

CHAPTER IV

RESULTS AND DISCUSSION

The dried roots of *Prismatomeris sessiliflora* Pierre ex Pitard (1.25 kg) were extracted with methanol (5x4 L) at room temperature. The methanol extract was partitioned between chloroform and water. The chloroform extract was then separated by repetitive chromatography to afford two compounds (PS-A and PS-B).

The dried roots of *Diospyros montana* Roxb. (2.6 kg) were extracted with petroleum ether (3x10 L), ethyl acetate (3x10 L), and methanol (3x10 L) to give a pet. ether extract (5 g), an ethyl acetate (12.34 g) and a methanol extract (90 g), respectively. The ethyl acetate and petroleum ether extracts were separated by various chromatographic techniques to afford four compounds (DM-A, DM-B, DM-C and DM-D).

The structures of all the isolates were determined through interpretation of their UV, IR, MS and NMR data, and subsequently confirmed by comparison of these values with those reported in the literature.

1. Structure Determination of Compound PS-A [1]

The EI mass spectrum of compound PS-A (Figure 3) exhibited a molecular ion at m/z 254, consistent with a molecular formula of $C_{15}H_{10}O_4$. The UV spectrum in methanol (Figure 4) demonstrated absorption maxima in methanol at λ_{max} ($\log \epsilon$) 244 (4.34), 278 (4.41), 412 (3.75) nm which were characteristics of the anthraquinone chromophore (Thomson, 1971: 57). The IR spectrum (Figure 5) exhibited maximum absorption bands at 3398 (O-H stretching), 2923 (C-H stretching), 1664-1624 (C=O stretching), 1589 (C-H stretching of aromatic ring), 1339-1311 (C-O stretching) cm^{-1} .

Compound PS-A could be assigned as the known compound rubiadin by analysis of its ^1H and ^{13}C NMR spectral properties. Its ^1H and ^{13}C NMR data (CD_3OD) have been earlier reported (Kusamba *et al.*, 1993: 18-22). In this investigation, the NMR studies were done in $\text{DMSO}-d_6$.

From the ^1H NMR spectrum (Figures 6a-6b), the presence of the following features was indicated: one methyl group at δ 2.04, five aromatic methine protons at δ 7.21 (1H, s), 7.85 (1H, ddd, $J=1.5, 7.5, 7.5$ Hz), 7.88 (1H, ddd, $J=1.5, 7.5, 7.5$ Hz), 8.09 (1H, dd, $J=1.5, 7.5$ Hz), 8.16 (1H, dd, $J=1.5, 7.5$ Hz), one H-bonded hydroxyl group at δ 13.08 (1H, s), respectively. The most downfield signal at δ 13.08 (1H, s) was assigned to 1-OH group because of its intramolecular hydrogen bonding to the C-9 carbonyl oxygen.

The ^{13}C NMR spectrum (Figures 7a-7b) showed fifteen carbons which could be classified by examination of the DEPT 135 and DEPT 90 spectra (Figures 8a-8b). The spectral data showed two carbonyl groups, five methine carbons, one methyl carbon and seven quaternary carbons.

Based on the information obtained from the HETCOR spectrum (Figures 9 a-9b), all protonated carbons of compound PS-A were assigned, as shown in Table 11.

Table 11 Carbon-proton correlations of compound PS-A observed in the HETCOR spectrum

Carbon	δ_c (ppm)	Correlation with proton at δ_H (ppm)
C-4	108.32	7.21
C-5	127.35	8.09
C-6	135.00	7.85
C-7	135.14	7.88

Table 11 (continued)

Carbon	δ_c (ppm)	Correlation with proton at δ_H (ppm)
C-8	127.01	8.16
2-CH ₃	9.06	2.04

The long-range C-H correlations of compound PS-A could be observed from the HMBC spectrum (Figures 10a-10f), as summarized in Table 12.

Table 12 Carbon-proton correlations of compound PS-A observed in the HMBC spectrum

Proton	δ_H (ppm)	Correlation with carbon
H-4	7.21	C-9a, C-2, and C-10
H-5	8.09	C-10, C-8a, and C-7
H-6	7.85	C-8 and C-10a
H-7	7.88	C-5 and C-8a
H-8	8.16	C-9, C-10a, and C-6
1-OH	13.08	C-9a, C-2, and C-1*
2-CH ₃	2.04	C-2*, C-1, and C-3

* Two-bond coupling.

The 2-CH₃ proton showed correlations with C-2, C-1, and C-3, suggesting the position of methyl substitution at C-2. The H-4 proton showed 3-bond correlations with C-9a, C-2, and C-10. The H-5 proton showed correlations with C-10, C-8a, and C-7. The H-6 proton showed correlations with C-8 and C-10a. The H-7 proton showed correlations with C-5 and C-8a. The H-8 proton showed correlation with C-9, C-10a, and C-6. The correlations of 1-OH proton with C-9a, C-2 and C-1 confirmed the assignments of C-1. The results from the HMBC experiment confirmed the structure of compound PS-A and all of the quaternary carbon resonances were assigned completely. The ¹H and ¹³C NMR data were compared with those rubiadin (Kusamba *et al.*, 1993: 18-22), as summarized in Table 13.

Table 13 ^1H and ^{13}C NMR spectral data of compound PS-A (in $\text{DMSO-}d_6$) and rubiadin* (in $\text{MeOH-}d_4$)

Position	Compound PS-A		Rubiadin* [1]	
	δ_{H} (ppm) (multiplicity, J in Hz)	δ_{C} (ppm)	δ_{H} (ppm) (multiplicity, J in Hz)	δ_{C} (ppm)
1	-	163.10	-	162.4 ^a
2	-	118.03	-	121.2
3	-	163.82	-	162.8 ^a
4	7.21 (s)	108.32	7.22 (s)	107.3
4a	-	132.36	-	131.6 ^b
5	8.09 (dd, $J=1.5, 7.5$)	127.35	7.86 (dd, $J=2, 8$)	126.2 ^c
6	7.85 (ddd, $J=1.5, 7.5, 7.5$)	135.00	8.15 (dd, $J=8, 8$)	134.3 ^d
7	7.88 (ddd, $J=1.5, 7.5, 7.5$)	135.14	8.15 (dd, $J=8, 8$)	134.4 ^d
8	8.16 (dd, $J=1.5, 7.5$)	127.01	7.86 (dd, $J=2, 8$)	126.6 ^c
8a	-	133.71	-	132.9 ^b
9	-	186.57	-	186.1
9a	-	109.53	-	108.8
10	-	182.35	-	181.7
10a	-	133.56	-	132.8 ^b
1-OH	13.08 (s)	-	-	-
2-CH ₃	2.04 (s)	9.06	2.06 (s)	7.9

* From Kusamba *et al*, 1993: 18-22.

a, b, c, d Assignments may be reversed in the same column.

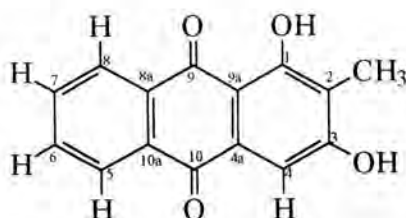


Figure 11 Structure of compound PS-A

2. Structure Determination of Compound PS-B [2]

The EI mass spectrum of compound PS-B (Figure 12) revealed its molecular ion at m/z 268, suggesting the molecular formula $C_{16}H_{12}O_4$. The UV absorption spectrum (Figure 13) showed maximal absorptions at λ_{max} 238, 279, and 361 nm, characteristic of the typical anthraquinone chromophore (Thomson, 1971: 57). The IR spectrum (Figure 14) indicated the presence of a hydroxyl group at 3315 (broad), carbonyl groups at 1673, aromatic rings at 1566 and an ether linkage at 1121 cm^{-1} .

Compound PS-B could be assigned as the known compound rubiadin-1-methyl ether by comparison of its 1H and ^{13}C NMR spectral properties with reported values (Kusamba *et al.*, 1993: 18-22). In this study, the unambiguous ^{13}C NMR assignments of rubiadin-1-methyl ether were obtained through careful analysis of the HETCOR and HMBC spectra.

From the 1H NMR spectrum (Figures 15a-15b), the structure of compound PS-B contained one methyl group at δ 2.13 (3H, s) and one methoxy group at δ 3.77 (3H, s). Five aromatic methine protons were found at δ 7.45 (1H, s), 7.80 (1H, ddd, $J=1.5, 7.5, 7.5$ Hz), 7.86 (1H, ddd, $J=1.5, 7.5, 7.5$ Hz), 8.08 (1H, dd, $J=1.5, 7.5$), 8.13 (1H, dd, $J=1.5, 7.5$ Hz), respectively.

The ^{13}C NMR spectrum (Figures 16a-16b) showed sixteen carbons which could be classified by examination of the DEPT spectra (Figures 17a-17b). These spectral data suggested the presence of two carbonyl groups, two methyl carbons, five methine carbons and seven quaternary carbons. Two of the most downfield signals were assigned to the carbonyl groups.

The HETCOR spectrum (Figures 18a-18b) revealed correlation between the directly coupled 1H and ^{13}C nuclei. According to the HETCOR spectrum, all protonated carbons of compound PS-B could be assigned. The directly coupled 1H and ^{13}C are summarized in Table 14.

Table 14 Carbon-proton correlations of compound PS-B observed in the HETCOR spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-4	110.20	7.45
C-5	126.84	8.08
C-6	134.09	7.80
C-7	135.33	7.86
C-8	127.45	8.13
1-OCH ₃	61.37	3.77
2-CH ₃	9.91	2.13

The HMBC spectrum (Figures 19a-19f) showed correlations of the long range coupling between ¹H and ¹³C nuclei, providing information for assignment of the quaternary carbons. The 2-CH₃ protons showed correlations with C-2, C-1 and C-3, suggesting the position of methyl substitution at C-2. This was confirmed by the correlation between H-4 and C-2. The 1-OCH₃ protons showed correlations with C-1, suggesting the right position of methoxy group at C-1. The H-4 proton showed 3-bond correlation with C-2, C-9a, and C-10 as expected. The information from the HMBC spectrum of compound PS-B is summarized in Table 15.

Table 15 Carbon-proton correlations of compound PS-B observed in the HMBC spectrum

Proton	δ_H (ppm)	Correlation with carbon
H-4	7.45	C-2, C-9a, and C-10
H-5	8.08	C-7, C-8a, and C-10
H-6	7.80	C-8 and C-10a
H-7	7.86	C-5 and C-8a
H-8	8.13	C-6, C-10a, and C-9
1-OCH ₃	3.77	C-1
2-CH ₃	2.13	C-2*, C-1, and C-3

* Two-bond coupling.

Therefore, the complete proton and carbon assignments of compound PS-B (Table 16) were obtained through analysis of HETCOR and HMBC spectra.

Table 16 ^1H and ^{13}C NMR spectral data of compound PS-B (in $\text{DMSO-}d_6$) and rubiadin-1-methyl ether* (in $\text{DMSO-}d_6$)

Position	Compound PS-B		Rubiadin-1-methyl ether* [2]	
	δ_{H} (ppm) (multiplicity, J in Hz)	δ_{C} (ppm)	δ_{H} (ppm) (multiplicity, J in Hz)	δ_{C} (ppm)
1	-	161.50	-	160.4 ^a
2	-	127.06	-	126.0
3	-	163.54	-	161.5 ^a
4	7.45 (s)	110.20	7.56 (s)	109.1
4a	-	134.58	-	131.9 ^b
5	8.08 (dd, $J=1.5, 7.5$)	126.84	8.07 (dd, $J=2, 8$)	125.6 ^c
6	7.80 (ddd, $J=1.5, 7.5, 7.5$)	134.09	7.80 (dd, $J=8, 8$)	133.1 ^d
7	7.86 (ddd, $J=1.5, 7.5, 7.5$)	135.33	7.80 (dd, $J=8, 8$)	134.3 ^d
8	8.13 (dd, $J=1.5, 7.5$)	127.45	8.07 (dd, $J=2, 8$)	126.3 ^c
8a	-	132.93	-	133.6 ^b
9	-	180.83	-	180.1 ^e
9a	-	117.81	-	117.7
10	-	183.64	-	182.4 ^c
10a	-	135.45	-	134.4 ^b
1-OCH ₃	3.77 (s)	61.37	3.86 (s)	60.2
2-CH ₃	2.13 (s)	9.91	2.20 (s)	8.6

* From Kusamba *et al.*, 1993: 18-22.

^{a, b, c, d, e} Assignments may be reversed in the same column.

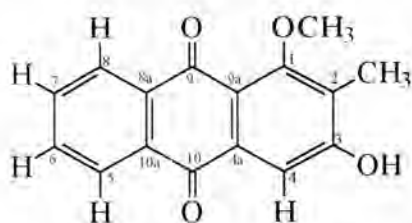


Figure 20 Structure of compound PS-B

3. Structure Determination of Compound DM-A [6]

Compound DM-A gave a molecular ion $[M^+]$ at m/z 374 in the EI mass spectrum (Figure 21), suggesting a tentative molecular formula of $C_{22}H_{14}O_6$. The UV spectrum (Figure 22) displayed UV absorptions at λ_{max} (log ϵ) 216 (4.48), 253 (4.08), 432 (3.65) nm, characteristic of a naphthoquinone dimer chromophore (Thomson, 1971: 49-52). The IR spectrum (Figure 23) showed two carbonyl peaks at 1670 and 1644 cm^{-1} , C-O stretching at 1259 cm^{-1} and the absence of a normal hydroxyl band, suggesting a chelated hydroxyquinone (Thomson, 1971: 64-67).

Comparison of its 1H and ^{13}C NMR spectra with reported data (Sankaram, Reddy, and Shoolery, 1987: 41-46) suggested that compound DM-A was identical with diospyrin [6]. Diospyrin was first isolated from the stem bark of *Diospyros montana* Roxb. (Kapil and Dhar, 1961: 498-500). The 1H and ^{13}C NMR spectral properties of this compound have been studied (Sankaram *et al.*, 1987: 41-46).

The results from several 1-D and 2-D NMR experiments in the present study, including HMQC and HMBC, suggested that the previous NMR assignments of H-8, H-8', C-8, C-8', C-9, C-9', C-10, and C-10' of diospyrin (Sankaram *et al.*, 1987: 41-46) should be revised.

The 1H NMR spectrum (Figure 24) indicated the presence of two hydroxyl groups at δ 11.86 and 12.12, two methyl groups at δ 2.29 and 2.44, and six methine

protons at δ 6.88 (1H, s), 6.94 (2H, s), 7.11 (1H, s), 7.49 (1H, d, $J=1$ Hz), 7.54 (1H, s), respectively.

The ^{13}C NMR spectrum (Figure 25) and DEPT spectra (Figure 26) provided signals for four carbonyl carbons, two methyl groups, six carbons and ten quaternary carbons.

All protonated carbons were assigned by analysis of the HMQC spectrum (Figures 27a-27c). The information from the HMQC spectrum of compound DM-A is summarized in Table 17.

Table 17 The carbon-proton correlations of compound DM-A observed in the HMQC spectrum

Position	δ_{C} (ppm)	correlation with proton at δ_{H} (ppm)
3	138.62	6.88
2	139.28	6.94
3'	138.68	6.94
6	124.12	7.11
8	121.17	7.49
8'	120.66	7.54
7'-CH ₃	21.21	2.29
7-CH ₃	22.40	2.44

The quaternary carbons could be assigned from the ^1H - ^{13}C long-range couplings displayed in the HMBC spectrum (Figures 28a-28h), as shown in Table 18.

Table 18 Carbon-proton correlations of compound DM-A observed in the HMBC spectrum

Proton	δ_H (ppm)	Correlation with carbon
H-3	6.88	C-9, C-6', and C-1
H-2', H-3'	6.94	C-1' and C-4'
H-6	7.11	7-CH ₃ , C-9, C-8, and C-5*
H-8	7.49	7-CH ₃ , C-9, C-6, and C-1
H-8'	7.54	7'-CH ₃ , C-9', C-6', and C-1'
7-CH ₃	2.44	C-8, C-6, and C-7*
7'-CH ₃	2.29	C-8', C-6', and C-7'
5-OH	11.86	C-9, C-6, and C-5*
5'-OH	12.12	C-9', C-6', and C-5'

* Two-bond coupling.

The results obtained from HMBC spectral examination suggested that the assignments of C-8 and C-8', C-9 and C-10, C-9' and C-10' in a previous report (Sankaram *et al.*, 1987 : 41-46) should be revised. The ¹H and ¹³C NMR data of DM-A are summarized in Table 19.

Table 19 ¹H and ¹³C NMR spectral data of compound DM-A (in CDCl₃) and diospyrin* (in CDCl₃)

Position	Compound DM-A		Diospyrin* [6]	
	δ_H (ppm) (multiplicity, <i>J</i> in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, <i>J</i> in Hz)	δ_C (ppm)
1	-	182.27	-	182.57
1'	-	183.85	-	184.12
2	-	145.57	-	145.89
2'	6.94 (s)	139.28	6.94 (s)	139.50
3	6.88 (s)	138.62	6.89 (s)	138.82

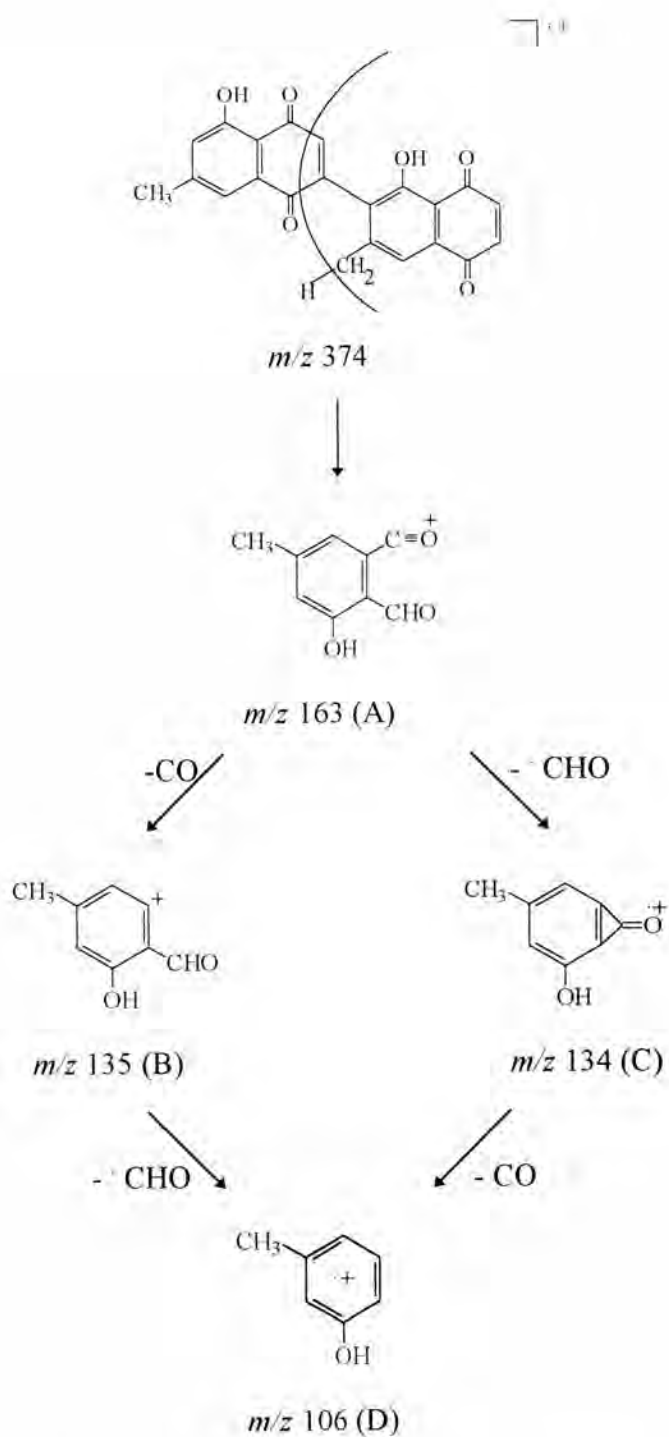
Table 19 (continued)

Position	Compound DM-A		Diospyrin* [6]	
	δ_{H} (ppm) (multiplicity, J in Hz)	δ_{C} (ppm)	δ_{H} (ppm) (multiplicity, J in Hz)	δ_{C} (ppm)
3'	6.94 (s)	138.68	6.94 (s)	138.88
4	-	188.64	-	188.98
4'	-	189.48	-	189.82
5	-	161.52	-	161.89
5'	-	158.96	-	159.28
6	7.11 (d, $J=1.0$)	124.12	7.12 (d, $J=1.5$)	124.28
6'	-	128.73	-	129.00
7	-	148.47	-	148.55
7'	-	146.30	-	146.51
8	7.49 (d, $J=1.0$)	121.17	7.55 (br s)	120.78
8'	7.54 (s)	120.66	7.50 (d, $J=1.5$)	121.28
9	-	113.10	-	131.82
9'	-	112.91	-	131.54
10	-	131.49 ^a	-	113.34
10'	-	131.22 ^a	-	113.15
7-CH ₃	2.44 (s)	22.40	2.45 (s)	22.23
7'-CH ₃	2.29 (s)	21.21	2.31 (s)	21.05
5-OH	11.86 (s)	-	11.85 (s)	-
5'-OH	12.12 (s)	-	12.11 (s)	-

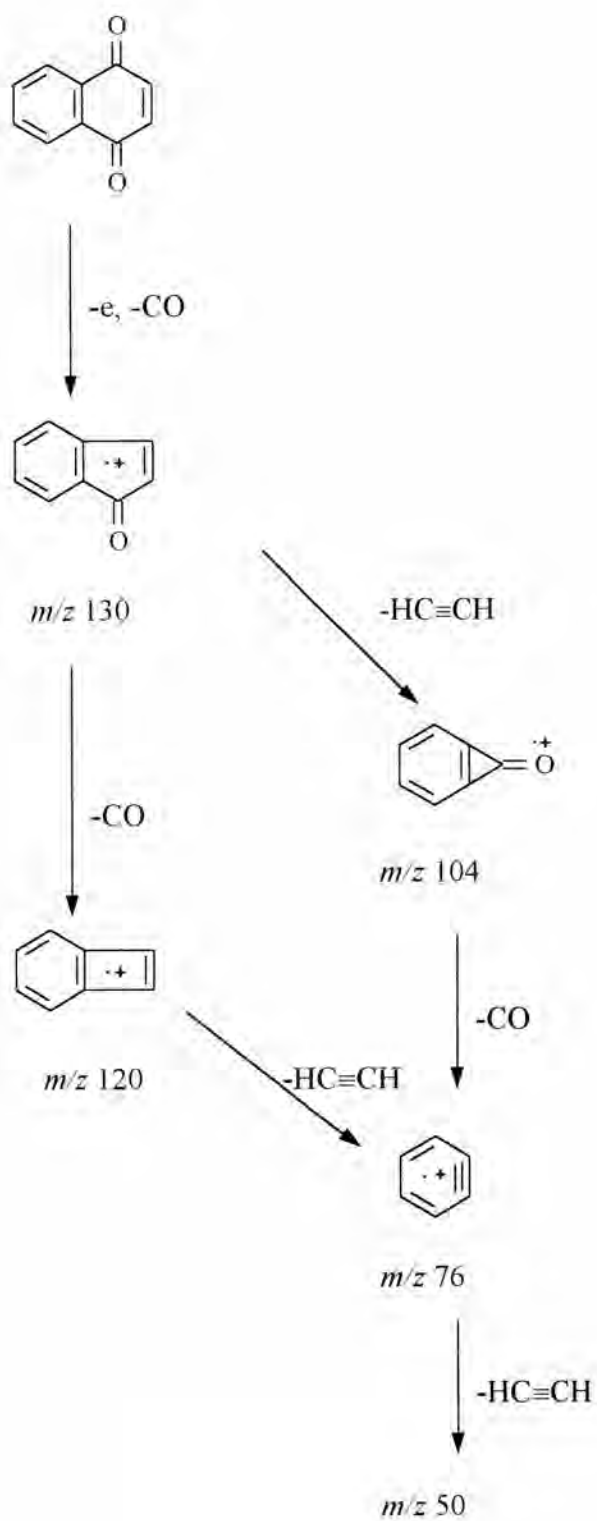
* Sankaram, *et al.* 1987: 41-46.

^a Assignments may be reversed in the same column.

The mass spectrum of compound DM-A shows four characteristic peaks at m/z 163, 135, 134, and 106 corresponding to ions A, B, C, and D (Scheme 3) (Sidhu, Pardhasaradhi, and Babu, 1976: 218-220). Fragment ions A, B, C and D arise from the cleavage of the inner quinone ring which carries an aryl substituent on C-2'. This fragmentation does not follow the normal course of expulsion of two moles of carbon monoxide resulting into a cyclobutene fragment (Scheme 4), as usually seen in naphthoquinone monomers (Bowie, Cameron, and Williams, 1965: 5094-5099).



Scheme 3 Mass fragmentation of compound DM-A



Scheme 4 Mass fragmentation of naphthoquinone monomer

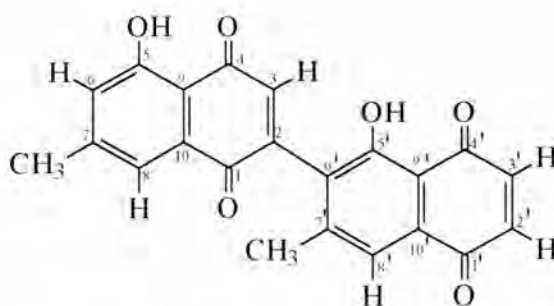


Figure 29 Structure of compound DM-A

4. Structure Determination of Compound DM-B

The EI mass spectrum of compound DM-B (Figure 30) showed the molecular ion $[M^+]$ at m/z 202, corresponding to $C_{12}H_{10}O_3$. The major fragmentation of compound DM-B in the mass spectrum showed the loss of a methyl group (m/z 187) followed by the expulsion of two molecules of carbon monoxide (m/z 159 and 131) (Harper, Kemp, and Tannock, 1970: 626-636). This compound showed typical naphthaldehyde UV absorptions (Figure 31) in methanol at λ_{max} (log ϵ) 217 (4.26), 257 (4.27), 299 (3.42) and 377 (3.57) nm (Harper *et al.*, 1970: 626-636). The IR spectrum (Figure 32) showed absorption bands at 3390 (O-H stretching), 1691 (C=O stretching), 1650-1450 (C=C stretching of aromatic ring), 1300-1000 (ether linkage) cm^{-1} .

Through comparison with earlier reported 1H NMR data (Harper *et al.*, 1970: 626-636), compound DM-B was identified as 5-hydroxy-4-methoxy-2-naphthaldehyde. This compound has been isolated from the heartwood of *Diopyros quiloensis* (Harper *et al.*, 1970: 626-636) and from the arial part of *Asarum himalaicum* (Hu *et al.*, 1989 :59-63). Regarding its NMR spectral data, only 1H NMR assignments have been reported. In this study, complete 1H and ^{13}C NMR assignments were obtained by analysis of the 1-D and 2-D NMR (HETCOR, COLOC, 1H - 1H COSY, and NOESY) spectra.

The ^1H NMR spectrum (Figures 33) showed signals for one methoxy group at δ 4.06 (3H, s), five aromatic protons at δ 7.02 (1H, dd, $J=2.57, 7.71$ Hz), 7.13 (1H, d, $J=1.03$ Hz), 7.41 (1H, m), 7.43 (1H, m), and 7.82 (1H, d, $J=1.03$ Hz), and one hydroxyl group at δ 9.24 (1H, s). The most downfield signal at δ 9.97 (1H, s) was assigned to the aldehydic proton.

The ^1H - ^1H COSY (Figures 34a-34b) showed coupling for the aromatic protons. To determine the positions of the aldehyde, methoxy, and hydroxyl groups, a NOESY experiment was carried out (Figures 35a-35b). The H-1 (δ 7.82) and H-3 (δ 7.13) signals showed NOE interactions with the resonance of 2-CHO at δ 9.97. The H-3 (δ 7.13) and 5-OH (δ 9.24) showed NOE interaction with the resonance of 4-OCH₃ (δ 4.06).

The NOE interactions observed in the NOESY spectrum of compound DM-B are summarized in Figure 36.

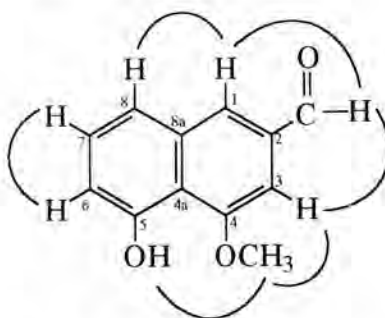


Figure 36 Results from the NOESY experiment of compound DM-B

Examination of the ^{13}C NMR spectrum (Figures 37) and DEPT spectra (Figure 38) provided signals for one carbonyl group, one methoxy carbon, five methine carbons, and five quaternary carbons.

Based on the information obtained from the HETCOR spectrum (Figure 39), all protonated carbons of compound DM-B were assigned, as shown in Table 20.

Table 20 Carbon-proton correlations of compound DM-B observed in the HETCOR spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-1	130.16	7.82
C-3	98.50	7.13
C-6	114.26	7.02
C-7	128.77	7.43
C-8	120.56	7.41
4-OCH ₃	56.38	4.06
2-CHO	191.35	9.97

The assignments of quaternary carbons were obtained from the COLOC correlations (Figures 40a-40b). The correlation of 2-CHO with H-3 and H-1 confirmed the location of aldehyde group at C-2. The correlation between 4-OCH₃ and C-4 confirmed the location of methoxy group on C-4. The information from the COLOC spectrum of compound DM-B is summarized in Table 21.

Table 21 Carbon-proton correlations of compound DM-B observed in the COLOC spectrum

Proton	δ_H (ppm)	Correlation with carbon
H-1	7.82	2-CHO, C-1*, C-4a, and C-3
H-3	7.13	2-CHO, C-1, C-4a, and C-3*
H-7	7.43	C-5, C-8a, C-7*, and C-6**
H-8	7.41	C-1, C-8, and C-4a
2-CHO	9.97	2-CHO* and C-2**
4-OCH ₃	4.06	C-4 and 4-OCH ₃ *
5-OH	9.24	C-5, C-4a, and C-6

* One-bond coupling; ** two-bond coupling.

The complete proton and carbon assignments of compound DM-B (in CDCl₃) and the previous proton assignments of 5-hydroxy-4-methoxy-2-naphthaldehyde (Harper *et al.*, 1970: 626-636) are summarized in Table 22.

Table 22 ¹H and ¹³C NMR spectral data of compound DM-B (in CDCl₃) and ¹H spectral data of 5-hydroxy-4-methoxy-2-naphthaldehyde* (in CDCl₃)

Position	Compound DM-B		5-Hydroxy-4-methoxy-2-naphthaldehyde*
	δ _C (ppm)	δ _H (ppm) (multiplicity, <i>J</i> in Hz)	δ _H (ppm) (multiplicity, <i>J</i> in Hz)
1	130.16	7.82 (d, <i>J</i> =1.03)	7.81 (d, <i>J</i> =1.5)
2	134.09	-	-
3	98.50	7.13 (d, <i>J</i> =1.03)	7.13 (d, <i>J</i> =1.5)
4	156.87	-	-
4a	117.32	-	-
5	154.48	-	-
6	114.26	7.02 (dd, <i>J</i> =2.57, 7.71)	7.03 (q, <i>J</i> =4.7)
7	128.77	7.43 (m)	7.43 (m, <i>J</i> =4.7)
8	120.56	7.41 (m)	7.43 (m, <i>J</i> =4.7)
8a	135.48	-	-
2-CHO	191.35	9.97 (s)	10.00 (s)
4-OCH ₃	56.38	4.06 (s)	4.06 (s)
5-OH	-	9.24 (s)	9.24 (s)

* Harper *et al.*, 1970: 626-636.



Figure 41 Structure of compound DM-B

5. Structure Determination of Compound DM-C [16]

Compound DM-C gave a violet color to anisaldehyde-sulphuric acid reagent, suggesting the presence of a triterpenoid nucleus. The EI mass spectrum of compound DM-C (Figure 42) showed the molecular ion $[M^+]$ at m/z 426, corresponding to $C_{30}H_{50}O$. The IR spectrum (Figure 43) showed absorption bands at 3350 (O-H stretching), 2944 and 2872 (C-H stretching), 1641 (C=C stretching), 1455 and 1382 (C-H bending) and 1042 (C-O stretching) cm^{-1} .

Comparison of its 1H and ^{13}C NMR spectra with reported data (Reynolds, McLean, and Poplawski, 1986: 3419-3428) suggested that compound DM-C was identical with lupeol [16].

The 1H NMR spectrum of compound DM-C (Figures 44a-44b) showed seven methyl signals at δ 0.73-1.65 ppm which could be assigned as shown in Table 23. The signals at δ 1.05-2.00 were the signals of methylene and methine protons. The signal at δ 3.16 (dd, $J=5.10, 10.50$ Hz) could be assigned to H-3 whereas the resonance at δ 2.35 (m) could be assigned to H-19. The signals at δ 4.54 (br s) and δ 4.66 (br s) could be assigned to H-29 (2H).

Table 23 ^1H NMR assignments of compound DM-C (in CDCl_3) and lupeol* (in CDCl_3)

Position	δ_{H} (ppm) (multiplicity, J in Hz)	
	Compound DM-C	Lupeol* [16]
C-23	0.94 (s)	0.98 (s)
C-24	0.73 (s)	0.77 (s)
C-25	0.80 (s)	0.84 (s)
C-26	1.00 (s)	1.04 (s)
C-27	0.92 (s)	0.97 (s)
C-28	0.76 (s)	0.97 (s)
C-30	1.65 (s)	1.69 (s)

* From Reynolds *et al.*, 1986: 3419-3428.

The ^{13}C NMR (Figures 45a-45b) and DEPT spectra (Figure 46) disclosed the presence of 30 carbon resonances which are in good agreement with those reported for lupeol whose unambiguous ^1H and ^{13}C NMR assignments were obtained by using the XCORFE pulse sequence (Reynolds *et al.*, 1986: 3419-3428). Their carbon assignments are shown in Table 24.

Table 24 The ^{13}C NMR assignments of compound DM-C (in CDCl_3) and lupeol* (in CDCl_3)

Carbon	δ_{C} (ppm)	
	Compound DM-C	Lupeol* [16]
1	38.66	38.67
2	27.36	27.35
3	78.95	78.94
4	38.82	38.81
5	55.25	55.25
6	18.28	18.28

Table 24 (continued)

Carbon	δ_c (ppm)	
	Compound DM-C	Lupeol* [16]
7	34.23	34.23
8	40.78	40.78
9	50.37	50.38
10	37.12	37.11
11	20.89	20.89
12	25.08	25.08
13	38.00	38.00
14	42.78	42.78
15	27.40	27.41
16	35.54	35.54
17	42.96	42.95
18	48.25	48.24
19	47.94	47.94
20	150.92	150.88
21	29.80	29.80
22	39.96	39.96
23	27.96	27.95
24	15.36	15.35
25	16.10	16.09
26	15.94	15.94
27	14.51	14.51
28	17.97	17.97
29	109.31	109.31
30	19.28	19.28

* From Reynolds *et al.*, 1986: 3419-3428.

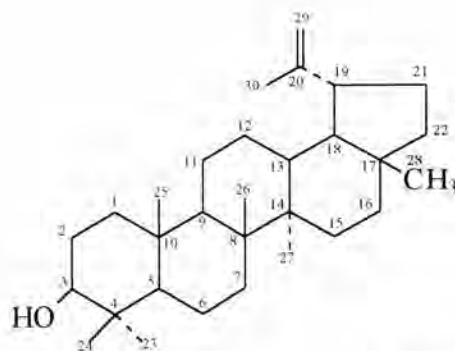


Figure 47 Structure of compound DM-C

6. Structure Determination fo Compound DM-D [17]

Compound DM-D gave a violet color to anisaldehyde-sulphuric acid reagent, suggesting the presence of a triterpenoid nuclues. The EI mass spectrum of compound DM-D (Figure 48) revealed a molecular ion at m/z 456, suggesting a molecular formula of $C_{30}H_{48}O_3$. The IR spectrum (Figure 49) revealed absorption bands at 3446 (O-H stretching), 2944 and 2871 (C-H stretching), 1712 (C=O stretching), 1642 (C=C stretching), 1453 and 1375 (C-H bending) and 1033 (C-O stretching) cm^{-1} .

By comparing the 1H and ^{13}C NMR spectra of compound DM-D with previously reported data (Siddiqui *et al.*, 1988: 229-233), compound DM-D was identified as betulinic acid.

The 1H NMR spectrum of compound DM-D (Figures 50a-50b) showed six methyl signals at δ 0.64-1.64 ppm which could be assigned and compared with betulinic acid as shown in Table 13. The signal at δ 3.17 (m) could be assigned to H-3 whereas the resonance at δ 2.97 (m) could be assigned to H-19. The signals at δ 4.55 (br s) and δ 4.68 (br s) could be assigned to the two vinyl protons of C-29.

Table 25 ^1H NMR assignments of compound DM-D (in $\text{DMSO-}d_6$) and betulinic acid* (in CDCl_3)

Position	δ_{H} (ppm) (multiplicity, J in Hz)	
	Compound DM-D	Betulinic acid* [17]
C-23	0.86 (s)	0.93 (s)
C-24	0.64 (s)	0.75 (s)
C-25	0.76 (s)	0.82 (s)
C-26	0.92 (s)	0.96 (s)
C-27	0.97 (s)	0.97 (s)
C-30	1.64 (s)	1.68 (s)

* From Siddiqui *et al.*, 1988: 229-233.

The ^{13}C NMR (Figures 51a-51b) and DEPT spectra (Figure 52) displayed carbon resonances which are in good agreement with those of betulinic acid. These data are quite similar to those of compound DM-C because of their closely related structures. The complete carbon assignments of compound DM-D and betulinic acid are shown in Table 26.

Table 26 The ^{13}C NMR assignments of compound DM-D (in $\text{DMSO-}d_6$) and betulinic acid* (in CDCl_3)

Carbon	δ_{C} (ppm)	
	Compound DM-D	Betulinic acid* [17]
1	38.34	38.7
2	27.24	27.4
3	76.78	78.9
4	38.57	38.8
5	54.93	55.3
6	18.09	18.3

Table 26 (continued)

Carbon	δ_c (ppm)	
	Compound DM-D	Betulinic acid* [17]
7	34.00	34.3
8	40.32	40.7
9	49.98	50.5
10	36.79	37.2
11	20.58	20.8
12	25.19	25.5
13	37.66	38.4
14	42.06	42.4
15	30.20	30.5
16	31.82	32.1
17	55.45	56.3
18	46.67	46.8
19	48.58	49.2
20	150.12	150.3
21	29.30	29.7
22	36.44	37.0
23	28.19	27.9
24	15.86	15.3
25	15.92	16.0
26	16.07	16.1
27	14.51	14.7
28	176.99	180.5
29	109.52	109.6
30	19.05	19.4

* From Siddiqui *et al.*, 1988: 229-233.

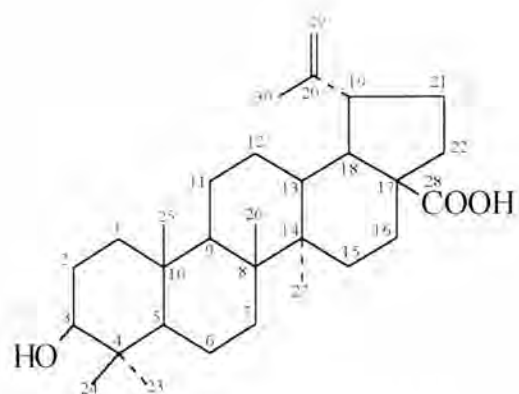


Figure 53 Structure of compound **DM-D**