CHAPTER IV

RESULTS AND DISCUSSION

The dried roots (1.3 kg) of *N. thorelii* Lec. were extracted with 95 % Ethanol. The EtOH extract was partitioned between water and chloroform. The dried chloroform fraction was separated between petroleum ether and methanol. The petroleum ether extract was then separated by chromatographic technique to afford five pure compounds, namely plumbagin (**18**), droserone (**19**), isoshinanolone (**102**), 2-methylnaphthazarin (**103**), and octadecyl caffeate ester (**104**). The structural characterizations of these compounds were based on data from the UV, IR, NMR and Mass spertra. The structures of these compounds were further confirmed by comparison of their physical properties with the data reported in the literature. The large amount of plumbagin (**18**) obtained in this study provided a good opportunity to study the chemistry and spectral properties of this group of compounds. Hence, several naphthoquinone-related compounds (**105-112**) were prepared using plumbagin as the first starting material.

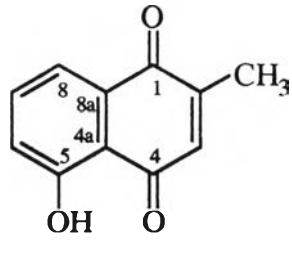
1. Structure characterization of isolated compounds

1.1 Identification of compound 18

Compound **18** was obtained as orange needles from fraction C-5 (Table 3). The EIMS of compound **18** (Figure 12) revealed its molecular ion peak at

m/z 188. The mass fragmentation pattern was shown in scheme 6 (Stensen and Jensen, 1995). The IR spectrum (Figure 13) indicated C=O stretching (1664 cm^{-1}), and olefinic combination bands of C-H bending (1364, 1335, 1231-1259 cm^{-1}). The UV absorption spectrum (Figure 14) showed maxima absorptions at λ_{max} 209, 264 and 418 nm (log ε 4.5, 4.06 and 3.58, respectively), suggesting a quinonoid and a benzenoid ring absorption.

This compound was assigned as a known 1,4-naphthoquinone, plumbagin (**18**). The ^{1}H and ^{13}C assignments of this compound have been reported by Bhattacharryya and De Carvalho, 1986; Sankaram, Narayana Reddy and Marthandamurthi, 1986. However, a controversy has arisen since in a recent study, different signal assignments were reported for C-2, C-6, C-7, C-8, C-4a and C-8a (Yue *et al*, 1994). To clarify this issue, a combination of 1- and 2-D NMR experiments, including DEPT, HMQC and HMBC (Figures 15-26) were performed. Results from careful analysis of the HMQC and HMBC spectra supported the earlier assignments by Bhattacharryya and De Carvalho, 1986; Sankaram *et al*, 1986. The ^{1}H and ^{13}C assignments are summarized in Table 4.

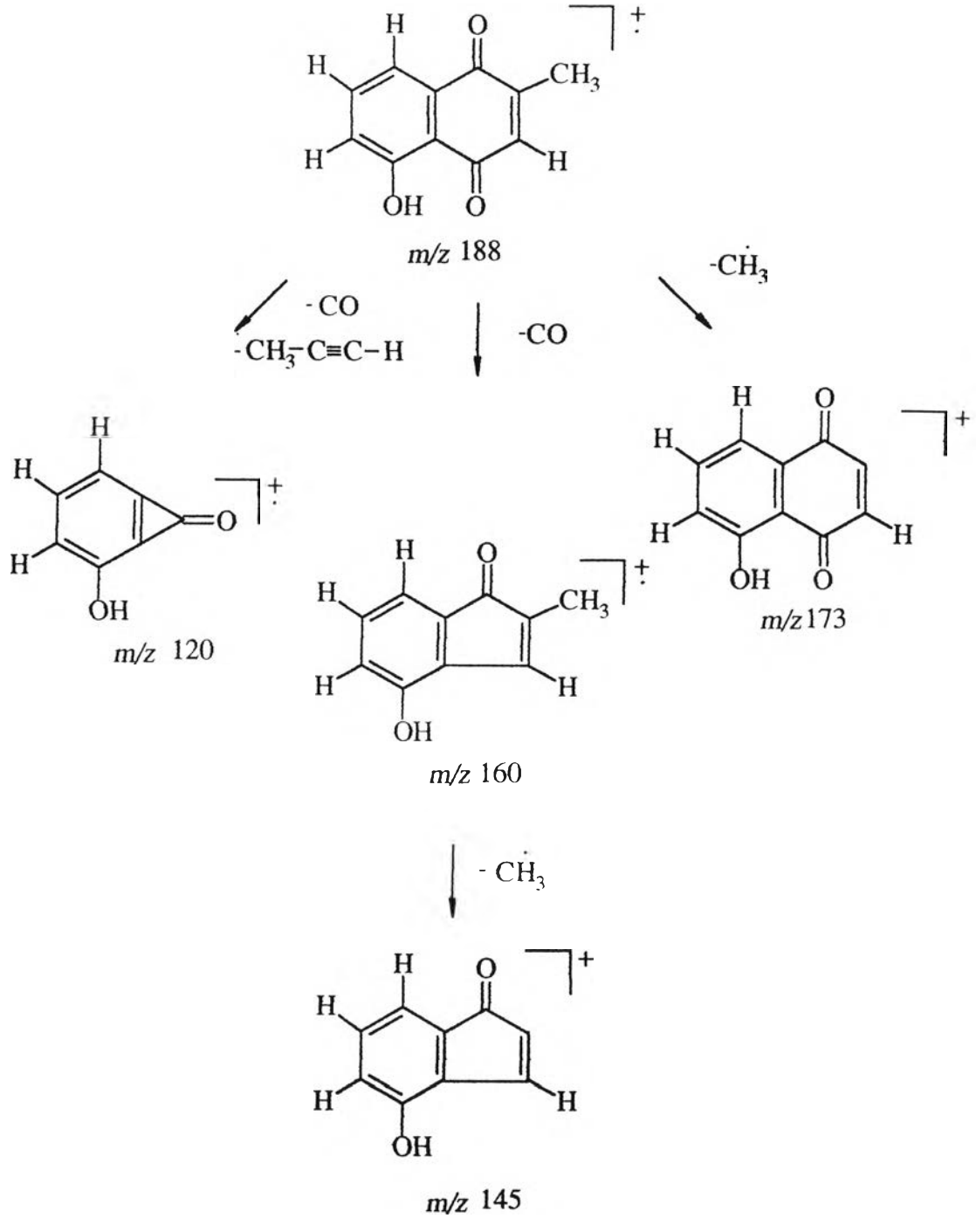


(18)

Table 4 The proton and carbon assignments of compound **18**

Table 4 The proton and carbon assignments of compound 18

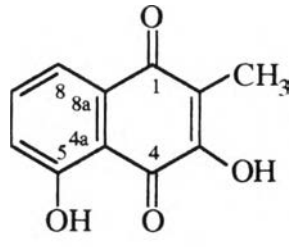
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	184.689
2	-	-	149.550
3	6.790	q, 1.53	135.385
4	-	-	190.201
5	-	-	161.098
6	7.236	dd, 1.83, 7.63	124.100
7	7.590	t, 7.63, 7.63	136.027
8	7.618	dd, 1.83, 7.63	119.214
4a	-	-	115.052
8a	-	-	131.996
2-CH_3	2.190	d, 1.53	16.460
5-OH	11.950	s	-



Scheme 6 Mass fragmentation of compound **18**

1.2 Identification of compound 19

Compound **19** was obtained as orange needles, from fraction C-12 (Table 3).



(19)

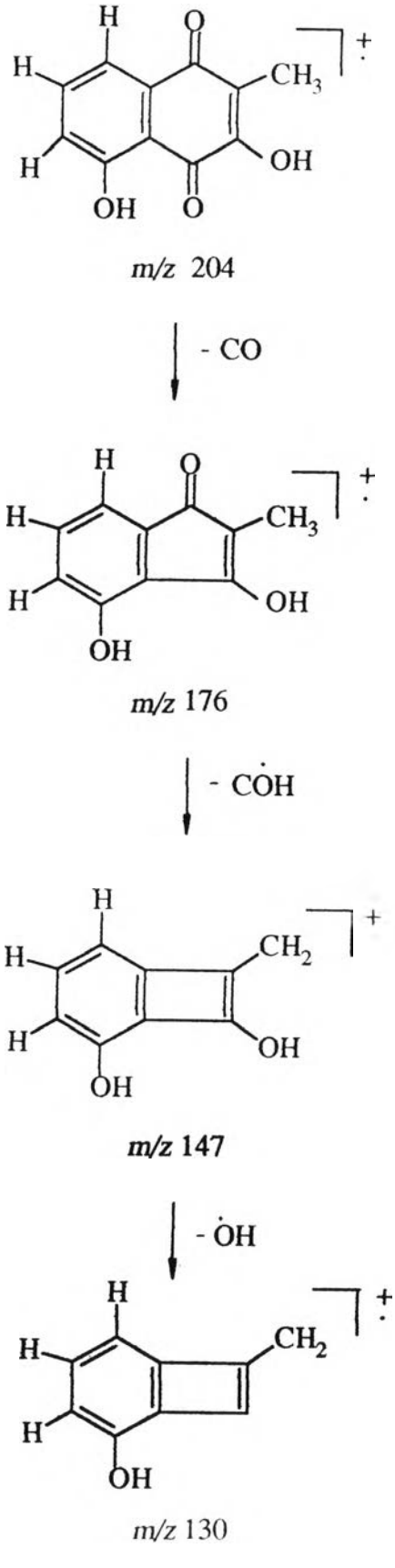
Compound **19** was identified as droserone (**19**). Its partial ^{1}H NMR assignments were reported by Gunaherath *et al*, 1983, and its ^{13}C NMR assignments were studied by Kreher, Neszmelyi and Wagner, 1989. The present work has revised the ^{13}C NMR assignments of this compound by using several 1-D and 2-D NMR techniques, including ^{1}H (Figures 30-31), ^{13}C (Figure 32), ^{1}H-^{1}H COSY (Figures 34-35), NOESY (Figures 36-37), HMQC (Figures 38-40) and HMBC (Figures 41-45) experiments. The complete ^{1}H and ^{13}C assignments of **19** are shown in Table 5.

The following data supported the identification of **19**. The EIMS of this compound (Figure 27) revealed the molecular ion peak at *m/z* 204. The mass fragmentation pattern is shown in scheme 7 (Stensen and Jensen, 1995). Absorptions in the IR spectrum (Figure 28) showed OH stretching at 3485 cm^{-1}, C=O stretching at 1626 cm^{-1}, C-H stretching at 1450 cm^{-1} and C-H bending at 1100 cm^{-1}. The UV absorption spectrum (Figure 29) showed quinonoid and benzenoid

ring absorptions at λ_{max} 228 nm (log ε 4.70), 279 nm (log ε 4.68) and 401 nm (log ε 4.15).

Table 5 The proton and carbon assignments of compound **19**

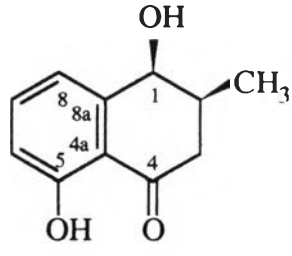
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	184.163
2	-	-	121.797
3	-	-	152.774
4	-	-	184.492
5	-	-	161.197
6	7.198	dd, 1.22, 8.24	123.146
7	7.621	t, 7.66, 7.66	137.507
8	7.660	dd, 1.22, 7.66	119.641
4a	-	-	112.913
8a	-	-	132.704
2-CH_3	2.098	s	8.760
3-OH	7.229	br, s	-
5-OH	11.099	s	-



Scheme 7 Mass fragmentation of compound **19**

1.3 Identification of compound 102

Compound **102** was obtained as a yellow semi-solid from fraction C-9 (Table 3), by repetitive chromatography using preparative TLC with 15% ethyl acetate in petroleum ether as the eluent.



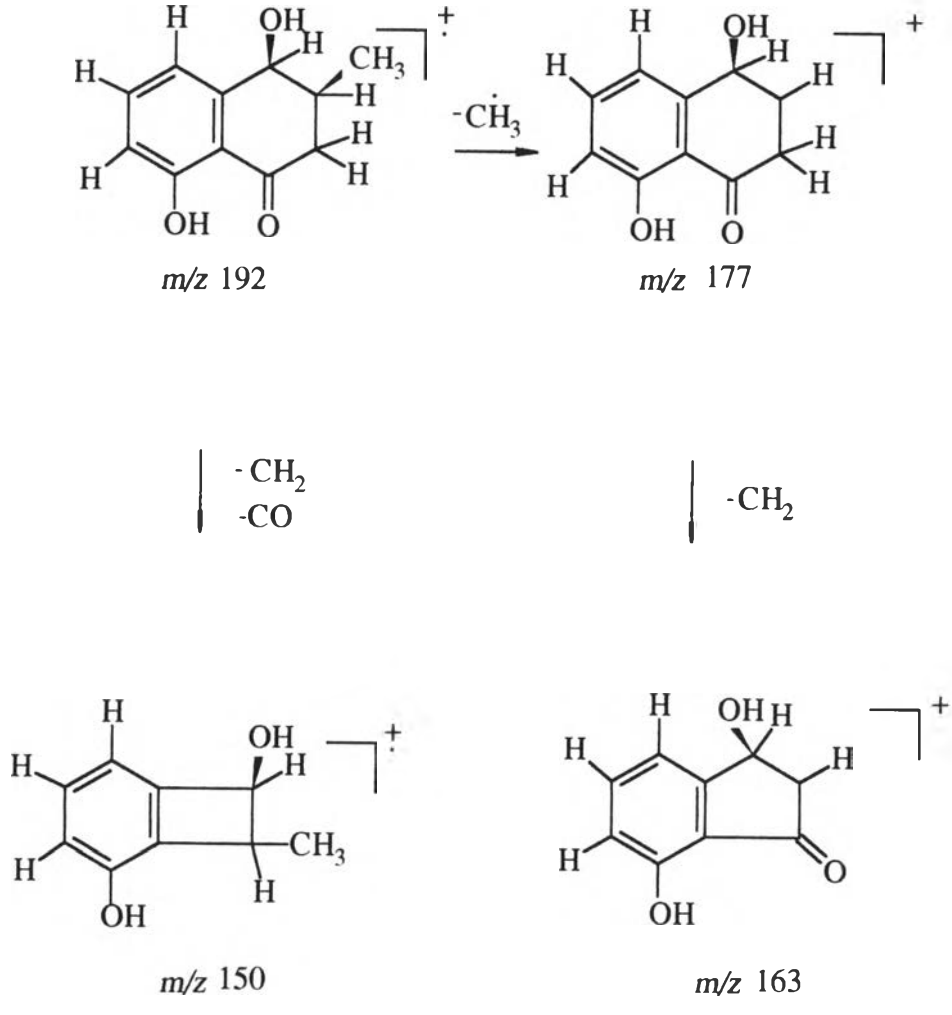
(102)

The EIMS of **102** (Figure 46) exhibited an $[M]^+$ ion at *m/z* 192. The proposed mass fragmentation is shown in scheme 8 (Silverstein, Bassler and Morril, 1991). The UV absorption bands (Figure 48) at λ_{max} 214 nm (log ε 3.99), 258 nm (log ε 3.80) and 332 nm (log ε 3.33) showed the quinone and benzenoid ring absorptions. The IR spectrum (Figure 47) displayed absorption bands for C=O stretching (1631 cm^{-1}), OH stretching (3101 cm^{-1}) and C-H bending (1243 cm^{-1}).

This compound was identified as isoshinanolone (**102**) (Tezuka *et al*, 1973, Bhattacharyya and De Calvalho, 1986). The present work completely assigned the ^1H (Figures 49-51) and ^{13}C NMR resonances (Figure 52) of this compound by DEPT, NOESY, ^1H-^1H COSY, HMQC and HMBC experiments, (Figures 53-67). The ^1H and ^{13}C assignments are shown in Table 6.

Table 6 The proton and carbon assignments of compound **102**

Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	4.737	d, 2.75	71.143
2	2.424	m	34.424
3e	2.562	dd, 17.7, 4.27	40.725
3a	2.857	dd, 17.7, 10.98	
4	-	-	204.727
5	-	-	162.678
6	6.932	d, 7.33	118.128
7	7.470	dd, 7.32, 7.33	136.899
8	6.917	d, 7.32	118.605
4a	-	-	114.936
8a	-	-	145.042
5-OH	12.400	s	-
2-CH_3	1.170	d, 6.71	16.131

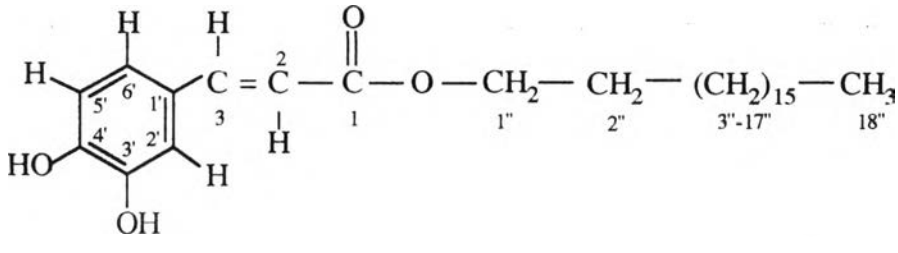


Scheme 8 Mass fragmentation of compound **102**

1.4 Identification of compound 103

Compound **103** was obtained as a white powder from the C-9 fraction through purification by silica gel preparative TLC, using 20% ethyl aceate in petroleum ether as the eluent. The EIMS (Figure 68) showed its molecular ion peak at *m/z* 432. The IR spectrum (Figure 69) exhibited OH stretching at 3500 cm^{-1}, C=O stretching at 2950 cm^{-1}, and C-O-C stretching at 1230, 1190 cm^{-1}.

Compound **103** could be assigned as octadecyl-3(3,4-dihydroxy)-2-propenoic acid ester or octadecyl caffeate (Iinuma, Ohyama and Tanaka, 1995). Complete ^{1}H (Figures 70-71) and ^{13}C NMR (Figure 72) assignments of this compound were obtained by ^{1}H-^{1}H COSY (Figures 76-78), NOESY (Figures 79-80), HMQC (Figures 81-85) and HMBC (Figures 86-90) experiments.

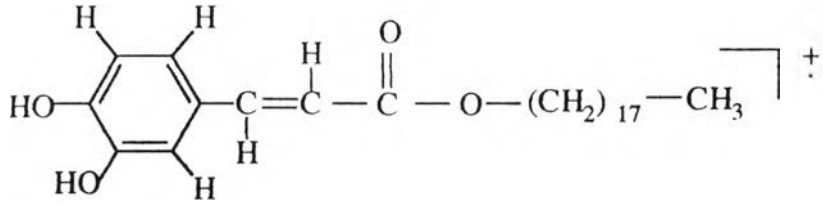


(103)

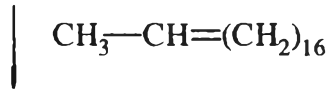
The proposed EI mass fragmentation of compound **103** is shown in scheme 9 (Silverstein *et al*, 1991). The ^{1}H and ^{13}C NMR assignments are summarized in Table 7.

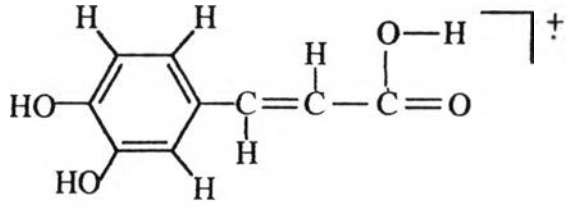
Table 7 The proton and carbon assignments of compound **103**

Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
3	7.520	d, 16.17	145.758
2	6.260	d, 16.17	115.126
1	-	-	167.556
1″	4.130	t, 6.71, 6.71	64.440
2″	1.670	m	29.564
3″-17″ (Thirty-CH_2 groups)	1.278	m	22.934, 26.290, 29.185, 29.613, 29.827, 29.876, 29.991, 32.130
18″	0.869	t, 7.02, 7.02	14.280
1′	-	-	126.905
2′	7.140	d, 1.83	115.784
3′	-	-	150.464
4′	-	-	147.733
5′	6.848	d, 8.24	122.019
6′	7.018	dd, 1.83, 8.24	116.722
4′-OH	-	-	-
5′-OH	-	-	-

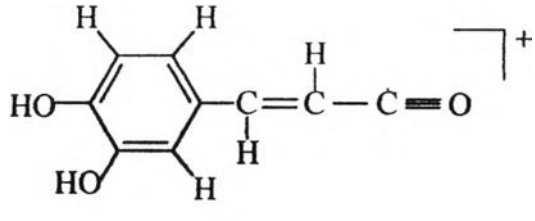


m/z 432





m/z 180

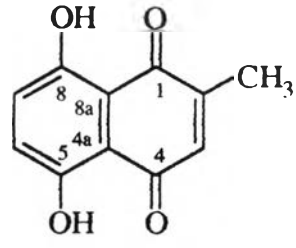


m/z 163

Scheme 9 Mass fragmentation of compound **103**

1.5 Identification of compound 104

Compound **104** was obtained from fraction C-6 (Table 4). It was separated by the Sephadex LH-20 gel filtration technique with chloroform and methanol, and then further purified by preparative TLC using 30% ethyl acetate in petroleum ether.

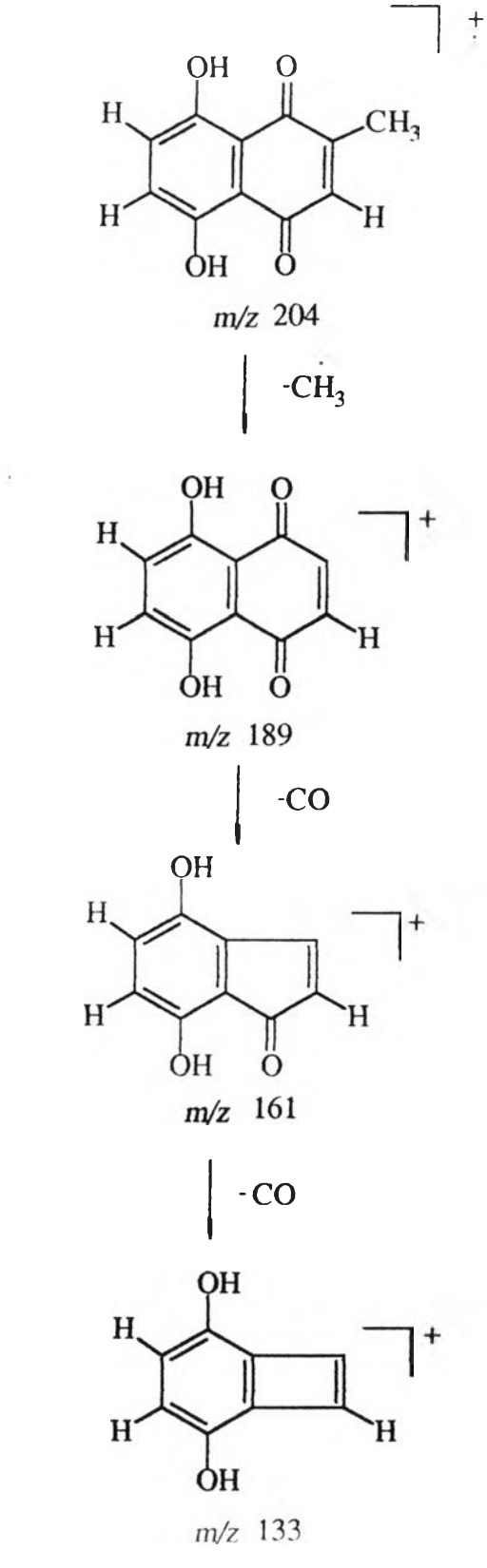


(104)

The EIMS (Figure 91) showed a molecular ion at *m/z* 204. The EI fragmentation pattern is shown in scheme 10 (Stensen and Jensen, 1995). The IR spectrum (Figure 92) showed C-H stretching of CH_3, CH_2 at 2922 cm^{-1}, C=O stretching at 1640 cm^{-1} and C-O stretching of phenol at 1230 cm^{-1}. Comparison of its ^1H NMR data with the literature values indicated that compound **104** was identical with 2-methylnaphthazarin (Ferreira, Costa and Alves, 1972). Regarding the NMR properties of compound **104**, only partial ^1H NMR assignments were reported. In this study complete ^1H (Figures 93-94) and unambiguous ^{13}C (Figure 95) assignments were obtained by careful examination of the COSY (Figures 96-97), NOESY (Figures 98-101), HMQC (Figures 102-104) and HMBC (Figures 105-109) spectra. The proton and carbon assignments of **104** are shown in Table 8.

Table 8 The proton and carbon assignments of compound **104**

Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	185.199
2	-	-	150.043
3	6.809	q, 1.53	134.941
4	-	-	190.513
5	-	-	161.362
6	7.291	d, 8.85	124.330
7	7.200	d, 8.85	137.985
8	-	-	135.566
4a	-	-	115.479
8a	-	-	128.213
CH_3	2.011	d, 1.53	16.641
5-OH	12.579	s	-
8-OH	12.579	s	-



Scheme 10 Mass fragmentation of compound **104**

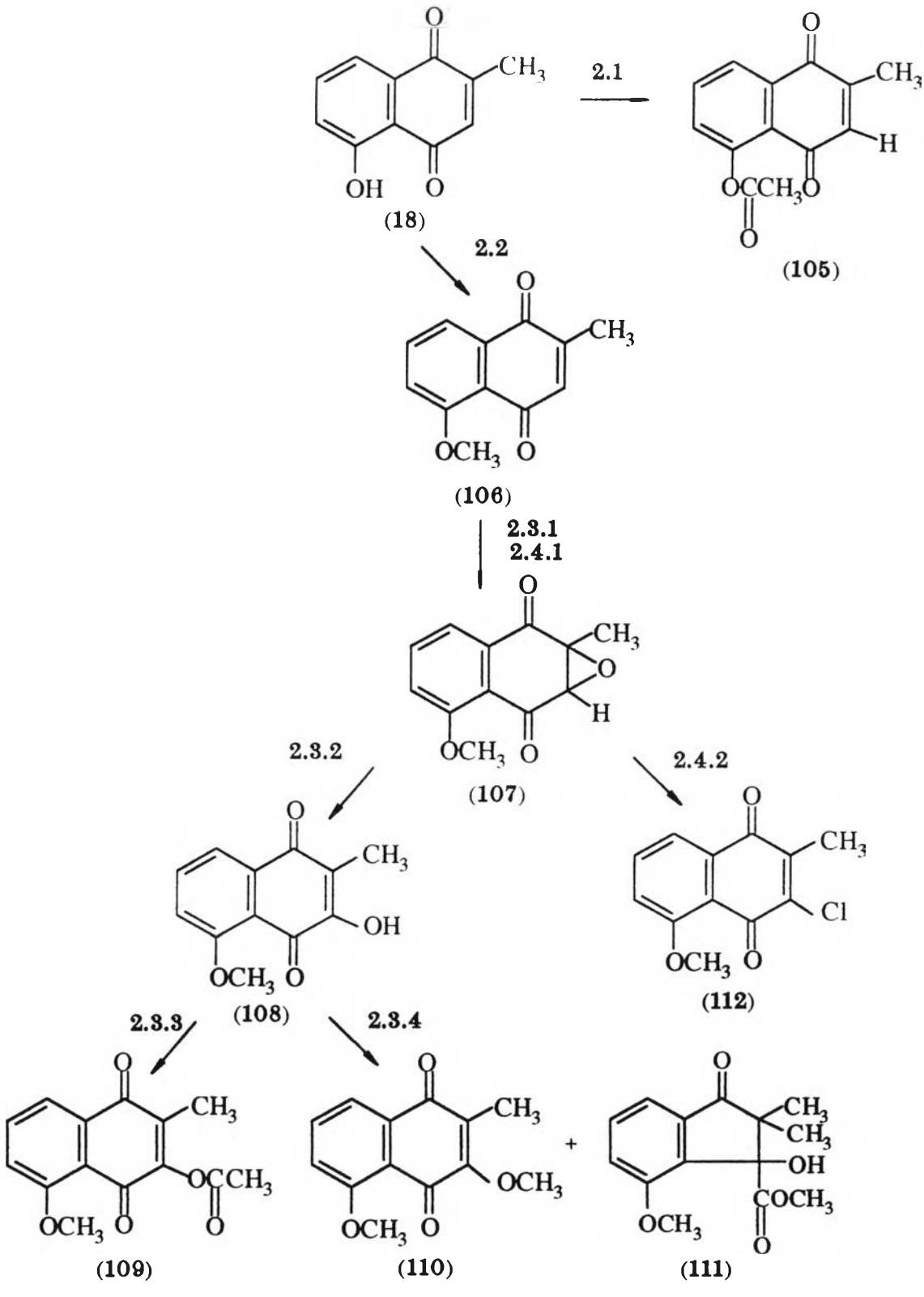
2. Chemical transformations of plumbagin (18)

Eight naphthoquinone-related compounds were prepared, using plumbagin (18) as the first starting material (Scheme 11). The ^{1}H and ^{13}C NMR properties of these transformation products were extensively studied, using several 1- and 2-D NMR techniques.

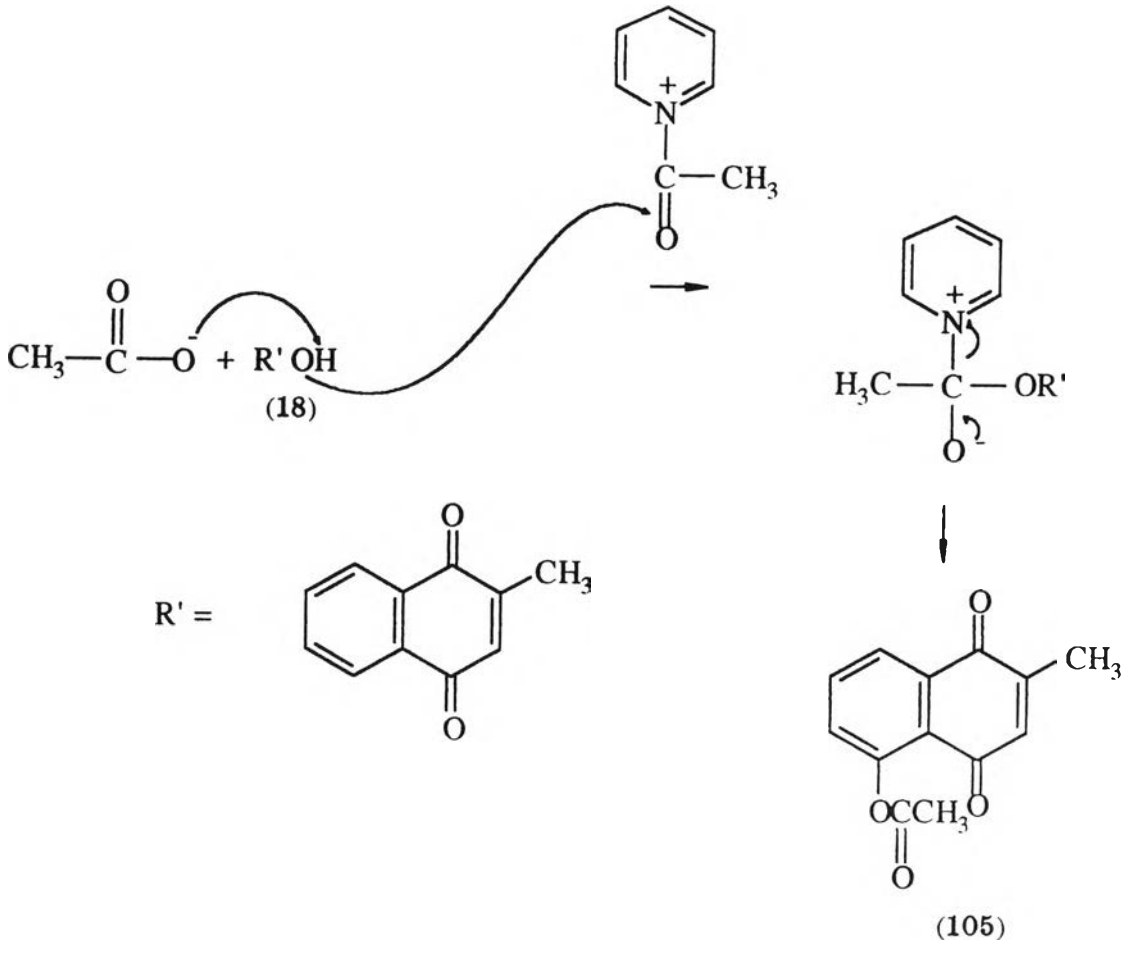
2.1 Acetylation of plumbagin (18)

Acetylation of plumbagin (18) was performed with acetic anhydride in pyridine to give compound **105**. The mechanism of this reaction is shown in Scheme 12. Acetic anhydride was a reactive acylating reagent because of the combination of the inductive effect of oxygen substituent on the reactivity of the carbonyl group and the ease with which the tetrahedral intermediate could expel such relatively good leaving group. Acylation of this compound was performed in the presence of an organic base, pyridine. This base served two purposes. It neutralized the protons generated in the reaction and prevented the development of high acid condition. Pyridine also became directly involved in the reaction as a nuclophile catalyst. It was more nucleophilic than the hydroxy of plumbagin, toward the carbonyl center of acetic anhydride. The product that resulted, an acylpyridinium ion, was in turn more reactive toward the hydroxy group than the original acetic anhydride (Carey and Sunberg, 1993).

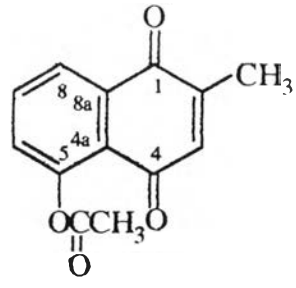
Compound **105** was obtained as yellow crystals from the acetylation of plumbagin in pyridine. This compound was plumbagin acetate.



Scheme 11 Chemical transformations of plumbagin (18)



Scheme 12 Acetylation of plumbagin (18)



(105)

The EIMS of compound **105** (Figure 110) showed the molecular ion peak at *m/z* 230 corresponding to the molecular formula $C_{13}H_{11}O_4$. The mass fragmentation (scheme 13) was proposed, based on the mass fragmentation of plumbagin. The IR absorption spectrum (Figure 111) showed C=O stretching at 1650 cm^{-1}, C-C stretching at 1580, 1400 cm^{-1} and C-H bending at 1000 cm^{-1}.

The protons and carbons of compound **105** were assigned by comparison of their ^{1}H NMR (Figure 112), ^{13}C NMR (Figure 113) data with those of plumbagin and are shown in Table 9.

Table 9 The proton and carbon assignments of compound **105**

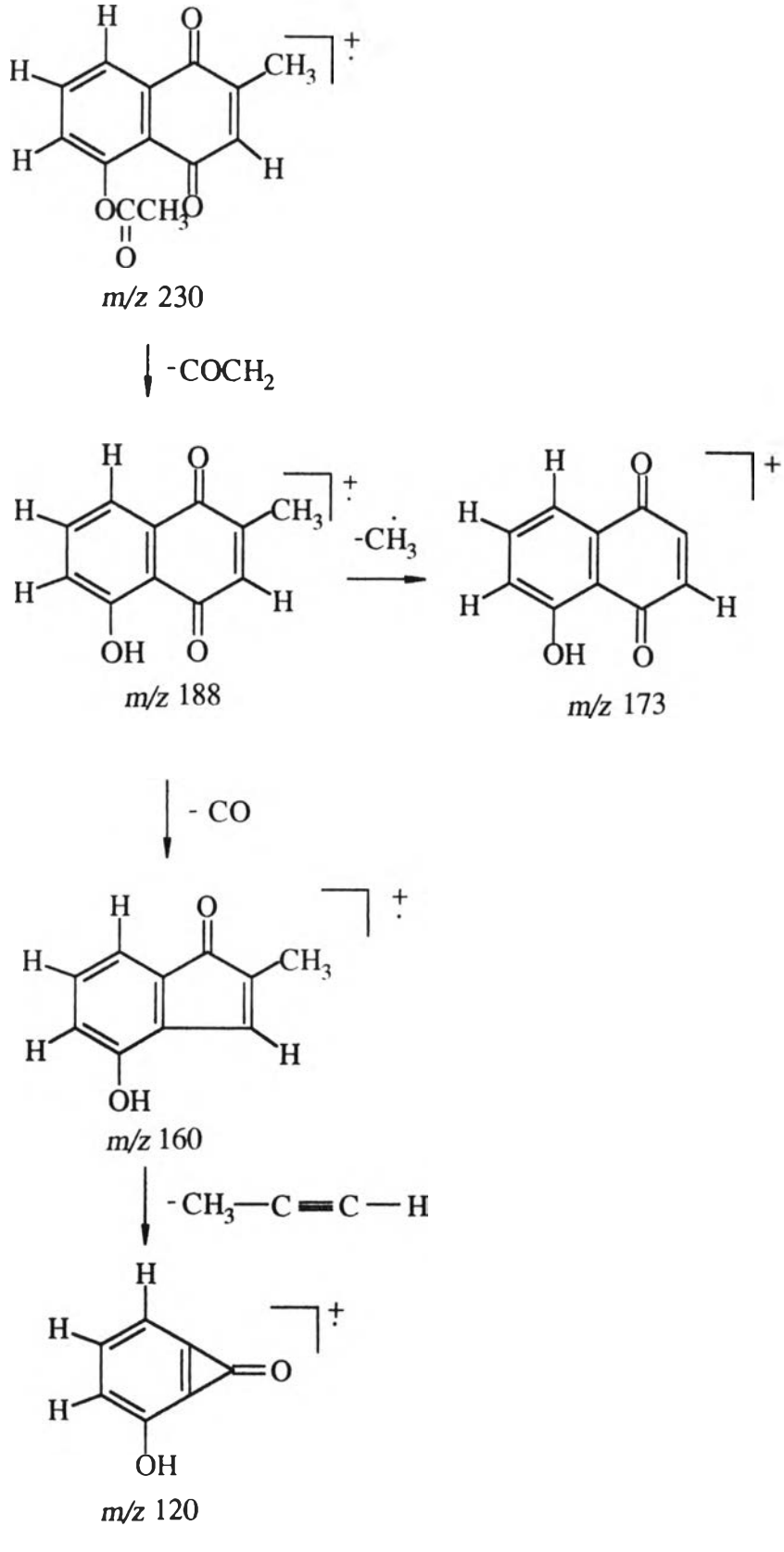
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	184.767
2	-	-	146.857
3	6.711	d, 1.526	134.409

Table 9 (continued)

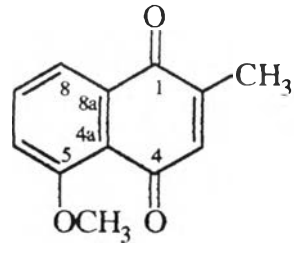
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
4	-	-	183.552
5	-	-	149.208
6	7.359	dd, 1.5, 8.39	125.066
7	7.730	t, 8.39, 8.39	136.848
8	8.060	dd, 1.5, 8.39	129.372
4a	-	-	123.426
8a	-	-	133.813
5-O<u>C</u>OCH$_3$	-	-	169.391
2-CH$_3$	2.162	d, 1.52	16.019
5-OCOC<u>H</u>$_3$	2.433	s	21.056

2.2 Methylation of plumbagin (18)

Methylation of plumbagin was achieved by using methyl iodide in the presence of silver (I) oxide. The mechanism of the reaction is shown in Scheme 14. The base (Ag_2O) deprotonated the hydroxy group to form a phenoxide ion. This ion preferred *O*-alkylation because *C*-alkylation would disrupt aromatic conjugation. Methyl iodide was an alkylating agent, giving a methyl group to the phenoxide ion (Carey and Sunberg, 1993).



Scheme 13 Mass fragmentation of compound **105**



(106)

Compound **106** (5-methoxyplumbagin) was obtained as yellow crystals from this reactions. The EIMS (Figure 115) showed the molecular ion at *m/z* 202 corresponding to the molecular formula $C_{12}H_{10}O_3$. The mass fragmentation of compound **106** is shown in scheme 15 (Stensen and Jensen, 1995). The IR spectrum (Figure 116) exhibited C=O stretching (1690 cm^{-1}), C-H stretching of CH_3, CH_2 (2947-2922 cm^{-1}), C-C stretching (1588 and 1451 cm^{-1}) and C-H bending (1344-1255 cm^{-1}).

Compound **106** was assigned as 5-methoxyplumbagin or plumbagin methyl ether by analysis of its ^{1}H (Figure 117) and ^{13}C NMR (Figure 118) spectra (Dinda, *et al* 1994 and Sankaram *et al*, 1986). The complete ^{1}H and ^{13}C assignments of this compound are shown in Table 10.

Table 10 The proton and carbon assignments of compound **106**

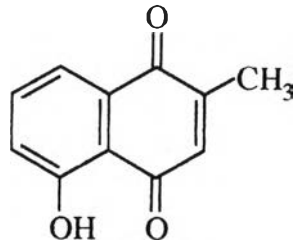
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	185.756
2	-	-	145.355

Table 10 (continued)

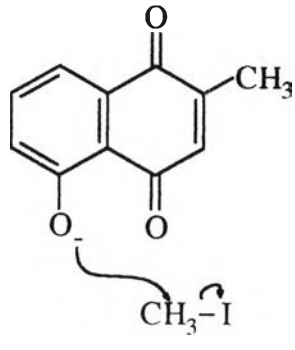
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
3	6.741	d, 1.52	134.602
4	-	-	184.465
5	-	-	159.379
6	7.291	d, 8.73	119.343
7	7.660	t, 8.73, 8.73	137.847
8	7.758	dd, 0.92, 8.73	119.947
4a	-	-	117.644
8a	-	-	134.372
2-CH_3	2.142	d, 1.52	15.756
5-OCH_3	4.010	-	56.443

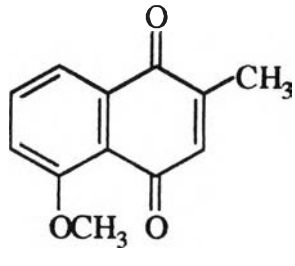
2.3 Hydroxylation of 5-methoxyplumbagin (106) at C-3

5-methoxyplumbagin (**106**) was the starting material in this reaction. A hydroxyl group could not be directly added to C-3 of 5-methoxyplumbagin because of high electron density in the unsaturated bond. Therefore, a 2,3-epoxide of compound **106** was prepared. This intermediate (**107**) was then converted to 3-hydroxy-5-methoxyplumbagin (**108**) by acid-catalyzed opening of the epoxide ring. Each of those two steps was described in section **2.3.1** and **2.3.2**, respectively.



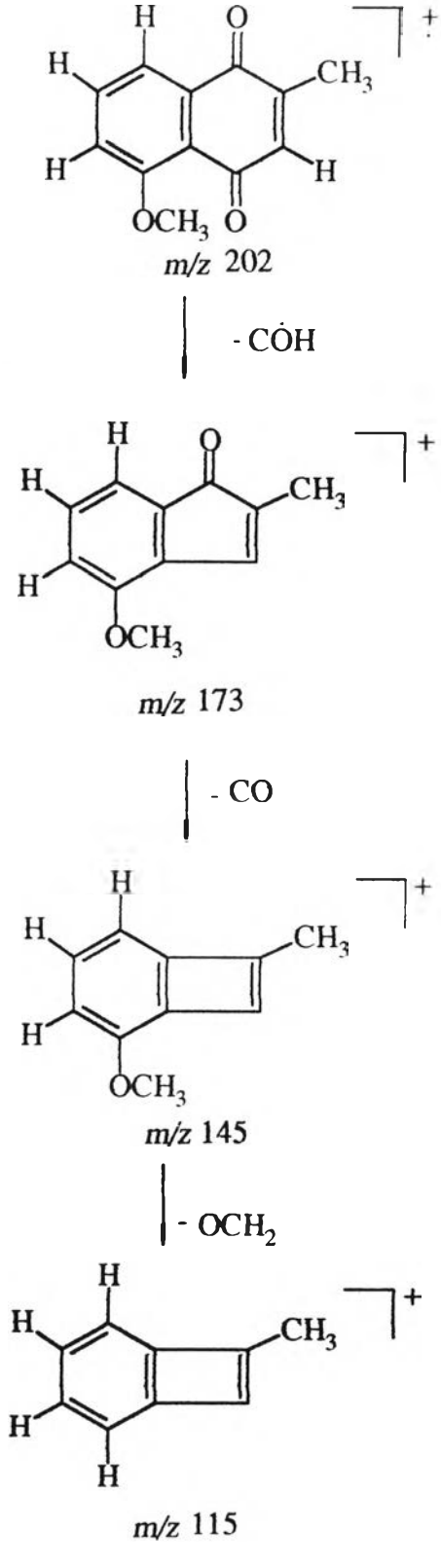
(**18**)





(**106**)

Scheme 14 Methylation of plumbagin (**18**)



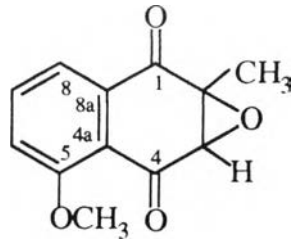
Scheme 15 Mass fragmentation of compound **106**

2.3.1 Epoxidation of 5-methoxyplumbagin (106)

Sodium carbonate was used to generate a peroxide anion which then added to the α,β unsaturated position of compound **106** (Scheme 16) to give 5-methoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide (**107**) (Smith, 1994). This compound was the intermediate compound which would be used for the preparation of compound **108**.

Compound **107** was obtained as a white powder. The IR spectrum (Figure 121) exhibited C-H stretching of CH_3 at 2922 cm^{-1}, C=O stretching at 1690 cm^{-1}, C-C stretching at 1451 cm^{-1}, C-H bending at 1344 cm^{-1} and ether linkage at 1255 cm^{-1}. The EIMS of compound **107** (Figure 120) exhibited the molecular peak at *m/z* 218. The mass fragmentation pattern (Scheme 17) was proposed based on mass fragmentation of compound **106** (Stensen and Jensen, 1995).

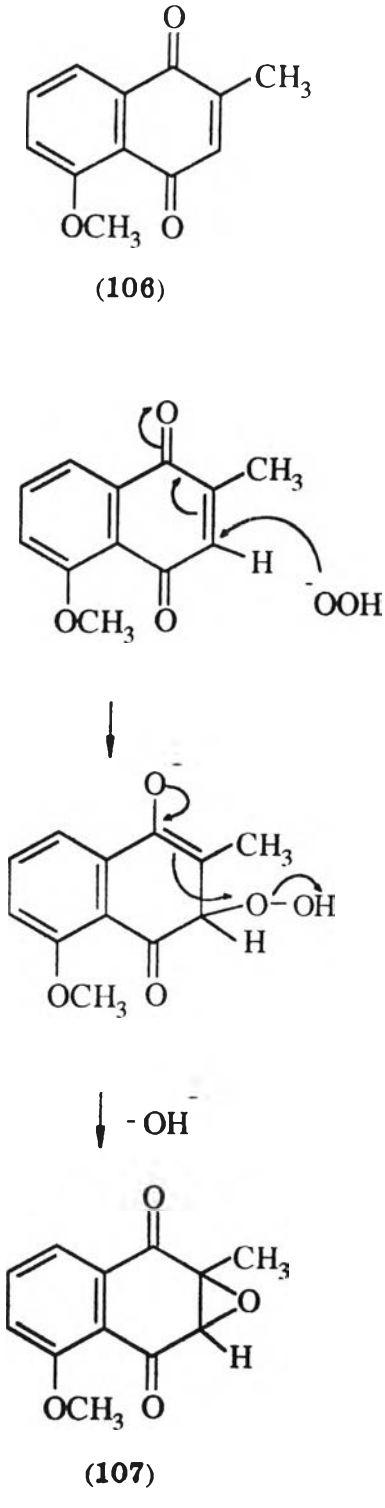
Although compound **107** has been prepared previously (Pluim and Wynberg, 1980), its ^{1}H and ^{13}C NMR properties have never been described. Results from analysis of the COSY (Figures 126-127), NOESY (Figure 128), HMQC (Figures 129-131) and HMBC (Figures 132-138) spectra of compound **107** in this study provided the first and complete report of ^{1}H (Figures 122-123) and ^{13}C NMR (Figure 124) assignments of this compound.



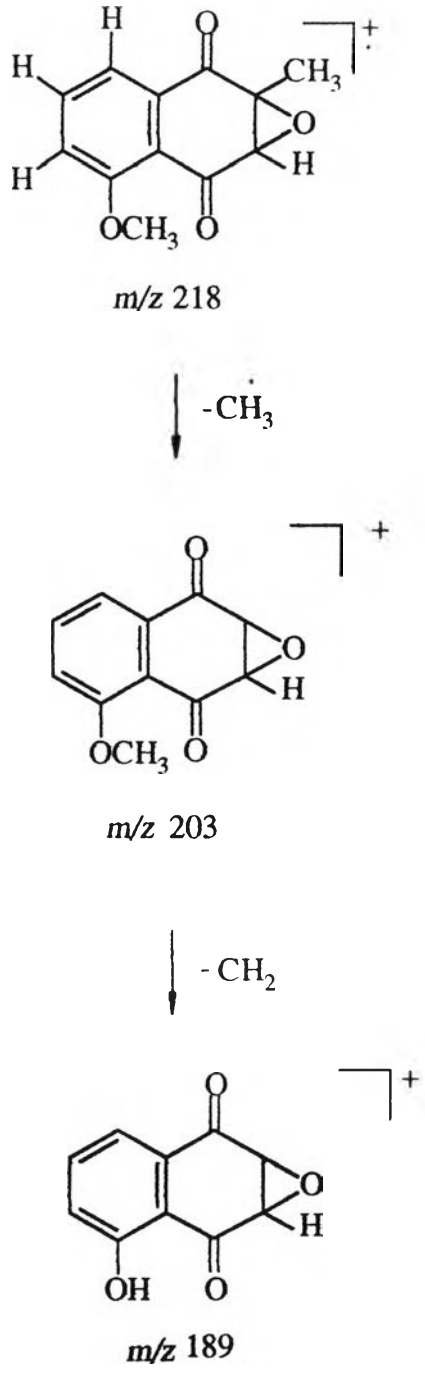
(**107**)

Table 11 The proton and carbon assignments of compound **107**

Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	192.915
2	-	-	61.454
3	3.830	s	61.552
4	-	-	191.172
5	-	-	158.861
6	7.267	dd, 0.92, 7.94	117.585
7	7.648	t, 7.63, 7.63	134.991
8	7.559	dd, 0.92, 7.63	119.477
4a	-	-	120.464
8a	-	-	134.300
5-OCH_3	3.953	s	56.436
2-CH_3	1.712	s	14.387



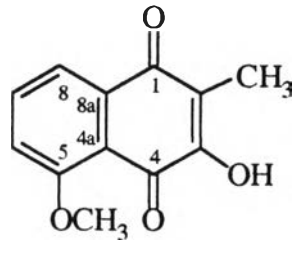
Scheme 16 Epoxidation of 5-methoxyplumbagin (**106**)



Scheme 17 Mass fragmentation of compound **107**

2.3.2 Treatment of compound 107 with concentrated sulfuric acid

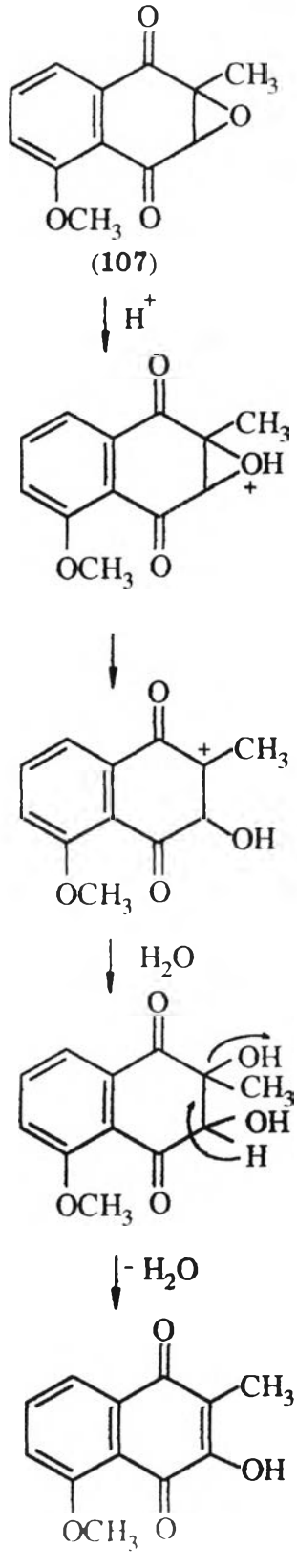
Compound **107** was first converted into a protonated epoxide, which then underwent nucleophilic attack by water to yield a 2,3-diol intermediate (Smith, 1994). The final step was acid dehydration which gave 3-hydroxy-5-methoxy-1,4-naphthoquinone (**108**), (Scheme 18).



(**108**)

Compound **108** was obtained as a yellow powder from this reaction. The EIMS of compound **108** (Figure 139) showed the molecular ion peak at *m/z* 218, consistent with the molecular formula $C_{12}H_{10}O_4$. The EI fragmentation pattern was proposed as shown in scheme 19 (Stensen and Jensen, 1995). The IR spectrum (Figure 140) exhibited OH stretching (3255 cm^{-1}), C-H stretching (2811 cm^{-1}) and C=O stretching (1658 cm^{-1}). Its UV and ^{1}H NMR data were identical with those reported for droserone methyl ether (Ghera and Ben-David, 1985).

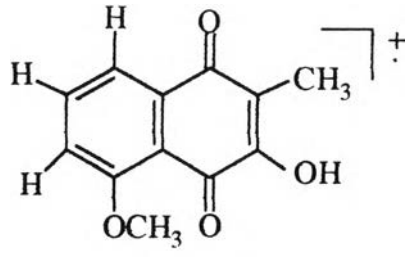
The complete proton and carbon assignments of compound **108** (Table 12) were obtained by analysis of its ^{1}H (Figure 141) and ^{13}C NMR chemical shifts (Figure 142) and their correlations observed in the HETCOR (Figure 144) and HMBC spectra (Figures 145-149).



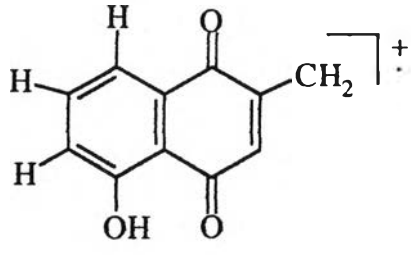
Scheme 18 Treatment of compound **107** with concentrated sulfuric acid

Table 12 The proton and carbon assignments of compound **108**

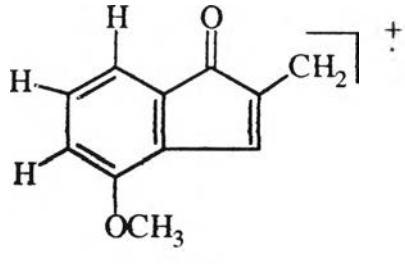
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicty, *J* (Hz)	δ (ppm)
1	-	-	184.788
2	-	-	117.996
3	-	-	153.580
4	-	-	179.622
5	-	-	160.013
6	7.240	dd, 0.83, 8.39	116.647
7	7.694	t, 8.01, 8.01	136.191
8	7.790	dd, 0.83, 8.01	119.592
4a	-	-	116.861
8a	-	-	135.171
3-OH	7.705	br, s	-
5-OCH_3	4.034	s	56.452
2-CH_3	2.066	s	8.431



m/z 218



m/z 200

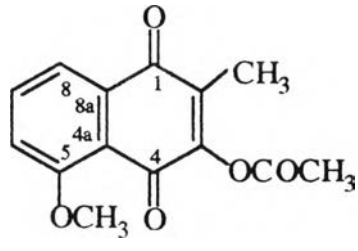


m/z 172

Scheme 19 Mass fragmentation of compound **108**

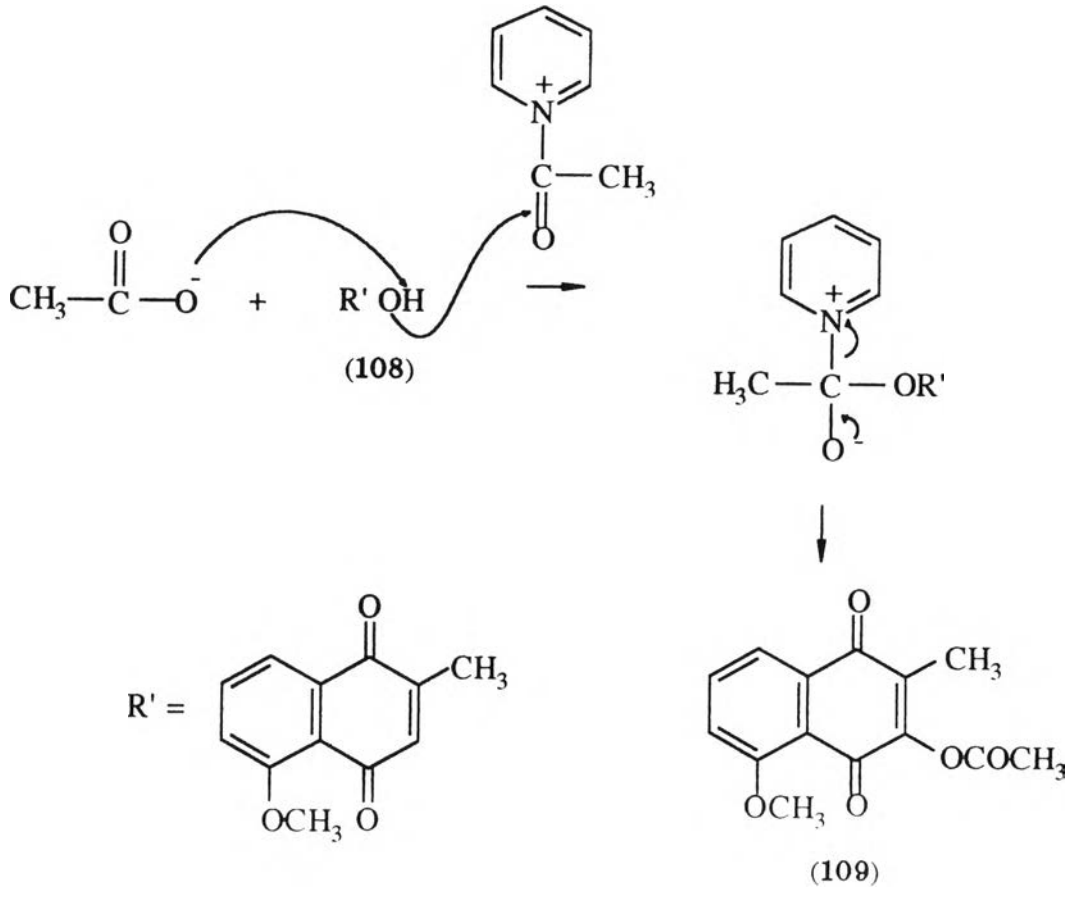
2.3.3 Acetylation of 3-hydroxy-5-methoxyplumbagin (108)

This reaction was conducted to further confirm the presence of the hydroxy group of C-3. The product from the reaction would also be used to exemplify the influence of the acyloxy group at C-3 on the neighboring carbons such as C-2, C-3, and 2-CH_3. Compound **108** was acylated by acetic anhydride in pyridine (Scheme 20) to give compound **109**. The mechanism of reaction was the same as that of plumbagin acetylation.



(109)

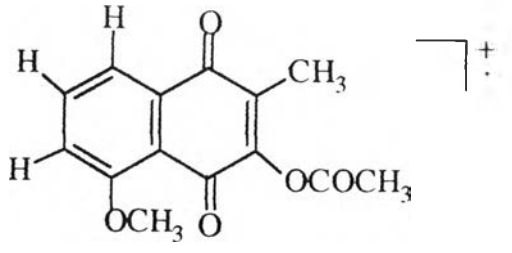
The EIMS of compound **109** (Figure 150) revealed the molecular ion peak at *m/z* 260, consistent with the molecular formula $C_{14}H_{12}O_5$. The proposed mass fragmentation pattern (Scheme 21) was analogous to that of 5-methoxy-1,4-naphthoquinone (Stensen and Jensen, 1995). The IR spectrum (Figure 151) disclosed absorption bands due to C-H stretching (3454 cm^{-1}), and C=O stretching (1663 cm^{-1}). The ^{1}H (Figures 152-153) and ^{13}C (Figure 154) resonances were assigned by comparison with those of 3-hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone (**108**) as shown in Table 13.



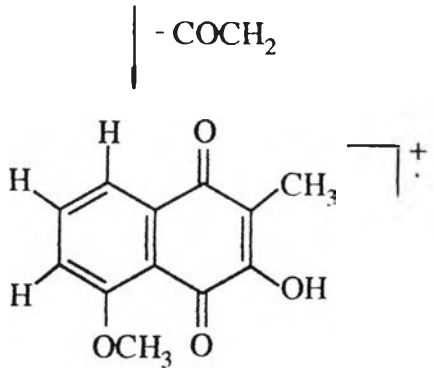
Scheme 20 Acetylation of 3-hydroxy-5-methoxyplumbagin (108)

Table 13 The proton and carbon assignments of compound **109**

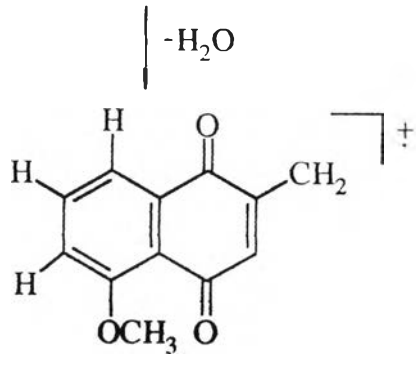
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	184.981
2	-	-	133.461
3	-	-	151.882
4	-	-	176.570
5	-	-	160.062
6	7.284	dd, 0.92, 7.94	117.774
7	7.674	dd, 7.63, 7.94	135.213
8	7.785	dd, 0.92, 7.63	119.404
4a	-	-	118.451
8a	-	-	134.272
2-CH_3	2.061	s	9.572
5-OCH_3	3.998	s	56.455
3-OCO	-	-	167.934
3-$OCOCH_3$	2.398	s	20.368



m/z 260



m/z 218



m/z 200

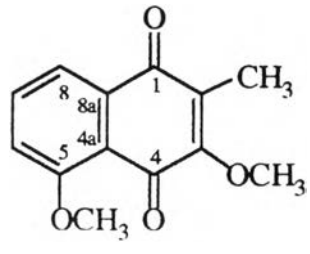
Scheme 21 Mass fragmentation of compound **109**

2.3.4 Methylation of 3-hydroxy-5-methoxyplumbagin (108)

Methylation of 3-hydroxy-5-methoxyplumbagin (**108**) with acidic MeOH was unsuccessful. Methylation of this compound by CH_3I and Ag_2O gave two products, compounds **110** and **111**.

2.3.4.1 Identification of compound 110

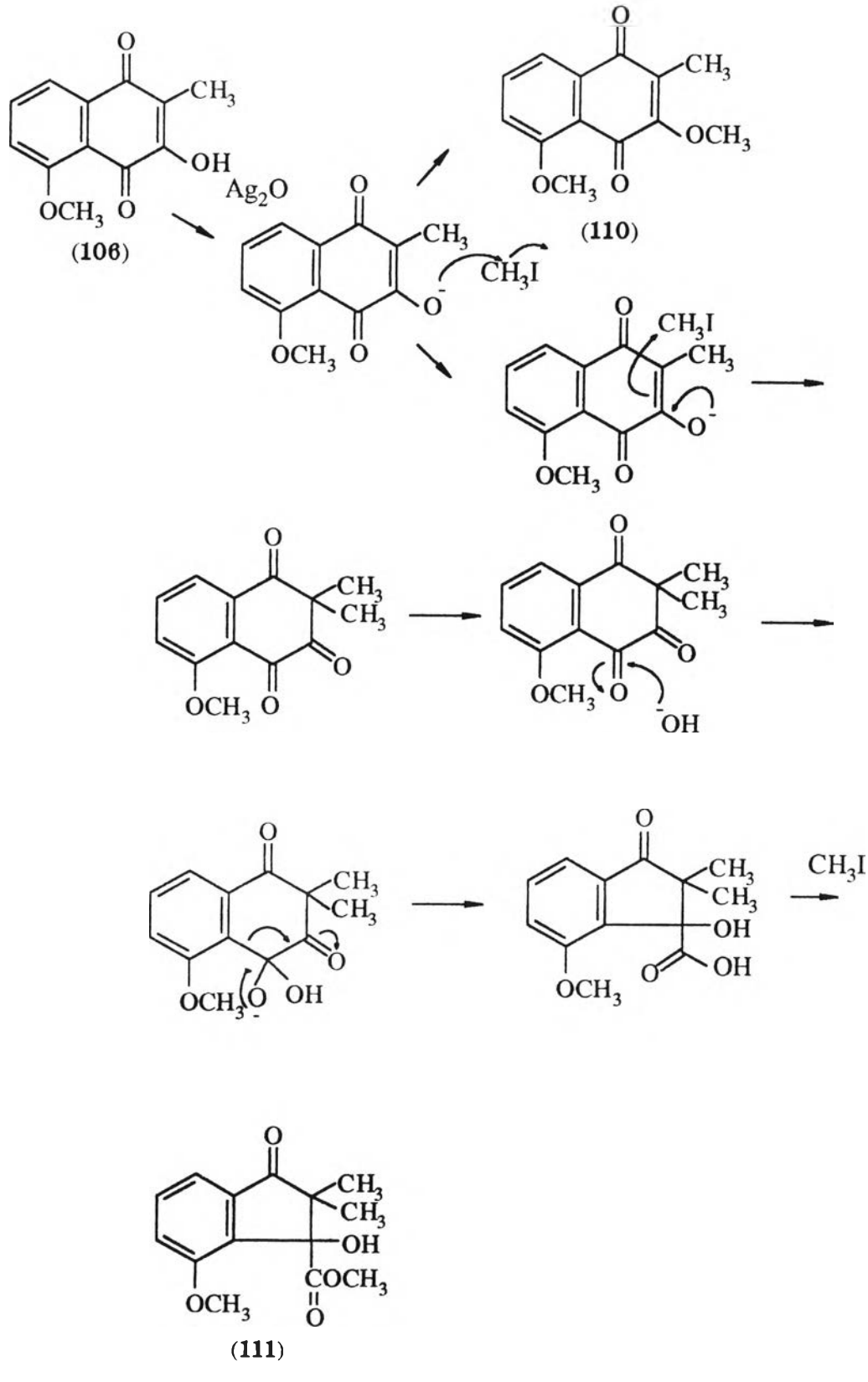
3,5-Dimethoxy-2-methyl-1,4-naphthoquinone (**110**) was obtained as a yellow powder from this reaction. It was also obtained as a by-product during the halogenation of compound **107** to compound **112** as described in **2.4**.



(110)

The reaction began with the deprotonation of compound **108** by Ag_2O to form an enolate anion. The alkylating agent methyl iodide was then attacked by the anion (*O*-alkylation) to give compound **110**. This reaction gave another product which was discussed in section **2.3.2** (Carey and Sunberg, 1993), (Scheme 22).

The IR spectrum (Figure 161) displayed C-H stretching (3471 cm^{-1}) and C=O stretching (1690 cm^{-1}). The mass fragmentation of this compound is shown in scheme 23 (Stenson and Jensen, 1995).

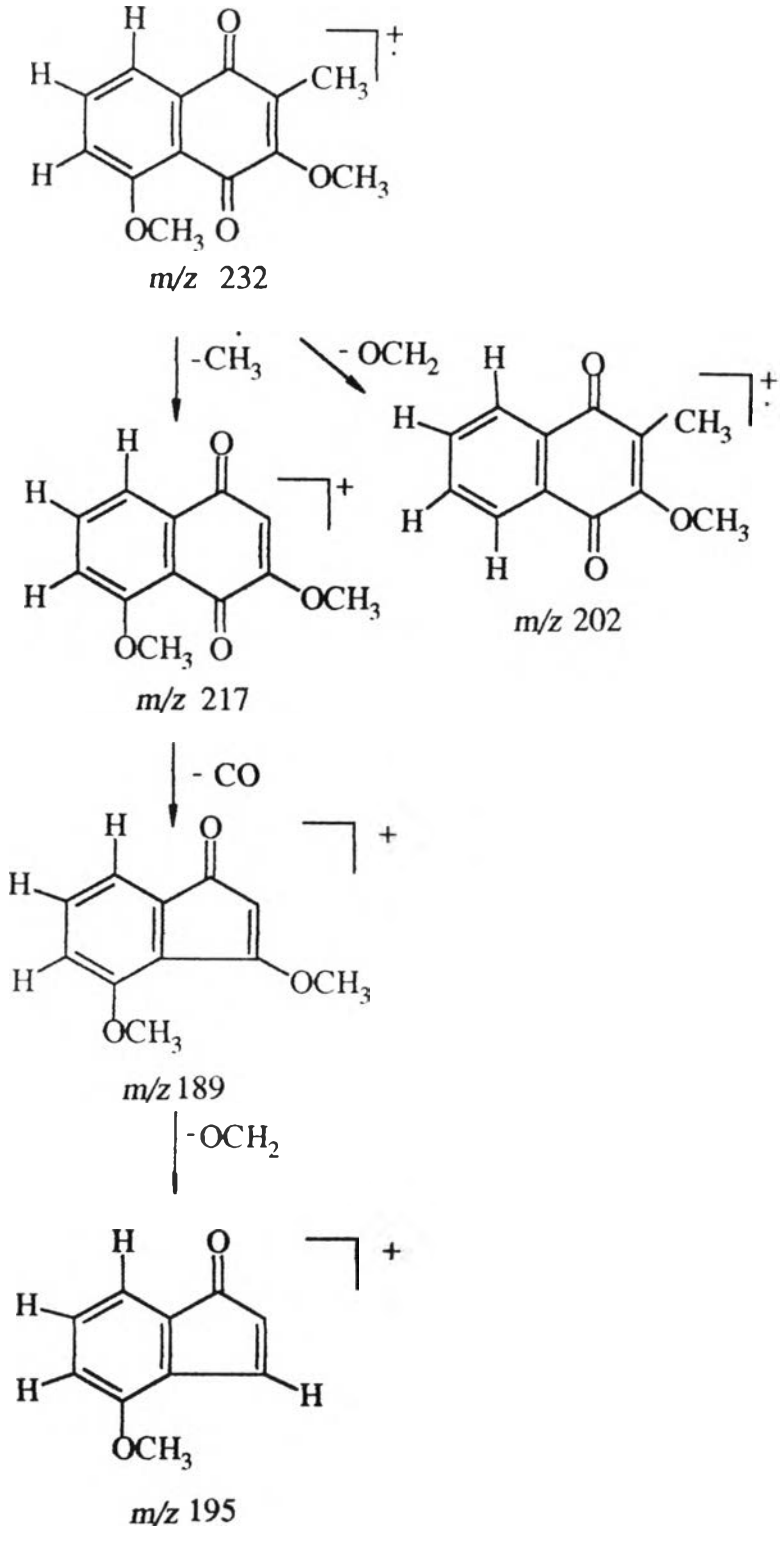


Scheme 22 Methylation of 3-hydroxy-5-methoxyplumbagin (**108**)

The ^{1}H NMR data of compound **110** were in the good agreement with 1.4-naphthoquinone, 3,5-dimethoxy-2-methyl-1,4-naphthoquinone (Giles and Roos, 1976). Its structure and ^{1}H (Figure 162) and ^{13}C NMR (Figure 163) assignments were thoroughly studied by the HMQC (Figures 165-167) and HMBC (Figures 168-172) experiments. Table 14 shows the complete ^{1}H and ^{13}C NMR assignments of compound **110.**

Table 14 The proton and carbon assignments of compound **110**

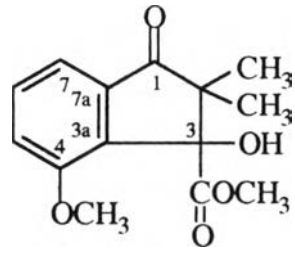
Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	185.759
2	-	-	128.903
3	-	-	158.746
4	-	-	180.248
5	-	-	159.519
6	7.251	dd, 1.14, 8.77	117.190
7	7.631	t, 8.77, 8.77	134.727
8	7.731	dd, 1.14, 8.77	118.918
4a	-	-	119.280
8a	-	-	134.349
2-CH_3	2.504	s	8.859
3-OCH_3	4.107	s	60.944
5-OCH_3	4.009	s	56.403



Scheme 23 Mass fragmentation of compound **110**

2.3.4.2 Identification of compound 111

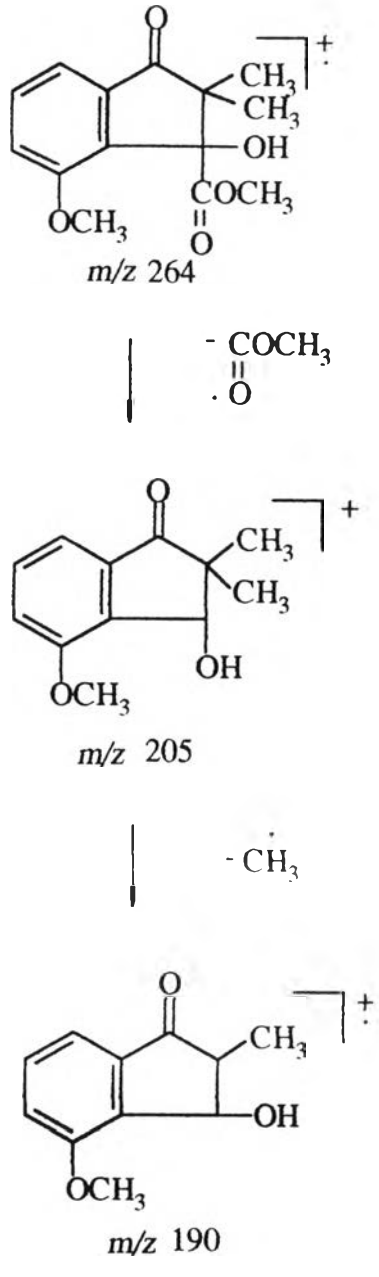
Compound **111** was obtained as a white powder during the methylation reaction of compound **108** by Ag_2O and methyl iodode.



(111)

The mechanism of the reaction invovled basic deprotonation at the 3-hydroxy position to generate an anion which underwent *C*-alkylation by CH_3I at C-2. The product was an α-diketo compound. The C-4 carbonyl carbon was then attacked by the base OH^-, leading to the migration of the aryl group to the C-3 carbonyl carbon. This was analogous to the benzil-benzilic rearrangement (March, 1977). Methylation at the COOH group gave the final product (**111**).

The HRFAB-MS (glycerol) of compound **111** (Figure 173) revealed the $[M+H]^+$ at *m/z* 265.1088 (calculated 265.1076) which therefore established the molecular formula $C_{14}H_{16}O_5$. The mass fragmentation pattern is shown in Scheme 24 (Silverstein *et al*, 1991). The IR spectrum (Figure 175) disclosed absorption bands due to O-H stretching (3443 cm^{-1}), asymmetric stretching of CH_3 (2961-2931 cm^{-1}) and C=O stretching (1726 cm^{-1}).



Scheme 24 Mass fragmentation of compound **111**

The structure was established as an indane derivative, 2,2-dimethyl-3-hydroxy-3-methoxycarbonyl-4-methoxy-1*H*-inden-1-one by analysis of the ^{1}H and ^{13}C NMR data. The ^{1}H spectrum (Figures 176-177) showed the proton resonances of 2-CH_3, 2-CH_3, 3-OCH_3 and 4-OCH_3 at δ 1.117 (3H, s), 1.216 (3H, s), 3.665 (3H, s) and 3.899 (3H, s), respectively. Three aromatic protons were shown at δ 7.108 (1H, d, J = 7.93 Hz), 7.401 (1H, dd, J = 0.62, 7.63 Hz) and 7.475 (1H, t, J = 7.63, 7.63 Hz). The signal at δ 7.108 was assigned to H-5 since it showed ortho coupling with H-6 and meta coupling with H-7. The signal at δ 7.401 was assigned to H-7 because it showed ortho coupling with H-6 and meta coupling with H-5. The signal at δ 7.475 was assigned to H-6 because it showed ortho coupling with H-5 and with H-7. These assignments were confirmed by the HMBC experiment. The ^{1}H NMR assignments are shown in Table 15.

In order to prove the structure and to obtain complete ^{1}H and ^{13}C NMR assignments of compound **111**, several 1-D and 2-D NMR techniques, including DEPT, HMQC and HMBC experiments, were used. The ^{13}C (Figure 178), DEPT (Figure 179) and HMQC (Figures 180-182) spectra showed all protonated carbons. Thus, the three aromatic carbons resonances at δ 115.759, 116.236 and 131.47 were assigned to C-7, C-5 and C-6, respectively. The four methyl signals at δ 55.794, 52.915, 23.024 and 18.319 were assigned to 4-OCH_3, 3-OCH_3, 2-CH_3 and 2-CH_3, respectively. The HMBC experiment was applied to analyze the quaternary carbons. From the HMBC spectrum (Figures 183-187), the two 2-CH_3 groups showed 3-bond coupling between each other. The protons of both 2-CH_3 groups showed 2-bond coupling with a quaternary carbon at δ 55.038 which could be assigned to C-2. The quarternary carbon at δ 82.083 was assigned to C-3 according to its 3-bond coupling

with the 2-CH_3 protons. H-7 exhibited 3-bond coupling with the quaternary carbon signal at δ 206.257 which could be assigned to C-1. This was confirmed by 3-bond coupling between the 2-CH_3 protons and this carbon (C-1). The carbonyl carbon signal at δ 174.111 belonged to the 3-methoxycarbonyl group demonstrating 3-bond connectivity to 3-OCH_3. The 4-OCH_3 protons showed 3-bond correlation to the quaternary carbon signal at δ 156.986 which was assigned to C-4. The 3-bond coupling between H-6 and this carbon (C-4) supported this assignment. The 3-bond correlation between H-7 and C-3a at δ 138.182 was observed. This was corroborated by the 3-bond coupling between C-3a and H-5. The final carbon signal at δ 137.228 was assigned to C-7a. This was confirmed by the 3-bond coupling observed between H-6 and C-7a. The HMBC correlations of this compound are illustrated in Figure 188. The ^{13}C NMR assignments are in Table 15.

Figure 188 The HMBC illustration of compound **111**

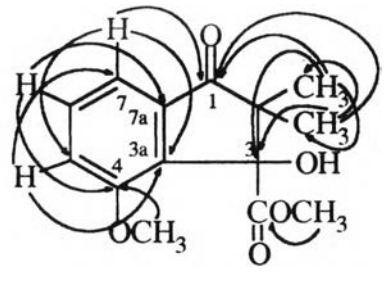


Table 15 The proton and carbon assignments of compound **111**

Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	206.257
2	-	-	55.038
3	-	-	82.083
3-$\underline{C}OOCH_3$	-	-	174.111
4	-	-	156.986
5	7.108	d, 7.93	116.236
6	7.475	t, 7.63, 7.63	131.470
7	7.401	dd, 0.62. 7.63	115.759
3a	-	-	138.182
7a	-	-	137.228
2-CH_3	1.117	s	18.319
2-CH_3	1.216	s	23.024
3-OCH_3	3.665	s	52.915
4-OCH_3	3.899	s	55.794

2.4 Halogenation of 5-methoxyplumbagin (106) at C-3

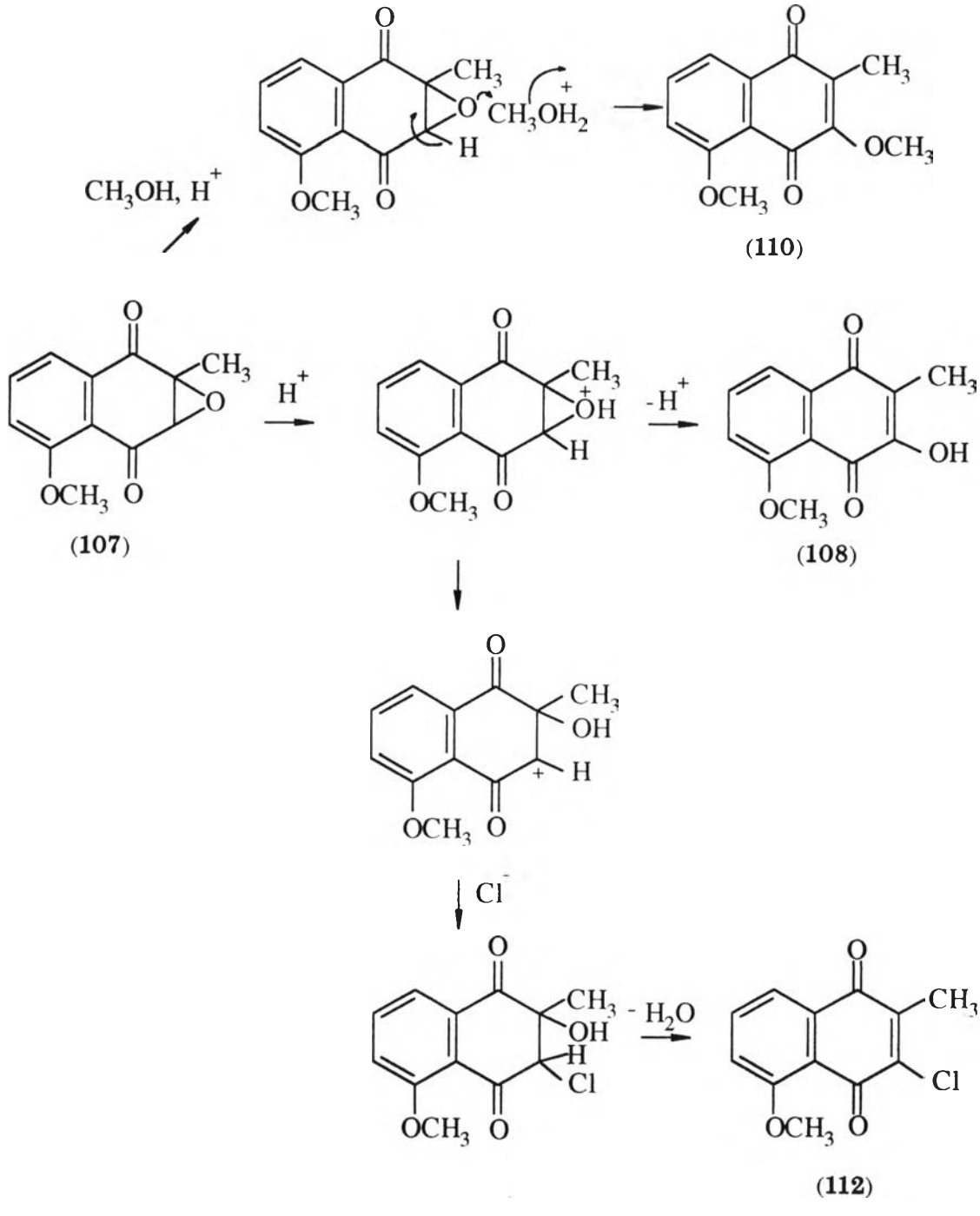
Hydrochloric acid was the halogenating agent in this reaction. First, 5-methoxyplumbagin (**106**) was converted to compound **107**. Then the intermediate **107** was allowed to react with HCl in MeOH to furnish the halogenation product **112**.

2.4.1 Preparation of compound 107

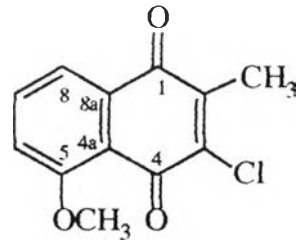
This reaction was the epoxidation of 5-methoxyplumgin to obtained compound **107**.

2.4.2 Chlorination of compound 107

Compound **107** (5-methoxy-2-methyl-1,4-naphthoquinone-2,3-epoxide) was the starting material. The mechanism of reaction is as follows. The acid (HCl) protonated compound **107**, to form a carbocation which then reacted with the halide ion (chloride), (Morrison and Boyd, 1983). This intermediate was subsequently dehydrated to give 3-chloro-5-methoxy-1,4-naphthoquinone (**112**), (Scheme 25). This reaction also gave compound **108** and 3,5-dimetoxy-2-methyl-1,4-naphthoquinone (**110**). The poor yield of the main product and the occurence of several by products suggested that MeOH was not a good solvent for this reaction and the formation of compound **110** suggested that the epoxide ring opening and the *O*-methylation were concerted reactions since in this study the reaction of compound **108** with 5% HCl in MeOH failed to yield compound **110**.



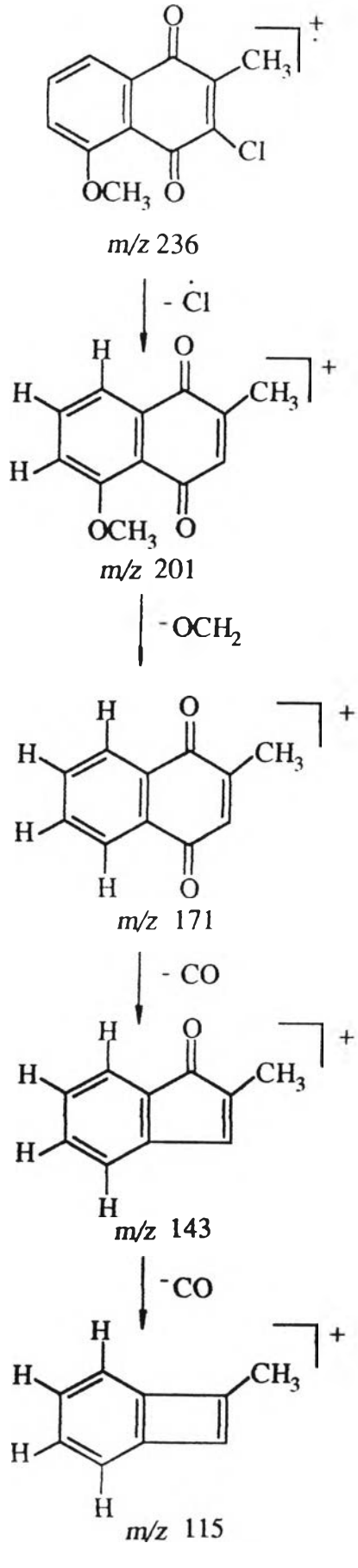
Scheme 25 Chlorination of compound **107**



(112)

Compound **112** was obtained as a yellow powder. The HRMS of compound **110** exhibited the $[M^+]$ ion peak at *m/z* 236.0228 (calculated 236.0238) and $[M+2]^+$, at *m/z* 238.0225 (calculated 238.0208), with a 3:1 ratio for $[M]^+$: $[M+2]^+$, establishing the molecular formula $C_{12}H_9O_3Cl$. The mass fragmentation of this compound was proposed by comparison with that of 5-methoxyplumbagin (**106**) (Scheme 26). The IR spectrum (Figure 190) showed C-H stretching (2800 cm^{-1}), C=O stretching (1656 cm^{-1}) and C-H bending (1321 cm^{-1}).

The structure of compound **112** was established as 3-chloro-5-methoxy-2-methyl-1,4-naphthoquinone. Its structure was hitherto unknown. The ^1H NMR spectrum of compound **110** (Figure 191) showed the proton resonances of 2-CH_3, 5-OCH_3 groups at δ 2.302 (3H, s) and 4.020 (3H, s), respectively. The three aromatic protons were found to resonate at δ 7.340 (1H, dd, J = 0.92, 8.39 Hz), 7.680 (1H, t, J = 7.63, 7.63 Hz) and 7.77 (1H, dd, J = 0.92, 7.63 Hz). The signal at δ 7.340 was assigned to H-6 because it showed meta coupling with H-8 and ortho coupling with H-7. The signal at δ 7.680 was assigned to H-7 because it showed ortho coupling with H-6 and H-8. The signal of H-8 at δ 7.770, exhibited meta coupling with H-6 and ortho coupling with H-7. These assignments were confirmed



Scheme 26 Mass fragmentation of compound **112**

by the HMBC experiment. The complete ^{1}H NMR assignments are shown in Table 16.

The assignments of the ^{13}C NMR resonances of this compound were achieved by 1-D and 2-D NMR experiments, including the DEPT, HETCOR and HMBC techniques. The ^{13}C (Figure 192), DEPT (Figure 193) and HETCOR (Figure 194) spectra allowed the assignments of all protonated carbons. Thus, the aromatic carbon resonances at δ 117.865, 119.658 and 135.139 were assigned to C-6, C-8 and C-7, respectively, while the methyl signals at δ 56.535 and 14.14 were assigned to 5-OCH_3 and 2-CH_3, respectively. Assignments of the quaternary carbons were then obtained by analysis of the HMBC spectrum (Figures 195-198). In the HMBC spectrum, the 2-CH_3 protons showed 2-bond correlation to a quaternary carbon at signal 144.976 which should be assigned to C-2 and 3-bond connectivity to a quaternary carbon signal 142.427 which should be assigned to C-3. The 3-bond coupling between these protons (2-CH_3) and C-1 at δ 182.864 was observed. The 3-bond coupling between H-8 and C-1 supported this assignment. H-8 also showed 3-bond correlation to the quaternary carbon at δ 119.164 which could be assigned to C-4a, and this was confirmed by the 3-bond coupling between C-4a and H-6. H-6 exhibited 3-bond coupling with C-8 (δ 119.658). The quaternary carbon at δ 133.938 was assigned to C-8a because it showed 3-bond connectivity to H-7, and this proton (H-7) also showed 3-bond correlation to a quaternary carbon at δ 160.046 which could be assigned to C-5. This was confirmed by the 3-bond coupling between C-5 and 5-OCH_3 protons. The last quarternary carbon at δ 176.003 was assigned to C-4. The illustration of HMBC correlations of compound

112 is displayed in Figure 199. The ^{13}C NMR assignments of compound **112** is shown in Table 16.

Figure 199 The HMBC illustration of compound **112**

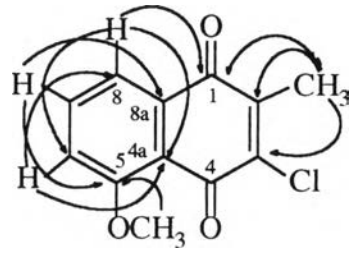


Table 16 The proton and carbon assignments of compound **112**

Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
1	-	-	182.864
2	-	-	144.976
3	-	-	142.427
4	-	-	176.003
5	-	-	160.046
6	7.340	dd, 0.92, 8.39	117.865
7	7.680	t, 7.63, 7.63	135.139
8	7.770	dd, 0.92, 7.63	119.658

Table 16 (Continued)

Carbon	^{1}H		^{13}C
	δ (ppm)	Multiplicity, *J* (Hz)	δ (ppm)
4a	-	-	119.164
8a	-	-	133.938
2-CH_3	2.302	s	14.140
5-OCH_3	4.020	s	56.535

3. Summary of proton and carbon assignments of isolated compounds and transformation products

Nine naphthoquinones derivatives were obtained from this study. They are plumbagin (**18**), droserone (**19**), 2-methylnaphthazarin (**104**), 5-acetyloxy-plumbagin (**105**), 5-methoxyplumbagin (**106**), 3-hydroxy-5-methoxyplumbagin (**108**), 3-acetyloxy-5-methoxyplumbagin (**109**), 3,5-dimethoxyplumbagin (**110**) and 3-chloro-5-methoxyplumbagin (**112**) The ^{1}H NMR and ^{13}C NMR data of these compounds could be discussed as summarized below.

3.1 Proton assignments of plumbagin (18) and related compounds

3.1.1 Chemical shifts of olefinic and aromatic protons

The H-3 proton was found at ~ δ 6.70-6.80 ppm. The H-6 proton was found at ~ δ 7.20-7.30 ppm. The H-7 proton was found at ~ δ 7.50-7.80 ppm (except for compound **104** in which the 8-OH group caused an upfield shift for H-

7). The H-8 proton was found ~ δ 7.60-8.00 ppm. In all cases, the chemical shifts of these protons are in the order :

H-8 > H-7 > H-6 > H-3.

3.1.2 Hydroxyl groups

The 5-OH group was always found at ~ δ 11.00-12.00 ppm, but the 3-OH group was found at ~ δ 7.20-7.70 ppm.

3.1.3 Methoxy substituents

The 5-OMe group was found at ~ δ 4.00 ppm, while the 3-OMe group was found at ~ δ 4.10 ppm.

3.1.4 Acetyloxy groups

The 3- or 5-OAc group was found at ~ δ 2.30-2.40 ppm.

3.1.5 2-Methyl group

The chemical shift of 2-CH_3 protons was found between δ 2.00 and 2.50 ppm. The presence of a hydroxyl or an acetyloxy group at C-3 caused a small upfield shift (~ 0.1 ppm) for the 2-CH_3 protons. For example, the chemical shift of 2-CH_3 of compound **19** was 0.092 ppm more upfield than that of compound **18**. Similar observations were found between compounds **108** and **106**, and between compounds **109** and **106**. On the other hand, the 3-OCH_3 or 3-Cl group moved the 2-CH_3 protons to a more downfield position (~ 0.2-0.3 ppm), as seen in the chemical shift of 2-CH_3 protons in compounds **110** and **112** when compared with that of compound **106**.

3.2 Carbon assignments of plumbagin (18) and related compounds

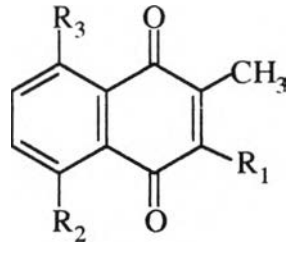
3.2.1 Effects of C-5 substituents

The 5-methoxy carbon was always found at ~ δ 56.00 ppm, while the 3-methoxy carbon was always found at ~ δ 61.00 ppm. The chemical shift of C-1 was more downfield than C-4 when C-5 was substituted with a methoxy or an acetyloxy group, but the reverse was true when C-5 was substituted with a hydroxyl group. The 5-methoxy or 5-acetyloxy group caused an upfield shift for C-5 when compared with the 5-hydroxy substituent (compounds **104**, **105** and **106**). The chemical shift of C-6 was moved to a more upfield position but C-4a was moved to a more downfield position if 5-OH was replaced by 5-OMe (compounds **104** and **106**). Both C-6 and C-4a were moved to a more downfield position if the 5-hydroxyl group was replaced with an acetyloxy group (compounds **104** and **105**). Finally, the 2-methyl carbon absorbed at a more upfield position when C-3 has a substituent rather than a hydrogen.

3.2.2 Effects of C-3 substituents

The chemical shift of C-3 was moved to a more downfield position when H-3 was replaced with a hydroxyl, a methoxy, an acetyloxy or a chloride group because of the electronegativity of the oxygen or chlorine atom (compounds **106-112**). The hydroxyl, methoxy, acetyloxy or chloride group at C-3 caused an upfield shift (about 4-8 ppm) for C-4 and an upfield shift (up to 27 ppm) for C-2 (compounds **106-112**).

Figure 200 Structures of plumbagin (**18**) and related compounds



Compound	R_1	R_2	R_3
18	H	OH	H
19	OH	OH	H
104	H	OH	OH
105	H	$OCOCH_3$	H
106	H	OCH_3	H
108	OH	OCH_3	H
109	$OCOCH_3$	OCH_3	H
110	OCH_3	OCH_3	H
112	Cl	OCH_3	H

Table 17 Summary of the ^{1}H NMR data of compounds **18-19** and **102-112**

Carbon	^{1}H NMR, δ (ppm)								
	18	**19**	**104**	**105**	**106**	**108**	**109**	**110**	**112**
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	6.790	-	6.809	6.711	6.741	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-
6	7.236	7.198	7.291	7.359	7.291	7.240	7.284	7.251	7.340
7	7.590	7.621	7.200	7.730	7.660	7.694	7.674	7.631	7.680
8	7.618	7.660	-	8.060	7.758	7.790	7.785	7.731	7.770
4a	-	-	-	-	-	-	-	-	-
8a	-	-	-	-	-	-	-	-	-
2-CH_3	2.190	2.098	2.011	2.162	2.142	2.066	2.061	2.504	2.302
5-OH	11.950	11.099	12.579	-	-	-	-	-	-
8-OH	-	-	12.579	-	-	-	-	-	-
3-OH	-	7.229	-	-	-	7.705	-	-	-
5-$OCOCH_3$	-	-	-	2.433	-	-	-	-	-
3-$OCOCH_3$	-	-	-	-	-	-	2.398	-	-
5-OCH_3	-	-	-	-	4.010	4.034	3.998	4.009	4.020
3-OCH_3	-	-	-	-	-	-	-	4.107	-

Table 18 Summary of the ^{13}C NMR data of compounds **18-19** and **102-112**

Carbon	^{13}C NMR, δ (ppm)								
	18	**19**	**104**	**105**	**106**	**108**	**109**	**110**	**112**
1	184.689	184.163	185.199	184.767	185.756	184.788	184.981	185.759	182.864
2	149.550	121.797	150.043	146.857	145.355	117.996	133.461	128.903	144.976
3	135.385	152.774	134.941	134.409	134.602	153.580	151.882	158.746	142.427
4	190.201	184.492	190.513	183.552	184.465	179.622	176.570	180.248	176.003
5	161.098	161.197	161.362	149.208	159.379	160.013	160.062	159.519	160.046
6	124.100	123.146	124.330	125.066	119.343	116.647	117.774	117.190	117.865
7	136.027	137.507	137.985	136.848	137.847	136.191	135.213	134.727	135.139
8	119.214	119.641	135.566	129.372	119.947	119.592	119.404	118.918	119.658
4a	115.052	112.913	115.479	123.426	117.644	116.861	118.451	119.280	119.164
8a	131.996	132.704	128.213	133.813	134.372	135.171	134.272	134.349	133.938
2-CH_3	16.460	8.760	16.641	16.019	15.756	8.431	9.572	8.859	14.140
5-$OCOCH_3$	-	-	-	169.391	-	-	-	-	-
5-$OCOCH_3$	-	-	-	21.056	-	-	-	-	-
3-$OCOCH_3$	-	-	-	-	-	-	167.934	-	-
3-$OCOCH_3$	-	-	-	-	-	-	20.368	-	-
5-OCH_3	-	-	-	-	56.443	56.452	56.455	56.403	56.535
3-OCH_3	-	-	-	-	-	-	-	60.944	-