).

The ^{1}H NMR of PH3 showed a strong methoxyl singlet at 4.26 ppm and another singlet at 6.26 ppm, indicative of the methylenedioxy of an oxoaporphine. The four aromatic protons resonated at 7.27 - 7.76 ppm (H - 9, 10) and 8.39 - 8.51 ppm (H - 8,11). The H - 11 has the highest chemical shift compared to the other protons

due to the deshielding effect of the facing ring A and hydrogen bonding with the methylenedioxy group. The absence of a singlet at the aromatic region suggested that C - 3 was substituted with a methoxyl. In addition two sets of doublets with coupling constants of 5.4 Hz were observed at 8.08 ppm and 8.85 ppm which were attributable to H - 5 and H - 4.

Hence, the molecule was hypothesized to be **atherospermidine 42** due to direct comparison with authentic sample for TLC, UV, IR and NMR together with literature values to confirmed the hypothesis [54, 55].



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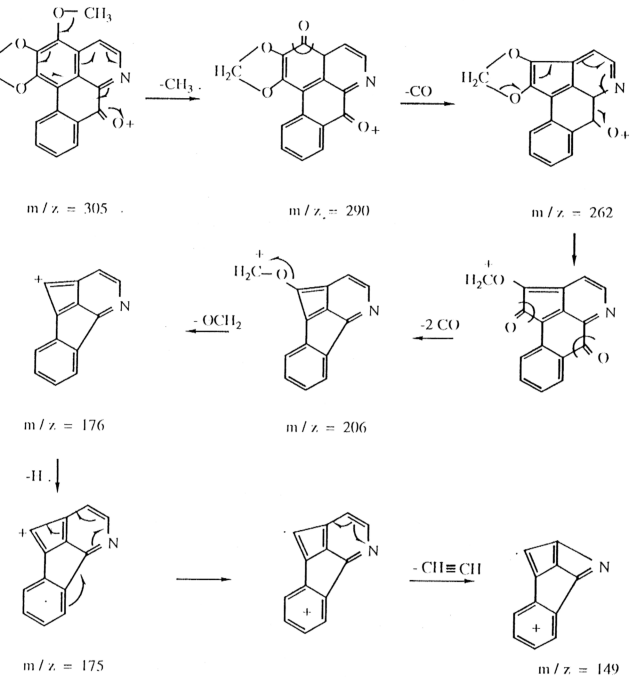


Scheme 15 : The MS Fragmentation of Compound Labelled PH3

(atherospermidine)

3.4 Conclusions

Two species, *Fissistigma lanuginosum* and *Polyalthia hookerian*, members of family Annonaceae have been fully studied for their chemical contents. Interestingly, some known and new flavonoids have been isolated only from *Fissistigma lanuginosum* and the simplest type of isoquinoline alkaloids in both species were also found.

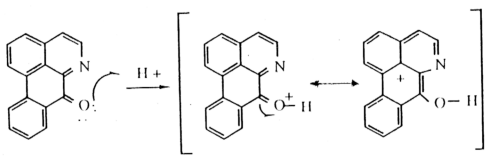
Fissistigma lanuginosum produces mostly flavonoids, namely pedicin **21,** 2, 5' - dihydroxy - 3', 4', 6' - trimethoxychalcone **22**, 5, 8 - dihydroxy - 6, 7 - dimethoxyflavone **23**, fissistin **33**, isofissistin **34**, 3', 4', 6' - trimethoxy - 2', 5' - quinochalcone **35** and alkaloids named as liriodenine **39** and lanuginosine **40**.

Three alkaloids, have been isolated from *Polyalthia hookerian*, namely lysicamine **41,** liriodenine and atherospermidine **42.**

The flavonoids found in *Fissistigma lanuginosum* can be confirmed by comparison with spectroscopic criteria especially by UV - Vis and NMR spectrum. Two new 'condensed' chalcones, fissistin **33** and isofissistin **34**, which showed cytotoxicity against KB cells were confirmed together with the inactive dihydropedicin **22** and 5, 8 - dihydroxy - 6, 7 - dimethoxyflavone **23**. In addition, the aminoquinone, **36**, **37** and **38** were isolated from the alkaloids extract. These compounds were artefacts, obtained by treatment of **33**, **21** and **22** with NH_4OH respectively.

The structure of the new compounds were elucidated by spectral methods, especially 2D NMR.

In this study of alkaloid compounds, generally compounds have been isolated and their structures have been fully elucidated, was in an oxoaporphine group. The oxoaporphine nature was deduced based on its deep red colouration produced in acid medium. Figure below shows the resonance structures of protonated oxoaporphine.



This was also supported by the data obtained from UV - Vis spectrum, indicating a highly unsaturated chromophoric systems.

From this study of compounds, one can observe and learn the variety of chemical nature in plants. Moreover, the discovery of cytotoxic compound is helpful in our strive to overcome the health problems encountered today such as cancer.