

Supplementary Material

Isolation and *In silico* prediction of potential drug-like compounds with a new dimeric prenylated quinolone alkaloid from *Zanthoxylum rhetsa* (Roxb.) root extracts targeted against SARS-CoV-2 (Mpro)

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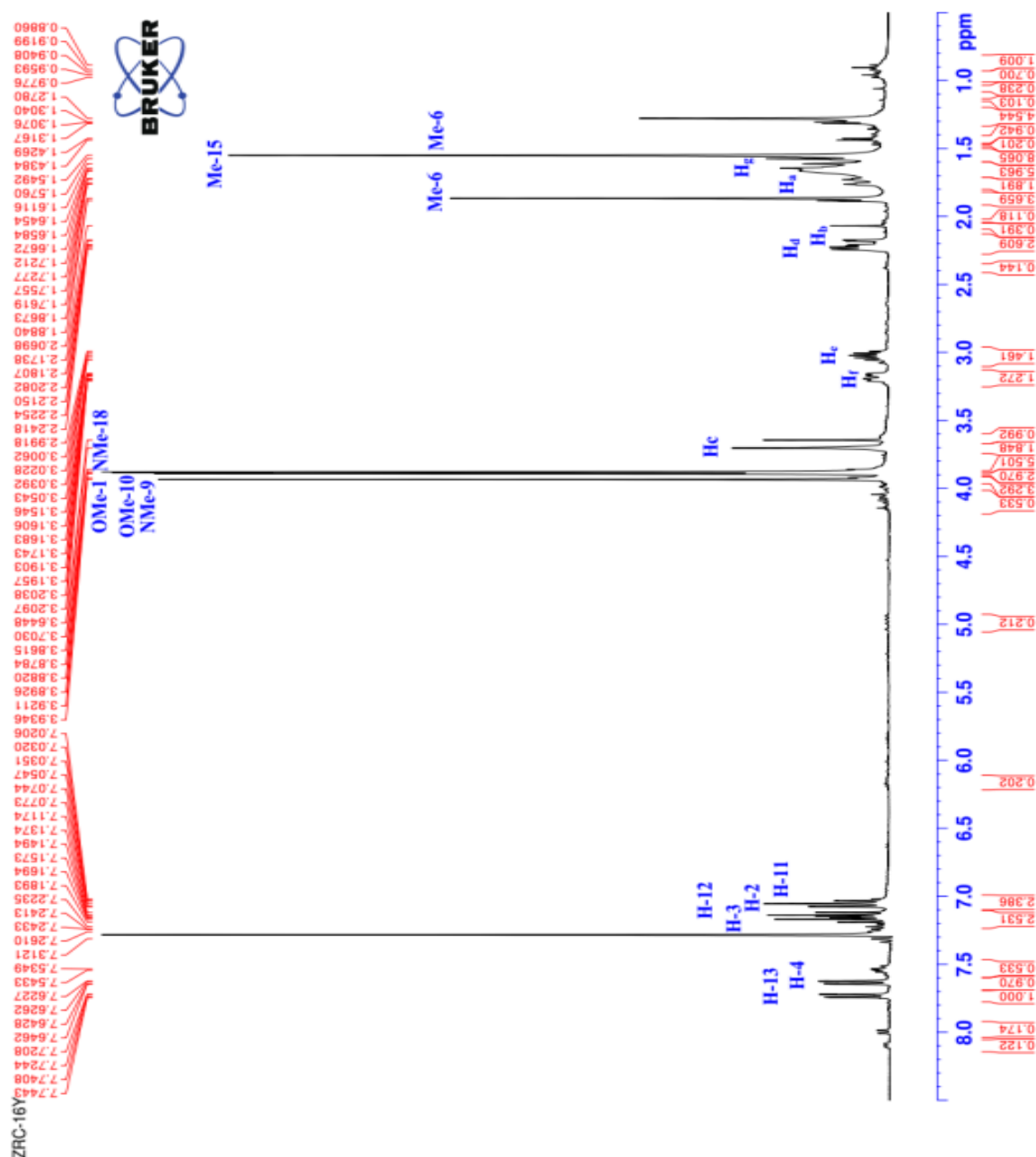
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1 Supplementary Data

2. NMR spectra of isolated compounds

2.1 NMR spectrum of compound 1 characterized as a new dimeric prenylated quinolone 2,11-didemethoxy-vepridimerine A:



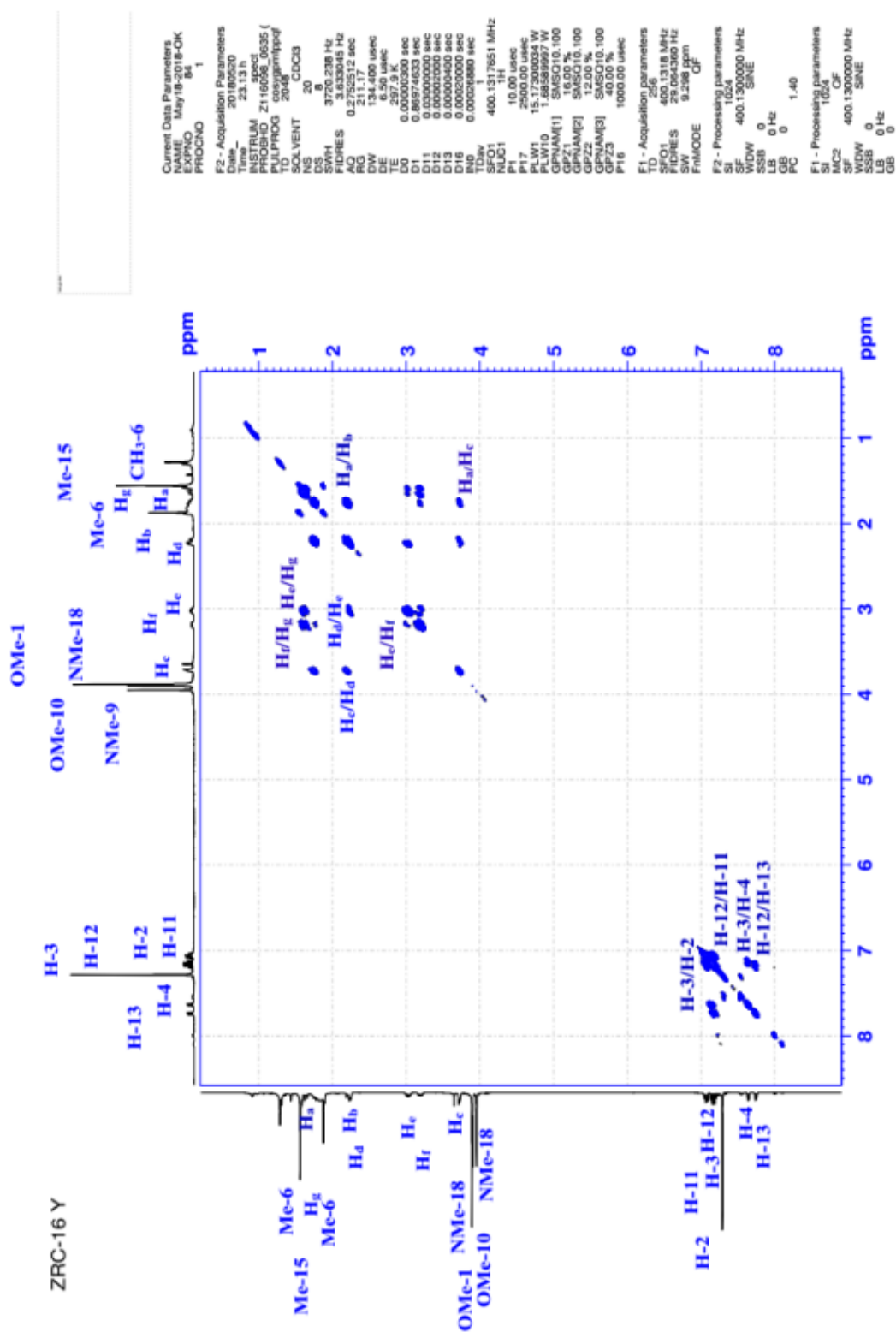
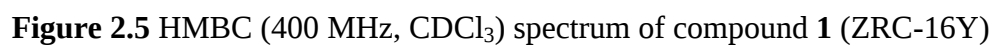


Figure 2.3 COSY (400 MHz, CDCl₃) spectrum of compound **1** (ZRC-16Y)



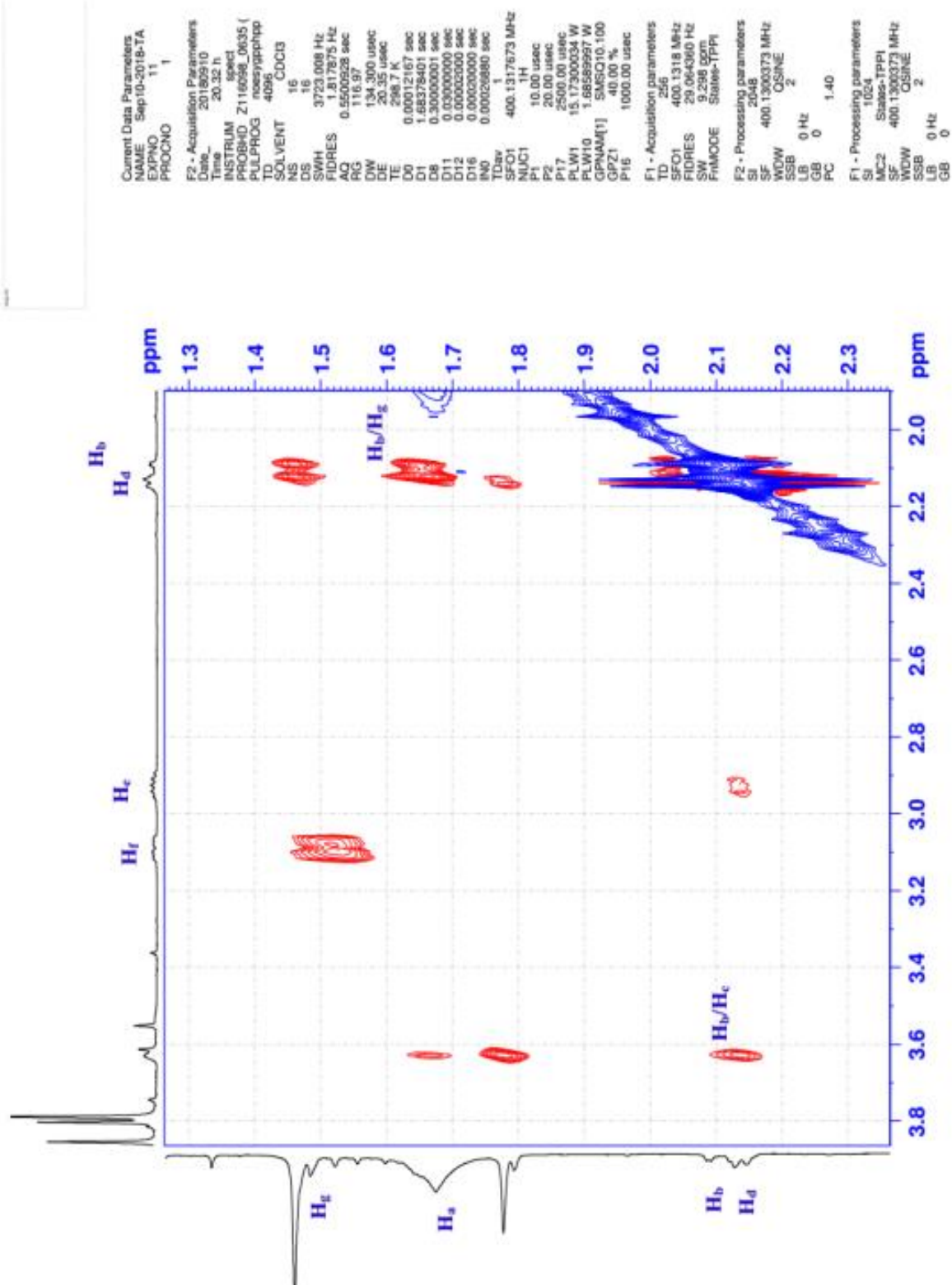


Figure 2.6 NOSEY (400 MHz, CDCl₃) spectrum of compound 1 (ZRC-16Y)

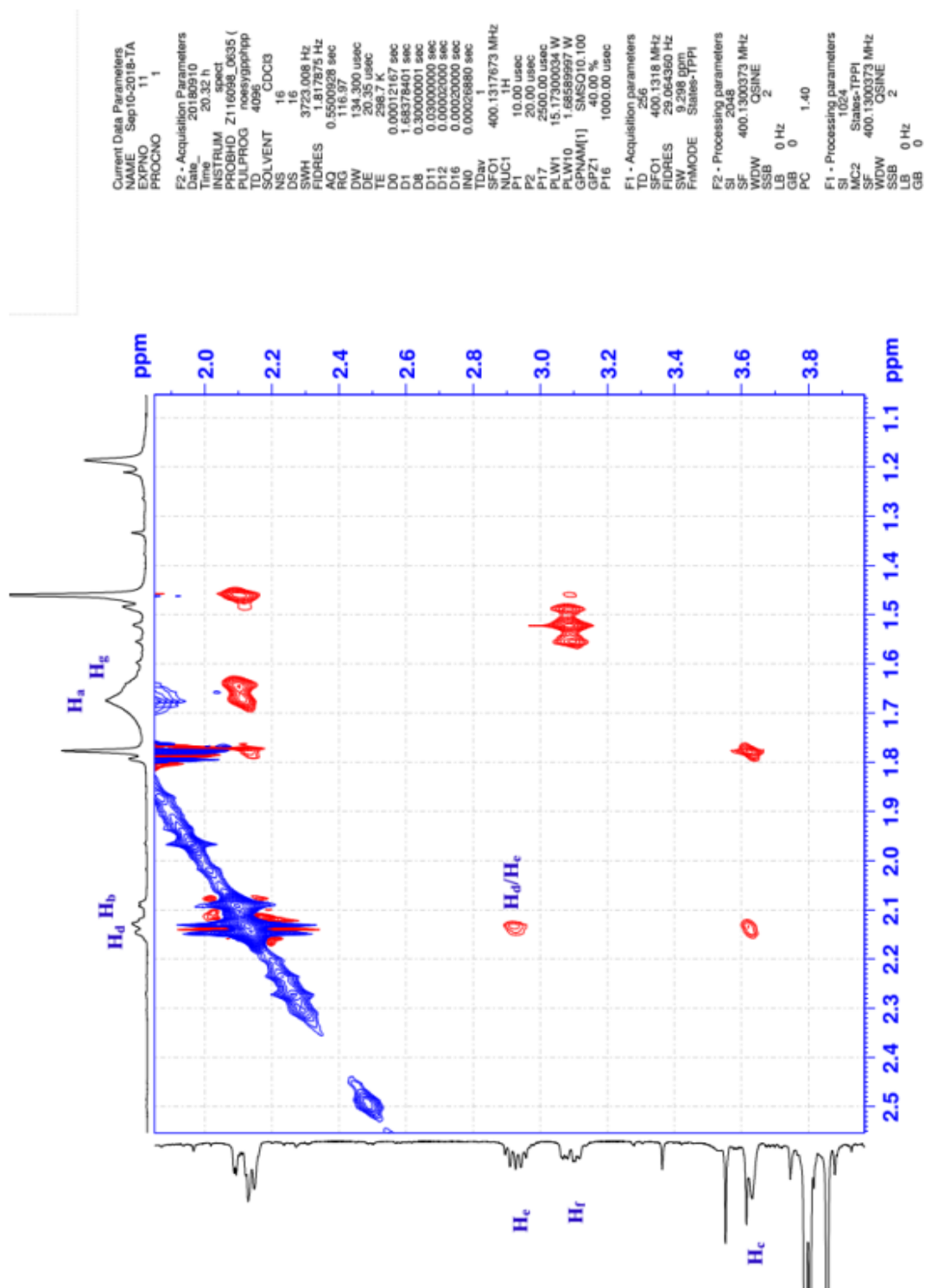


Figure 2.7 NOSEY (400 MHz, CDCl₃) spectrum of compound **1** (ZRC-16Y)

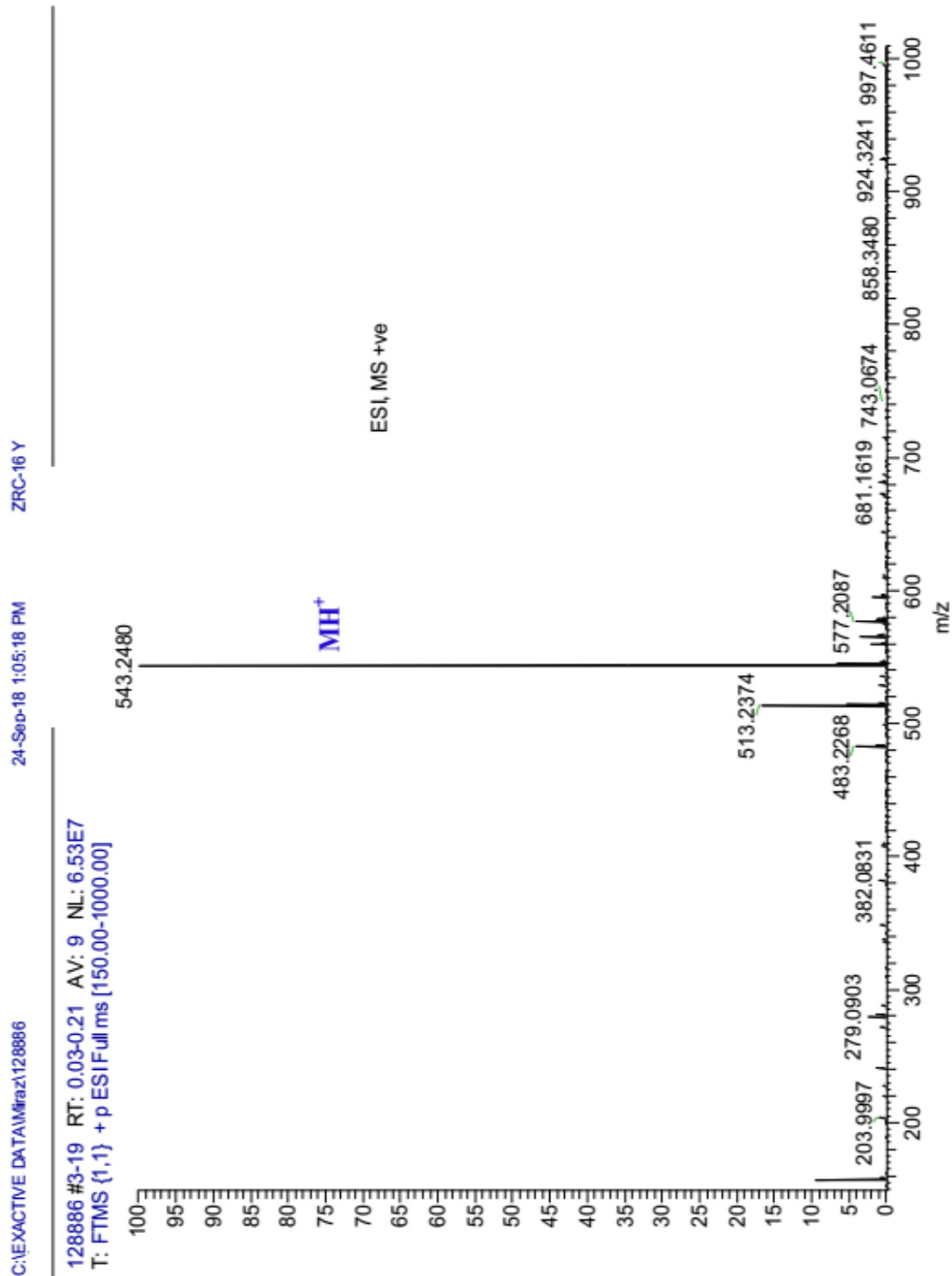
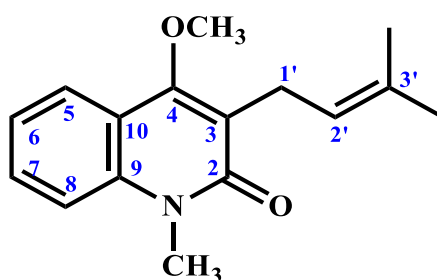


Figure 2.8 Mass spectrum of compound 1 (ZRC-16Y)

2.2 Characterization of Compound 2 and 3 (ZRP-14) as *N*-methyllatanine and 3-dimethylallyl-4,8-dimethoxy-1-methyl-2-quinolone

ZRP-14 was isolated as a greenish gum, showed a blue fluorescent spot under UV light at 366 nm on a TLC plate and did not give any color after spraying with vanillin-sulfuric acid reagent followed by heating for 2 minutes.

The ^1H NMR spectrum (400 MHz, CDCl_3 ; **Figure 2.9**) of ZRP-14 indicated a mixture of two prenylated 2-quinolones, compounds **2** and **3** which were confirmed by ^{13}C NMR (**Figure 2.10**), COSY (**Figure 2.11**), HSQC (**Figure 2.12**) and HMBC (**Figure 2.13**) experiments. The ^1H NMR spectrum (Table 1 & Table 2) displayed two olefinic protons at δ 5.20 (2H, t, $J = 7.0$ Hz), two methylene groups at δ 3.34 and 3.33 (2H, d, $J = 7.0$, each), four methyls at δ 1.62 (6H s) and δ 1.75 (6H s), indicating the presence of two prenyl (dimethylallyl) groups in the compounds. The spectrum further revealed four adjacent aromatic protons at δ 7.76 dd ($J = 8.0, 1.1$ Hz), 7.20 ddd ($J = 8.6, 8.0, 1.4$ Hz), 7.48 ddd ($J = (8.6, 8.4, 1.1)$ Hz) and 7.30 d ($J = 8.4$ Hz) comprising an ABCD ring system and three adjacent aromatic protons at δ 7.38 dd ($J = 8.0, 1.2$ Hz), 7.09 dd ($J = 8.0, 7.9$ Hz) and 6.97dd ($J = 7.9, 1.2$ Hz) comprising an ABC ring system, suggesting a di- and tri-substituted benzene rings. In addition, the ^1H NMR spectrum showed two *N*-methyl signals at δ 3.66 and 3.89 and three methoxy groups at δ 3.81, 3.82 and 3.85. The ^{13}C NMR spectrum showed 35 carbons including two carbonyl carbons at δ 164.0 and 165.0 and three oxygenated unsaturated carbons at δ 160.2, 160.0 and 148.8. Position of the prenyl groups in the two compounds was confirmed at C-3 by an HMBC experiment as methylene at 1' showed 2J correlation to C-3 and 3J correlation to C-2 and C-4 (Table 1 & Table 2). Further, the *N*-methyl groups in both of the compounds showed 3J correlations to both C-2 (C=O) and C-9. All of the above data strongly suggested that ZRP-14 is a mixture of two prenylated *N*-methyl 2-quinolones. The methoxy group at δ 3.85 showed 3J correlation to C-4 (160.2) of the di-substituted benzene ring while the remaining two methoxy groups at δ 3.81 and 3.82 showed 3J correlations to C-4 (δ 160.0) and C-8 (δ 148.8) of the tri-substituted benzene ring. On the basis of above spectral data compound **2** and compound **3** were identified as 3-dimethylallyl-4-methoxy-1-methyl-2-quinolone or *N*-methyllatanine and 3-dimethylallyl-4,8-dimethoxy-1-methyl-2-quinolone respectively. These two compounds (compounds **2** & **3**) were isolated for the first time from the genus *Zanthoxylum*.

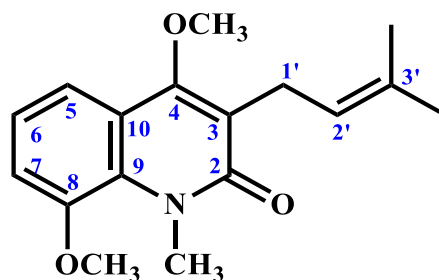


N-methylatanine

Table 1 NMR spectral data (CDCl₃) for compound 2 (ZRP-14)

Position	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	HSQC	HMBC
2	---	164.0		
3	---	122.6		
4	---	160.2		
5	7.76 dd ($J = 8.0, 1.1$ Hz)	123.4	123.4	130.1 (C-7), 139.0 (C-9)
6	7.20 ddd ($J = 8.6, 8.0, 1.4$ Hz)	121.9	121.9	122.4 (C-10)
7	7.48 ddd ($J = (8.6, 8.4, 1.1)$ Hz)	130.1	130.1	
8	7.30 d ($J = 8.4$ Hz)	114.1	114.1	122.4 (C-10)
9	---	139.0		
10	---	122.4		
1'	3.34 d (2H, $J = 7.0$ Hz)	24.3	24.3	122.6 (C-3), 132.5 (C-3'), 160.0 (C-4), 165.0 (C-2)
2'	5.20 t ($J = 7.0$ Hz)	121.5	121.5	
3'	---	132.5		
Me-3' <i>cis</i>	1.62, 3H s	25.7	25.7	132.5 (C-3'), 122.6 (C-3), 19.0 (Me-3' <i>trans</i>)
Me-3' <i>trans</i>	1.75, 3H s	19.0	19.0	132.5 (C-3'), 122.6 (C-3), 25.7 (Me-3' <i>cis</i>)
N-Me	3.66, 3H s	29.8	29.8	139.0 (C-9), 164.0 (C-2)
OMe-4	3.85, 3H s	61.8	61.8	160.2 (C-4)

^a = measured in 400 MHz, ^b = measured in 100 MHz



3-dimethylallyl-4,8-dimethoxy-1-methyl-2-quinolone

Table 2 NMR spectral data (CDCl₃) for compound 3 (ZRP-14)

Position	δ_H^a	δ_C^b	HSQC	HMBC
2	---	165.0		
3	---	122.6		
4	---	160.0		
5	7.38 dd ($J = 8.0, 1.2$ Hz)	116.0	116.0	130.6 (C-9), 113.5 (C-7)
6	7.09 dd ($J = 8.0, 7.9$ Hz)	122.7	122.7	120.2 (C-10), 148.8 (C-8)
7	6.97 dd ($J = 7.9, 1.2$ Hz)	113.5	113.5	130.6 (C-9), 116.0 (C-5)
8	---	148.8		
9	---	130.4		
10	---	120.2		
1'	3.33 d (2H, $J = 7.0$ Hz)	24.4	24.4	122.6 (C-3), 132.5(C-3'), 160.0 (C-4), 165.0 (C-2)
2'	5.20 t ($J = 7.0$ Hz)	121.5	121.5	
3'	---	132.5		
Me -3' <i>cis</i>	1.62, 3H s	25.7	25.7	132.5 (C-3'), 122.6(C-3), 19.0 (Me-3' <i>trans</i>)
Me-3' <i>trans</i>	1.75, 3H s	19.0	19.0	132.5 (C-3') , 122.6 (C-3), 25.7(Me-3' <i>cis</i>)
N-Me	3.89, 3H s	35.5	35.5	130.4 (C-7), 165.0 (C-2)
OCH ₃ -4	3.81, 3H s	61.6	61.6	160.0 (C-4)
OCH ₃ -8	3.82, 3H s	56.7	56.7	148.8 (C-8)

^a = measured in 400 MHz, ^b = measured in 100 MHz

NMR spectrum of compound 2&3:

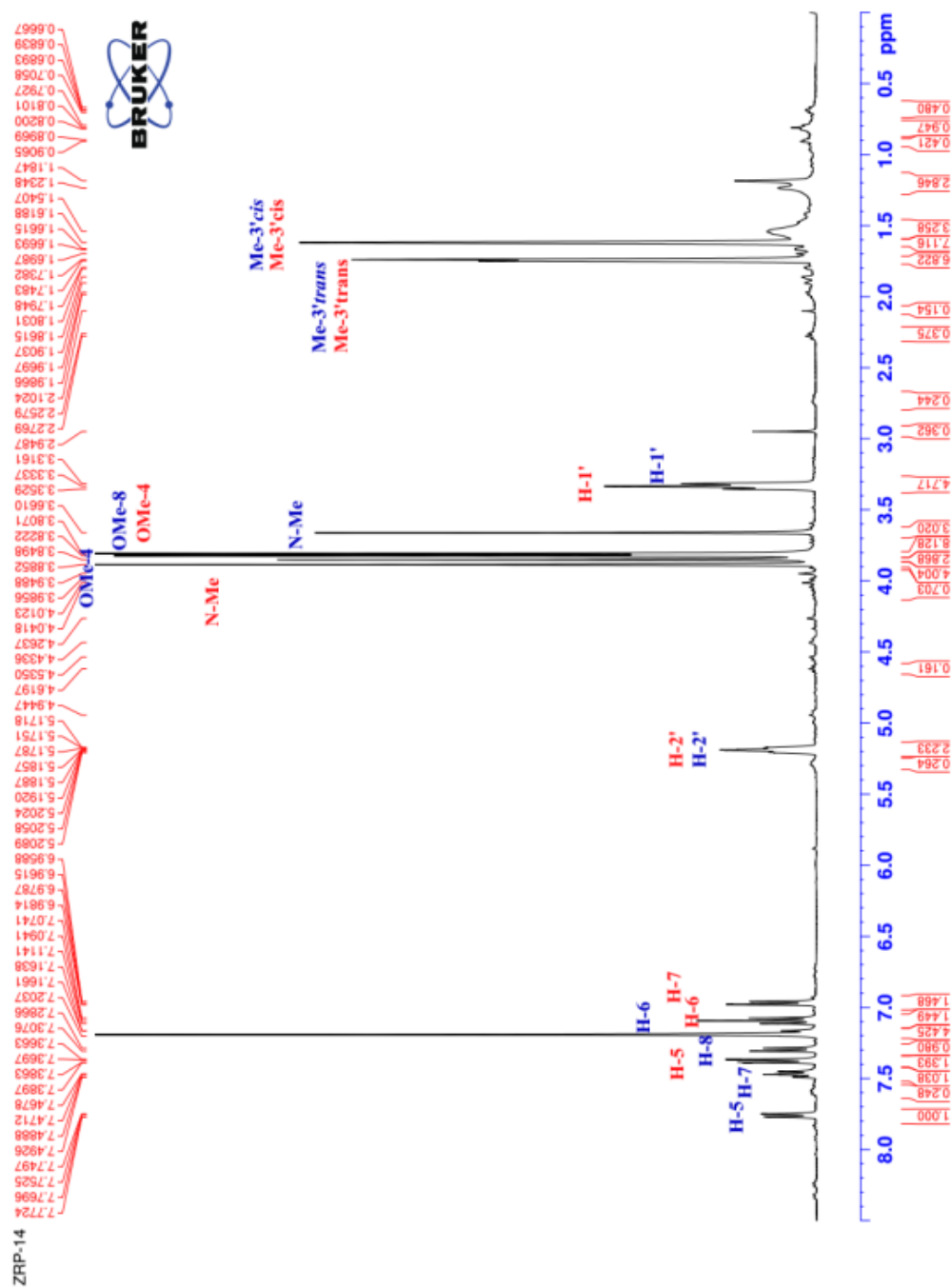


Figure 2.9 ^1H NMR (400 MHz, CDCl_3) spectrum of compound 2 and 3 (ZRP-14)

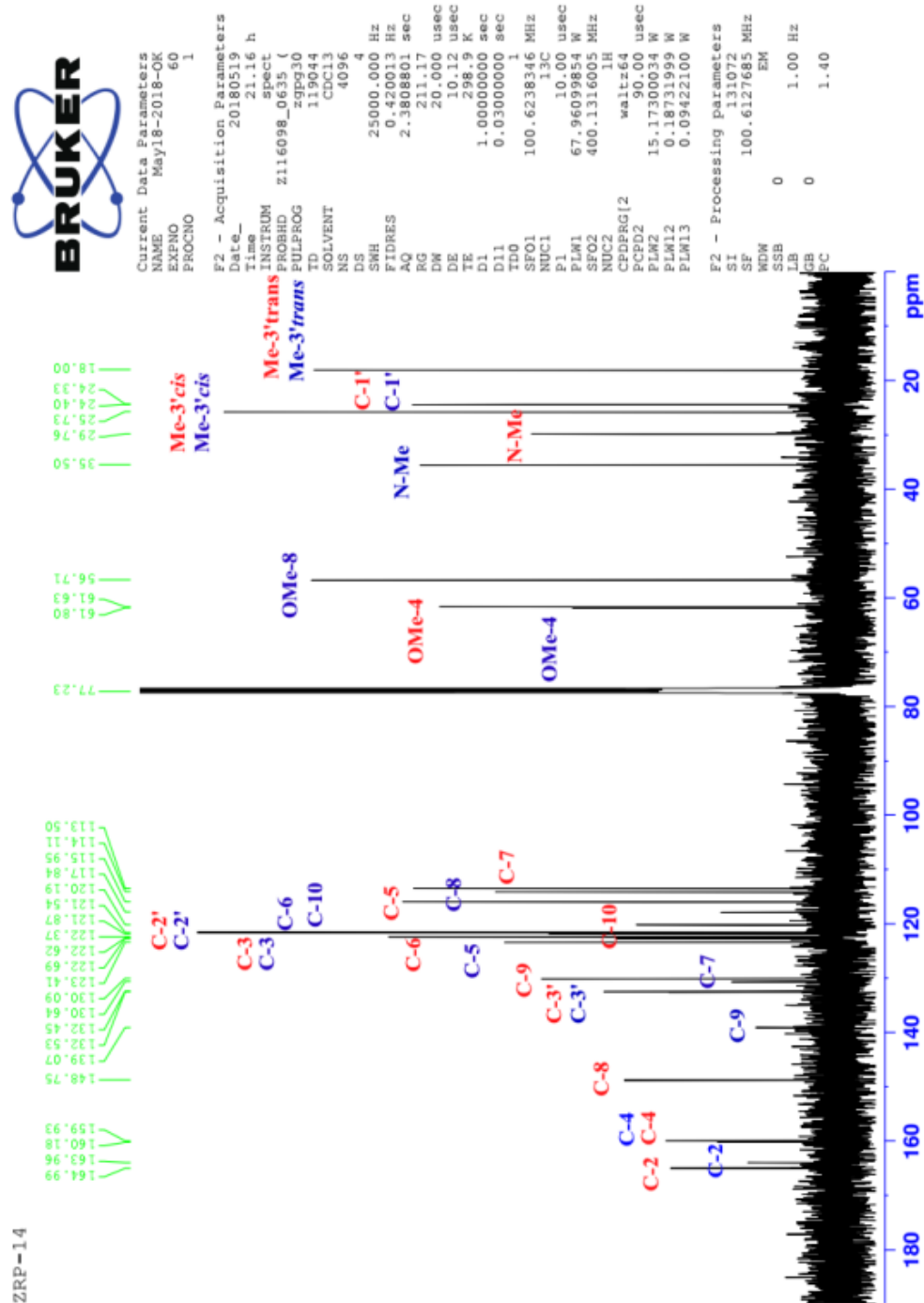
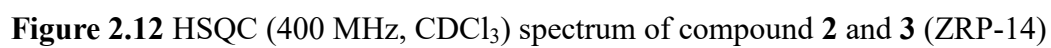


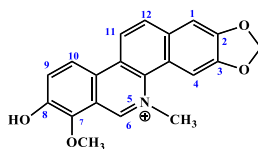
Figure 2.10 ^{13}C NMR (100 MHz, CDCl_3) spectrum of compound **2** and **3** (ZRP-14)



2.3 Characterization of compound 4 (ZRC-7) as 8-O-demethylchelerythrine

Compound **4**, isolated as yellow mass, showed dark quenching spot under UV light at 254 nm on a TLC plate and gave yellow color when sprayed with vanillin-sulfuric acid reagent followed by heating at 110 °C for 2 minutes.

The ^1H NMR spectrum (Table 3, Figure 2.14) of this compound displayed two sets of ortho-coupling doublets at δ 7.99 ($J = 9.0$ Hz), 8.67 ($J = 9.0$ Hz), 8.67 ($J = 9.0$ Hz) and 8.24 ($J = 9.0$ Hz) and two singlets at δ 7.58 and 8.31 attributable to H-9, H-10, H-11, H-12, H-1 and H-4 respectively. In addition, the spectrum showed one methoxy group at δ 4.28, an N-methyl at δ 5.50 and a methylenedioxy group at δ 6.29. A highly deshielded proton at δ 9.71 could be assigned to H-6, the deshielded effect may be due to presence of neighbouring nitrogen atom. On the basis of above data compound **4** was identified as 8-O-demethylchelerythrine. The ^1H NMR data were formed similar to those reported in the literature (Tolkachev et al., 1999). 8-O-demethylchelerythrine is reported for the first time from *Z. rhetsa*.



8-O-demethylchelerythrine

Table 3 ^1H NMR spectral data (400 MHz, CD_3OD) for compound 9 (ZRC-7)

Compound 9		8-O-demethylchelerythrine* (Tolkachev et.al, 1999)
Position	δ_{H}	δ_{H}
1	7.58 s	7.50 s
4	8.31 s	7.94 s
6	9.71 s	9.69 s
9	7.99 d ($J = 9.0$ Hz)	8.05 d ($J = 9.1$ Hz)
10	8.67 d ($J = 9.0$ Hz)	8.50 d ($J = 9.1$ Hz)
11	8.67 d ($J = 9.0$ Hz)	8.50 d ($J = 9.1$ Hz)
12	8.24 d ($J = 9.0$ Hz)	8.19 d ($J = 9.1$ Hz)
OMe-7	4.28 3H s	4.25 3H, s
N-Me	5.51 3H s	5.00 3H, s
-OCH ₂ O-	6.29 2H s	6.21 2H, s

* = spectrum recorded in CDCl_3 -TFA

2.3.1 NMR spectrum of compound 4 characterized as 8-O-demethylchelerythrine:

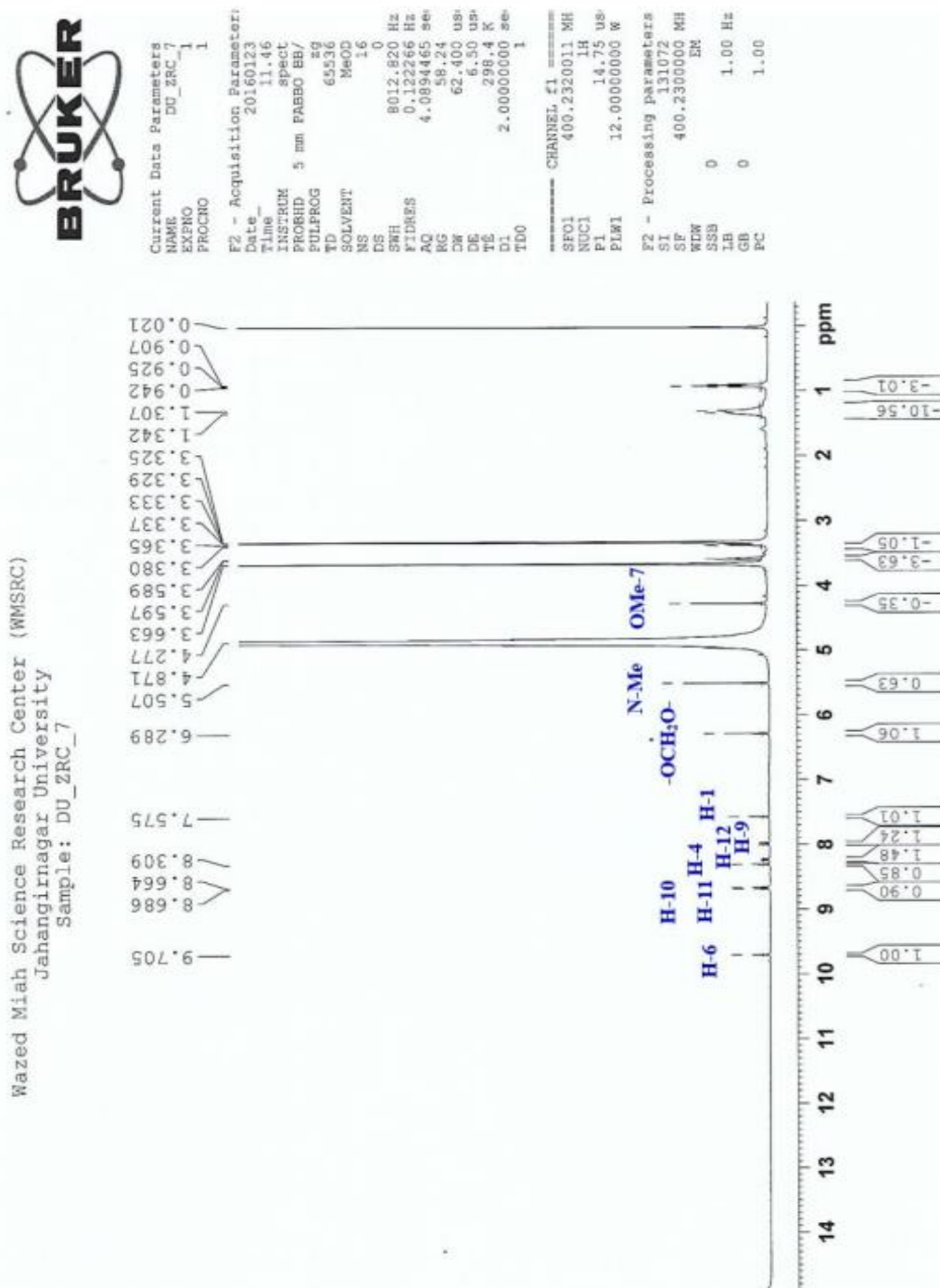


Figure 2.14 ¹H NMR (400 MHz, CDCl₃) spectrum of compound 4 (ZRC-7)

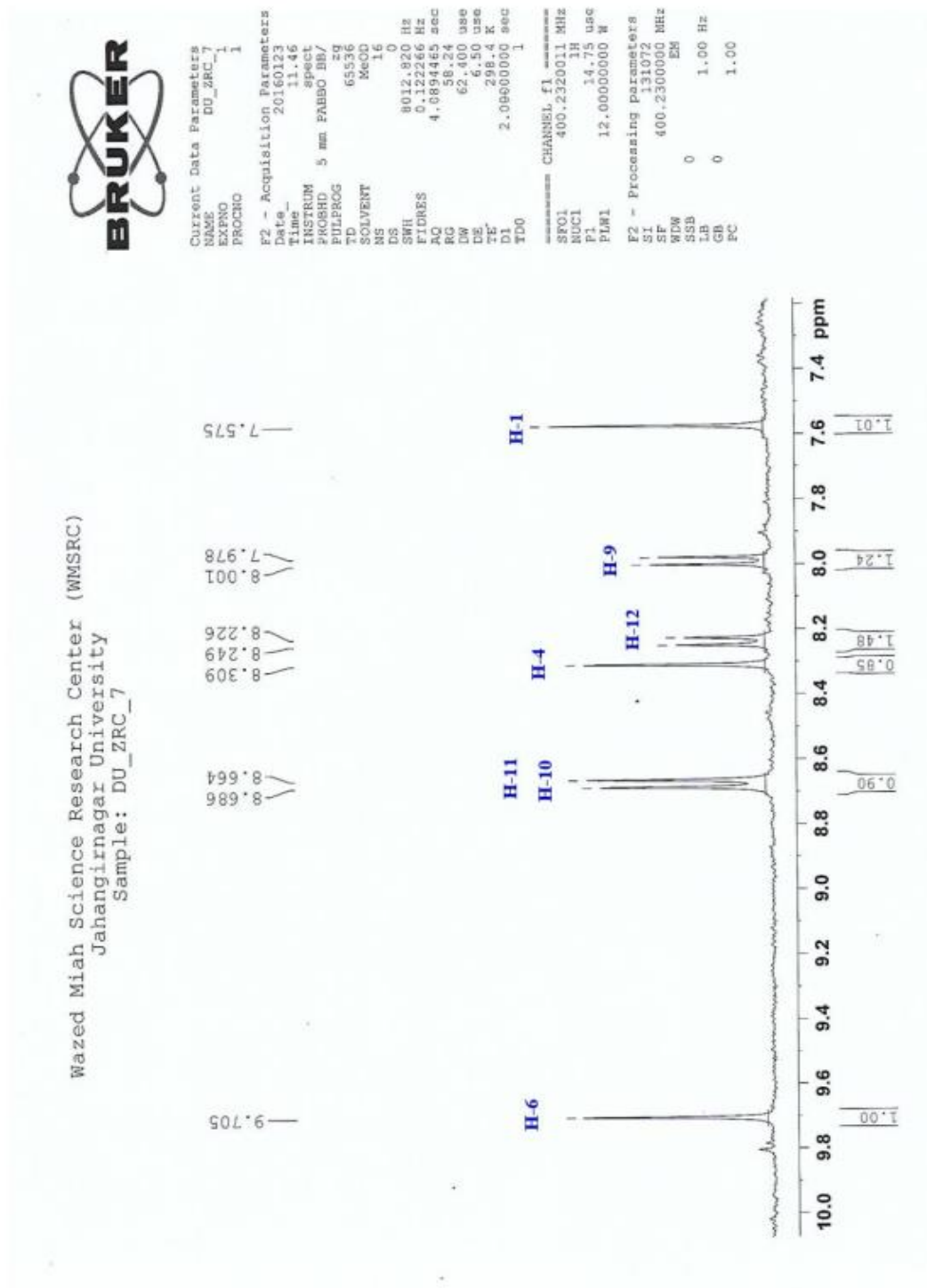


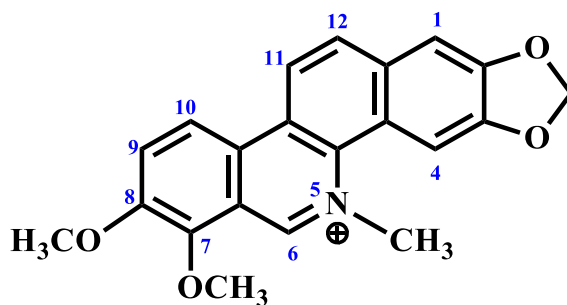
Figure 2.15 partially expanded ^1H NMR (400 MHz, CDCl_3) spectrum of compound **4** (ZRC-7)

2.4 Characterization of compound 5 and 6 (ZRC-79) as mixture of chelerythrine and 7-methoxynitidine

ZRC-79, isolated as yellow mass, presented a deep yellow fluorescent spot at 366 nm UV light on a TLC plate and produced reddish brown color with Dragendorff's reagent.

The ^1H NMR spectrum (400 MHz, CDCl_3 ; **Table 4**; **Figure 2.16**) of ZRC-79 indicated the presence of two compounds in 2:1 ratio. The major signals include two pairs of ortho coupling doublets at δ 7.85, 8.34, 8.32 and 7.98, two aromatic proton singlets at δ 7.32 & 8.02, two methoxy groups at δ 4.41 and 4.04, a methylenedioxy group at δ 6.18 (2H, s), a very deshielded singlet at δ 10.72 and an N-methyl group resonating at δ 5.27 (3H, s). All these ^1H NMR signals were found identical to those reported for chelerythrine (Miao et al., 2011).

The rest of the ^1H NMR signals (minor compound) (**Table 5**) include two ortho-coupling doublets at δ 8.23 and 7.85, three aromatic proton singlets at δ 7.82, 8.04 and 8.42, three methoxy groups at δ 4.41, 4.04 and 3.88, a methylenedioxy group at δ 6.22 (2H, s), a deshielded proton singlet at δ 10.72 and an N-methyl at δ 5.24 (3H, s). All these latter signals indicated the minor compound similar to chelerythrine (major one) but with additional methoxy group instead of the proton at C-9. Thus ZRC-79 was identified as a mixture of chelerythrine (compound **5**) and 7-methoxynitidine, (compound **6**). 7-methoxynitidine is a previously undescribed naturally occurring benzophenanthredine alkaloid, however, it was synthesized by Ishii et al in 1985.

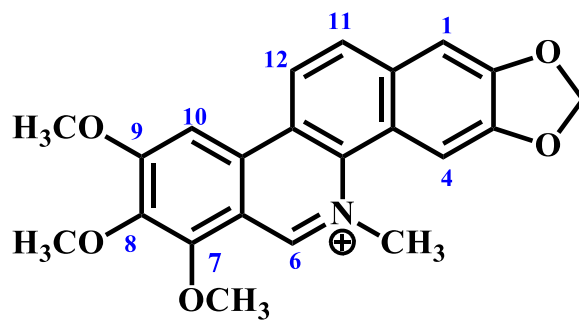


Chelerythrine

Table 4 ^1H NMR spectral data (400 MHz, CDCl_3) for compound 5 (ZRC-79)

Position	Compound 5	Chelerythrine (Miao et al., 2011)
	δ_{H}	δ_{H}
1	7.32 s	7.49 s
4	8.02 s	8.08 s
6	10.72 s	9.92 s
9	7.85 d ($J = 9.0$ Hz)	8.01 d ($J = 9.0$ Hz)
10	8.34 d ($J = 9.0$ Hz)	8.60 d ($J = 9.0$ Hz)
11	8.32 d ($J = 9.0$ Hz)	8.56 d ($J = 9.0$ Hz)
12	7.98 d ($J = 9.0$ Hz)	8.10 d ($J = 9.0$ Hz)
OMe-7	4.41 3H s	4.27 3H s
OMe-8	4.04 3H s	4.12 3H s
N-Me	5.27 3H s	4.97 3H s
-OCH ₂ O-	6.18 2H s	6.26 2H s

*= spectrum recorded in CD_3OD



7-methoxynitidine

Table 5 ^1H NMR spectral data (400 MHz, CDCl_3) for compound 6 (ZRC-79)

Position	δ_{H}
1	7.82 s
4	8.04 s
6	10.72 s
10	8.42 s
11	8.23 d ($J = 9.0$ Hz)
12	7.85 d ($J = 9.0$ Hz)
OMe-7	4.41 3H s
OMe-8	4.04 3H s
OMe-9	3.88 3H s
N-Me	5.24 3H s
-OCH ₂ O-	6.22 2H s

2.4.1 NMR spectrum of compound 5 and 6 characterized as a mixture of chelerythrine and 7-methoxynitidine:

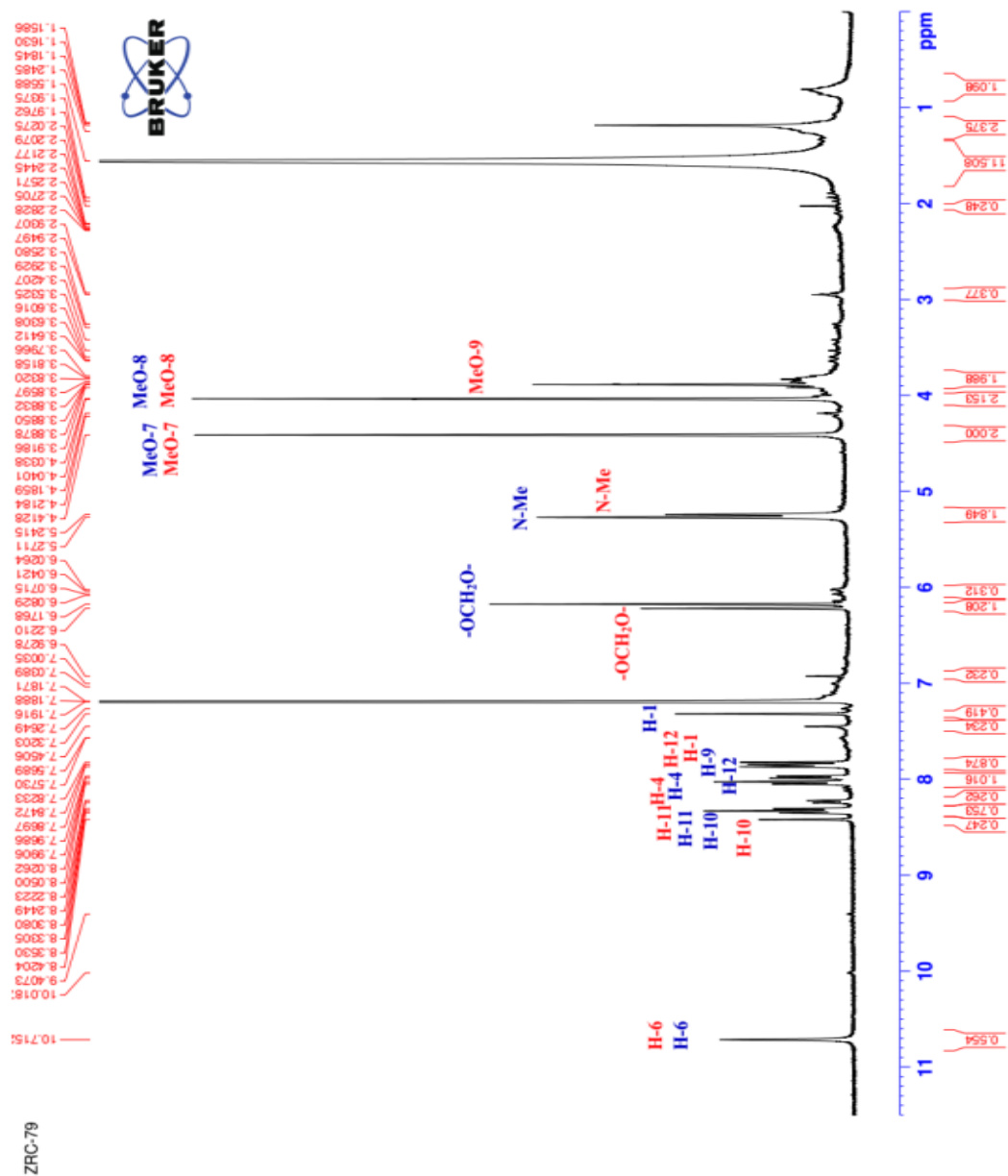
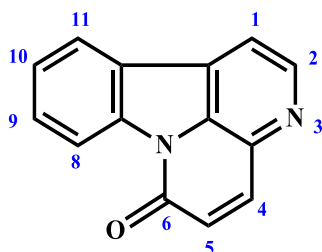


Figure 2.16 ^1H NMR (400 MHz, CDCl_3) spectrum of compound 5 & 6 (ZRC-79)

2.5 Characterization of compound 7 (ZRC-35) as canthin-6-one

Compound 7, isolated as colorless crystals, provided blue fluorescent spot on a TLC plate at 366 nm UV light. The ^1H NMR spectral (400 MHz, CDCl_3 ; **Table 6**, **Figure 2.17**) data of compound 7 demonstrated the presence of four aromatic protons at δ 8.17 (d, $J = 8.0$ Hz), 7.59 (dd, $J = 8.0, 7.5$ Hz), 7.81 (dd, $J = 8.4, 7.5$ Hz) and 8.65 (d, $J = 8.4$ Hz) suggesting a disubstituted benzene ring and could be assigned to H-8, H-9, H-10 and H-11 respectively. Two ortho coupled aromatic protons resonating at δ 8.20 (d, $J = 5.9$ Hz) and δ 8.73 (d, $J = 5.9$ Hz) were attributable to H-1 and H-2 respectively. Another set of doublets at δ 8.49 ($J = 10$ Hz) and δ 7.09 ($J = 10$ Hz) were attributable to H-4 and H-5 of the conjugated lactam ring of canthin-6-one. The structure of compound 7 was identified as canthin-6-one by compairing its spectroscopic data with those published for the compound (Nafiah et al., 2011).

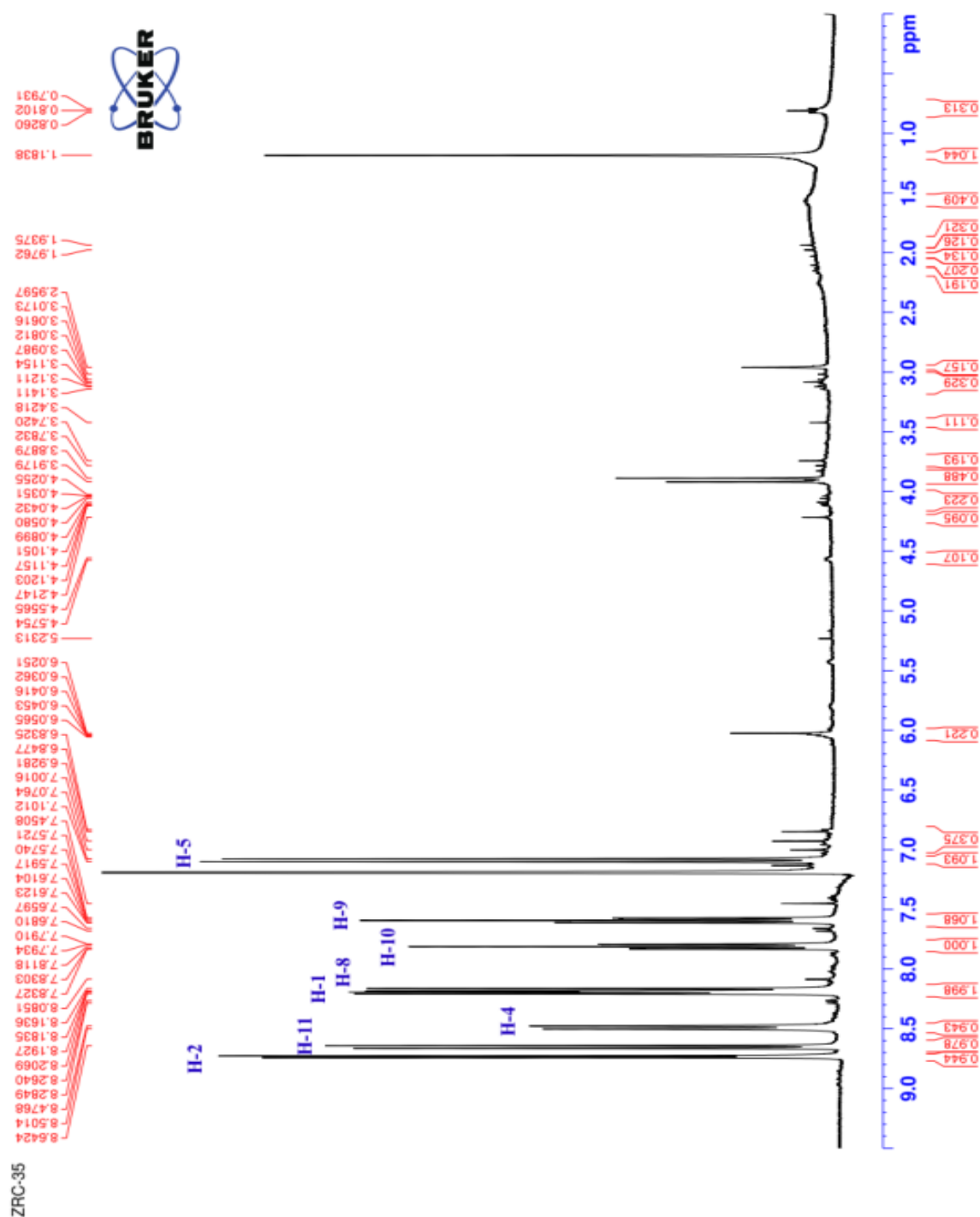


Canthin-6-one

Table 6 NMR spectral data (400 MHz; CDCl_3) for compound 7 (ZRC-35)

Compound 7		Canthin-6-one (Nafiah et al., 2011)
Position	δ_{H}	δ_{H}
1	8.20 d ($J = 5.9$ Hz)	8.35 d ($J = 4.8$ Hz)
2	8.73 d ($J = 5.9$ Hz)	8.86 d ($J = 4.8$ Hz)
4	8.49 d ($J = 10$ Hz)	8.7 d ($J = 9.7$ Hz)
5	7.09 d ($J = 10$ Hz)	7.02 d ($J = 9.7$ Hz)
8	8.17 d ($J = 8.0$ Hz)	8.55 d ($J = 8.1$ Hz)
9	7.59 dd ($J = 8.0, 7.5$ Hz)	7.79 t ($J = 7.6$ Hz)
10	7.81 dd ($J = 8.4, 7.5$ Hz)	7.62 t ($J = 7.6$ Hz)
11	8.65 d ($J = 8.4$ Hz)	8.42 d ($J = 7.8$ Hz)

2.5.1 NMR spectrum of compound 7 characterized as canthin-6-one:

Figure 2.17 ^1H NMR (400 MHz, CDCl_3) spectrum of compound 7 (ZRC-35)

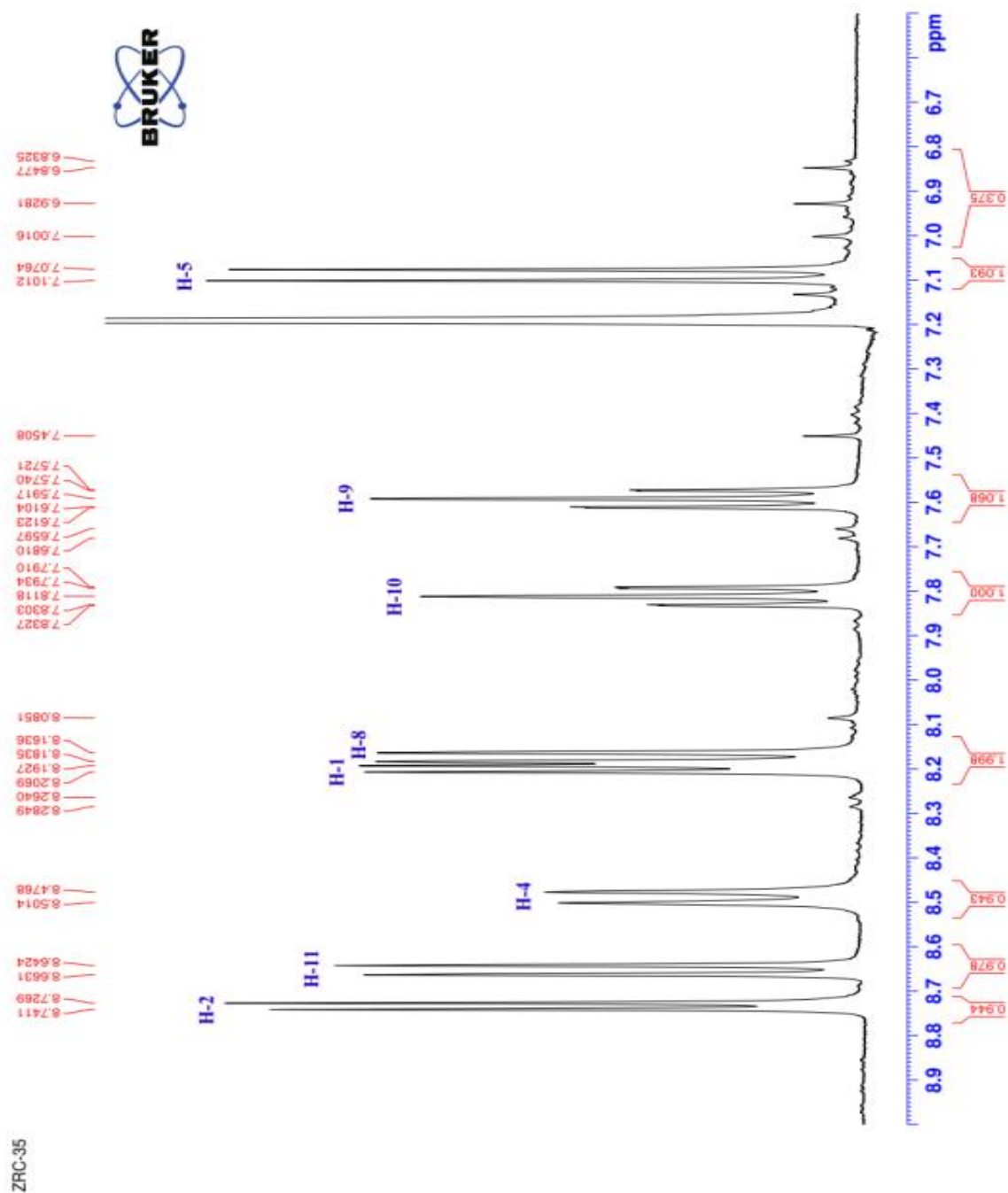
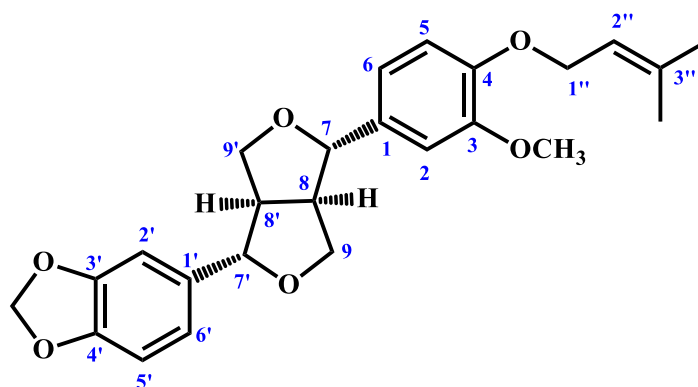


Figure 2.18 Partially expanded ^1H NMR (400 MHz, CDCl_3) spectrum of compound 7 (ZRC-35)

2.6 Characterization of compound 8 [ZRP-14(2)] as (+)-piperitol- γ - γ - dimethylallylether

Compound **15**, isolated as a yellow mass, showed blue fluorescence at 366 nm UV light on a TLC plate and produced no colour after spraying with vanillin-sulfuric acid reagent.

The ^1H NMR spectrum (400 MHz, CDCl_3 ; **Table 7**, **Figure 2.19**) of compound **15** displayed six aromatic protons at δ 6.97, 6.71, 6.76, 6.83, 6.74 and 6.76, the coupling constant of which indicated two trisubstituted benzene rings. A two proton singlet at δ 5.88 and three proton singlet at δ 3.80 indicating respectively a methylenedioxy and a methoxy groups. An oxymethylene group at δ 4.50 (2H, d, $J = 9.6$ Hz), an olefinic proton δ 5.44 (1H, brt, $J = 6.0$ Hz) and two methyls at δ 1.69 and 1.65 indicated the presence of a 3-methyl-but-2-enyloxy (prenyloxy) chain in the molecule. In addition the spectrum showed eight olefinic protons at δ 3.09 to 4.44, suggesting a lignan with a furofuran ring. The ^{13}C NMR spectrum (100 MHz, CDCl_3 ; **Table 7**, **Figure 2.20**) showed twenty four carbons including a methylenedioxy carbon at δ 101.1, a methoxy carbon at δ 56.0 and five carbinol carbon at δ 85.9, 85.8, 71.7, 71.7 and 65.8. A methylenedioxy group at δ 5.88 with three aromatic protons suggested the presence of a 3, 4-methylenedioxyphenyl (i.e piperonyl) group. The rest three aromatic protons, together with the 3-methyl-but-2-enyloxy (prenyloxy) and the methoxy group indicated the presence of a 3-methoxy-4-prenyloxyphenyl group. The HSQC (**Figure 2.21**) and HMBC (**Figure 2.22**) experiment allowed assignment of all the protons and carbons in the molecule. All these data enabled the identity of ZRP-14(2) as a furofuran lignan with a piperonyl group and a 3-methoxy-4-prenyloxy group. On the basis of above spectral data, compound **8** was identified as (+)-piperitol- γ - γ - dimethylallylether.



(+)- Piperitol- γ - γ -dimethylallylether

Table 7 NMR spectral data (CDCl₃) for compound 8 [ZRP-14(2)]

Position	$\delta_{\text{H}}^{\text{a}}$	$\delta_{\text{C}}^{\text{b}}$	HSQC	HMBC
1		135.1		
2	6.97 d ($J = 1.2$ Hz)	106.5	108.4	148.0 (C-4)
3		147.9		
4		148.0		
5	6.71 d ($J = 7.0, 9.0$ Hz)	112.9	114.2	135.1 (C-1)
6	6.76 dd ($J = 8.0, 2.0$ Hz)	119.4	118.4	
7	4.66 d ($J = 5.2$ Hz)	85.8	82.1	71.7 (C-9), 71.7 (C-9')
8	3.09 m	54.4	50.2	
9 α	4.25 dd ($J = 9.6, 6.0$ Hz)	71.7	69.8	85.8 (C-7), 85.9 (C-7')
9 β	3.94 m			85.8 (C-7), 85.9 (C-7')
1'		133.5		
2'	6.83br s	109.4	106.5	149.8 (C-4')
3'		147.1		
4'		149.8		
5'	6.74 d ($J = 8.8$ Hz)	108.2	108.2	133.5 (C-1')
6'	6.76 d ($J = 8.0$ Hz)	118.2	119.5	
7'	4.44 d ($J = 4$ Hz)	85.9	87.7	71.7 (C-9), 71.7 (C-9')
8'	3.09 m	54.1	54.6	
9' α	4.25 dd ($J = 9.6, 6.0$ Hz)	71.7		85.8 (C-7), 85.9 (C-7')
9' β	3.94 m			85.8 (C-7), 85.9 (C-7')
OMe-3	3.80 3H s	56.0	56.0	147.9 (C-3)
3',4'-OCH ₂ O-	5.88 2H s	101.1	101.0	147.1 (C-3'), 149.8 (C-4')
1''	4.50 ($J = 9.6$ Hz)	65.8		120.0 (C-2''), 137.6 (C-3'')
2''	5.44 ($J = 6$ Hz)	120.0		
3''		137.6		
Me-3'' <i>cis</i>	1.69 3H s	25.8		18.2 (Me-3'' <i>trans</i>), 120.0 (C-2''), 137.6 (C-3'')
Me-3'' <i>trans</i>	1.65 3H s	18.2		25.8 (Me-3'' <i>cis</i>), 120.0 (C-2''), 137.6 (C-3'')

^a = measured in 400 MHz, ^b = measured in 100 MHz

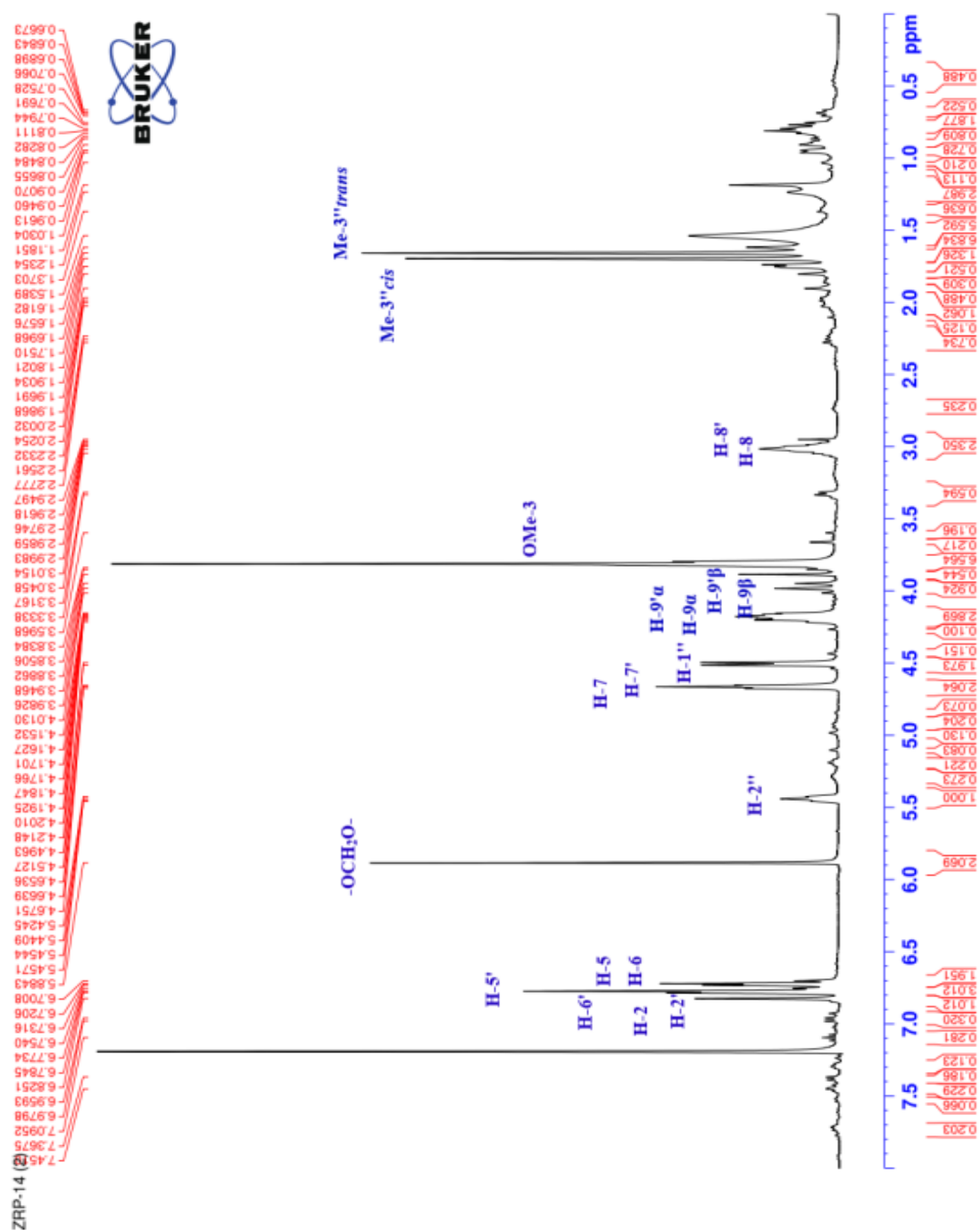


Figure 2.19 ^1H NMR (400 MHz, CDCl_3) spectrum of compound **8** [ZRC-14 (2)]

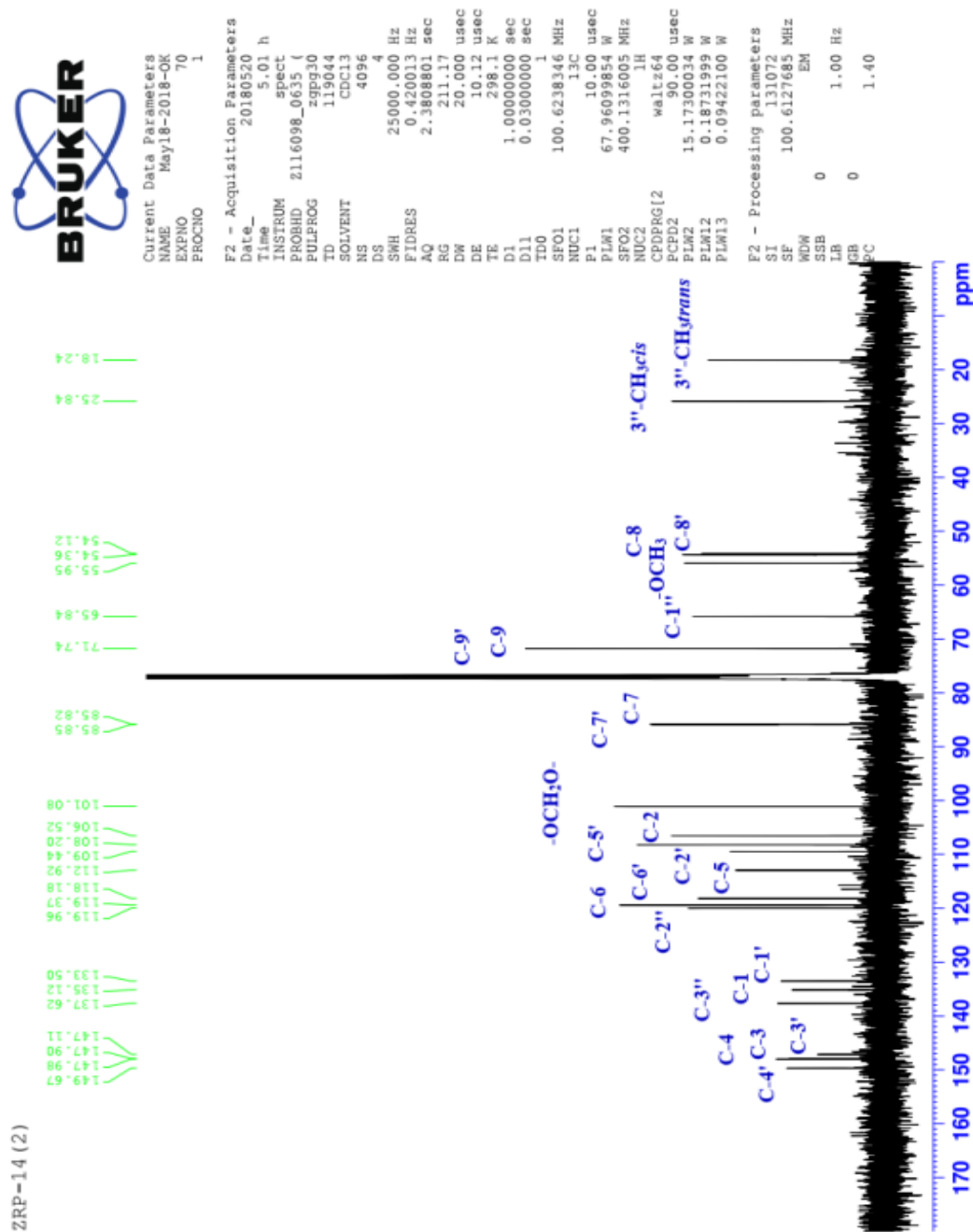


Figure 2.20 ¹³C NMR (100 MHz, CDCl₃) spectrum of compound **8** [ZRC-14 (2)]

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2.7 Characterization of compound 9 and compound 10 (ZRP-51) as mixture of β -sitosterol and stigmasterol

ZRP-51 was isolated as colorless crystals and produced purple color when sprayed with vanillin in sulphuric acid reagent, followed by heating for 2 minutes. The compounds were appeared as a single spot on a TLC plate and therefore could not be separated from each other.

The ^1H NMR spectrum (**Table 8; Figure 2.22**) showed two singlets at δ 0.70 and 1.03, assignable to H-18 and H-19 and the other three doublets at δ 0.95, 0.84 and 0.86 having a coupling constant of $J = 6.4$, 7.2 and 7.2 Hz which could be attributed to H-21, H-26 and H-27 respectively. Similarly, in the spectrum a triplet at δ 0.87 ($J = 7.2$ Hz) was assigned to H-29. The spectrum also showed an olefinic proton at δ 5.37 with coupling constant $J = 5.2$ Hz and a multiplet at δ 3.55, assignable to H-6 and H-3 of a sterol moiety. Thus compound **10** was identified as β -sitosterol. The structure was further confirmed by comparing its ^1H NMR data with those published (Aurang, 2017).

In addition to the signals discussed for β -sitosterol, the ^1H NMR spectrum (**Table 8, Figure 2.22**) also displayed two olefinic protons at δ 5.18 and 5.04 (dd, $J = 15.2$, 8.6 Hz each) respectively assigned to H-22 and H-23. Three doublets at δ 1.04, 0.83 and 0.88 with coupling constant $J = 7.5$, 7.0 and 6.3 Hz attributed to H-21, H-26 and H-27 respectively. The ^1H NMR also showed a triplet at δ 0.83 ($J = 7.0$ Hz) was assigned to H-29 and two singlets at δ 0.72 and 1.03, assignable to H-18 and H-19. The spectrum as well showed a doublet of an olefinic proton at δ 5.37 (d, $J = 5.2$ Hz) and a multiplet at δ 3.55, assignable to H-6 and H-3 of a sterol moiety. These ^1H NMR data were found to be in close agreement to those reported for stigmasterol (Aurang, 2017). Thus compound **9** was identified as stigmasterol.

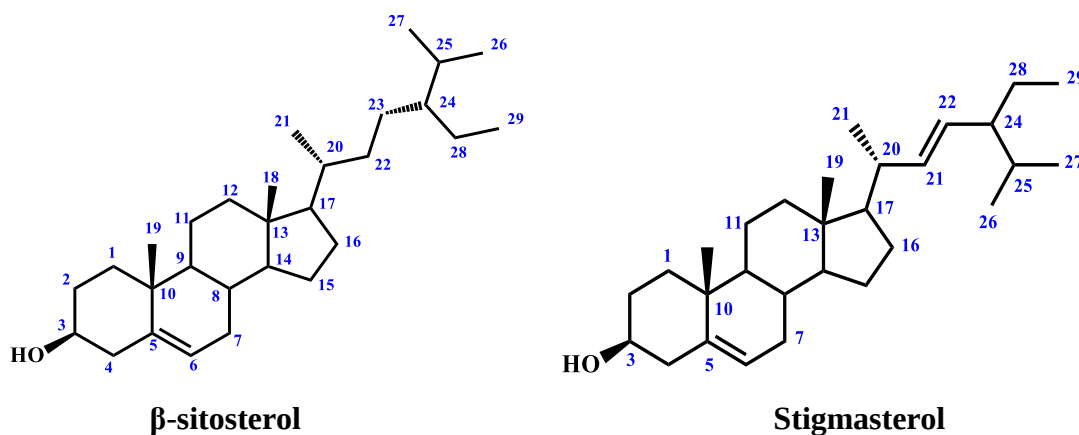


Table 8 ^1H NMR spectral data (400 MHz, CDCl_3) for compound 10 and 9 (ZRP-51)

Position	Compound 10	β -sitosterol (Aurang, 2017)	Compound 9	Stigmasterol (Aurang, 2017)
	δ_{H}	δ_{H}	δ_{H}	δ_{H}
3	3.55 m	3.53 m	3.55 m	3.52 m
6	5.37 d ($J = 5.2$ Hz)	5.37 br s	5.37 d ($J = 5.2$ Hz)	5.38 br s
18	0.70 3H, s	0.68 3H, s	0.72 3H, s	0.69 3H, s
19	1.03 3H, s	1.01 3H, s	1.03 3H, s	1.01 3H, s
21	0.95 d ($J = 6.4$ Hz)	0.92 d ($J = 6.4$ Hz)	1.04 d ($J = 7.5$ Hz)	1.02 d ($J = 7.5$ Hz)
22			5.18 dd ($J = 15.2, 8.6$ Hz)	4.98 1H, m
23			5.04 dd ($J = 15.2, 8.6$ Hz)	5.14 1H, m
26	0.84 d ($J = 7.2$ Hz)	0.81 d ($J = 6.4$ Hz)	0.83 d ($J = 7.0$ Hz)	0.79 d ($J = 6.5$ Hz)
27	0.86 d ($J = 7.2$ Hz)	0.83 d ($J = 6.4$ Hz)	0.88 d ($J = 6.3$ Hz)	0.85 (d, $J = 6.5$ Hz)
29	0.87 t ($J = 7.2$ Hz)	0.85 t ($J = 7.5$)	0.83 t ($J = 7.0$ Hz)	0.80 (t, $J = 7.5$ Hz)

2.7.1 NMR spectrum of compound 9 and 10 as a mixture of β -sitosterol and stigmasterol:

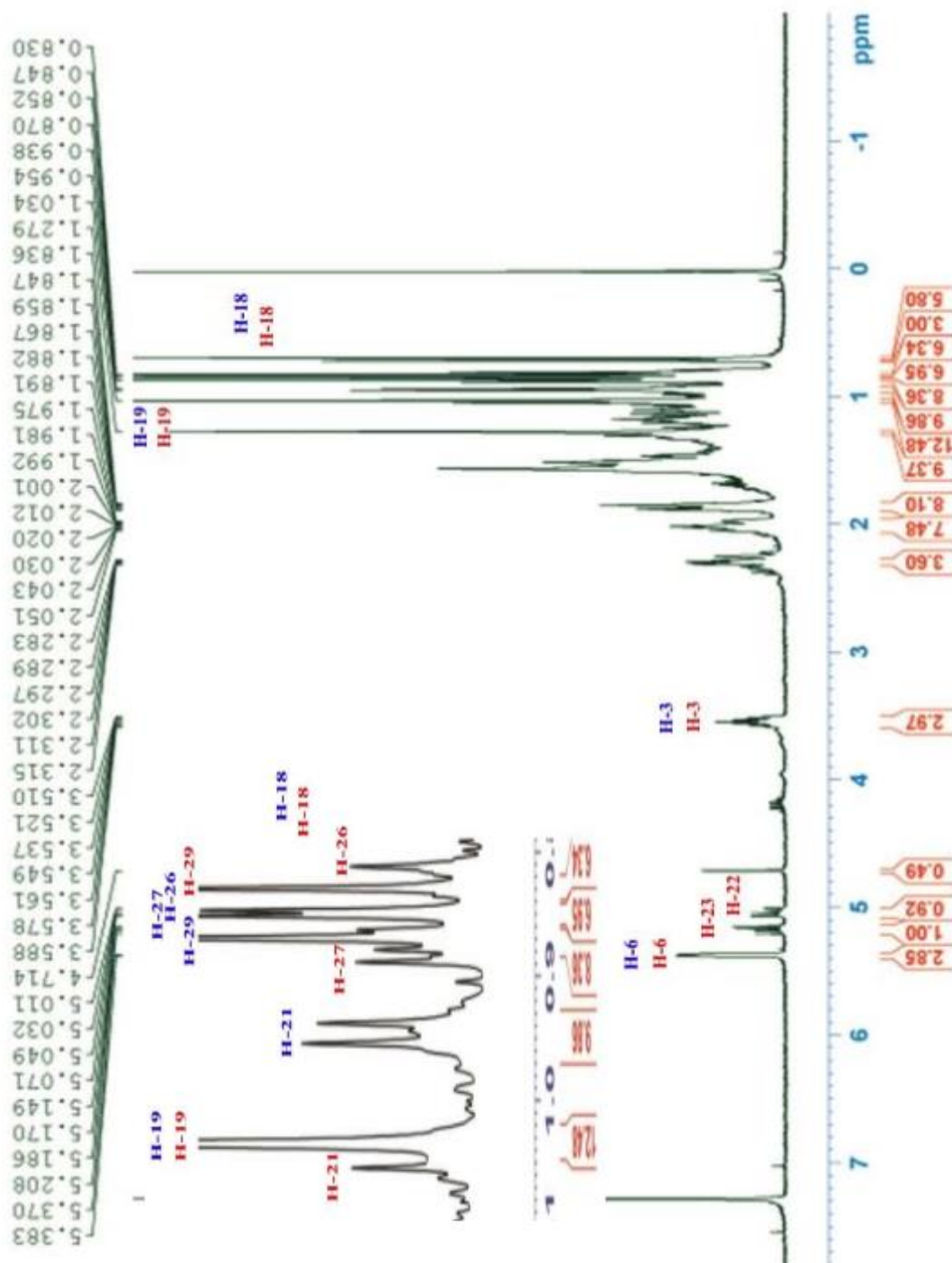
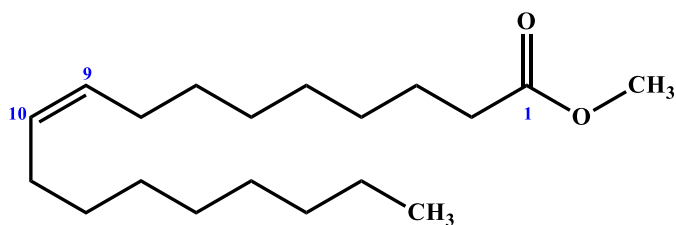


Figure 2.22 ^1H NMR (400MHz, CDCl_3) spectrum of compound **9** & **10** (ZRP-51)

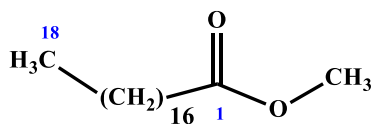
2.8 Characterization of compound 11, compound 12 and compound 13 (ZRP-3) as a mixture of methyl oleate, methyl stearate and stearic acid

Compound **11**, **12** and **13** were obtained as light yellowish liquid, and produced grayish yellow color on a TLC plate when sprayed with vanillin in sulphuric acid reagent followed by heating for 2 minutes.

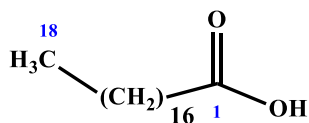
The ^1H NMR spectral data (500 MHz, CDCl_3 ; **Table 9**, **Figure 2.23**) showed two olefinic proton multiplets at δ 5.36 and a methyl triplet at δ 0.88, which could be assigned to at H-9, H-10 and the terminal methyl group H-18 respectively. The protons resonating at δ 2.03 (4H, m) are the allylic protons ($\text{CH}_2\text{-CH=CH}$) H-8 and H-11. The protons directly adjacent to the carbonyl group resonated at δ 2.32 (2H, t, $J = 7.4$ Hz, H-2) and the $\text{HOOC-CH}_2\text{-CH}_2$ protons resonated at δ 1.63 (2H, m, H-3). The methylene protons of the fatty chain appeared at δ 1.27 (20H, m) assignable to H-4 to H-7 and H-12 to H-17. A methoxy group at δ 3.68 indicated an esterified fatty acid. All these data permitted the identification of ZRP-3 as methyl oleate (compound **11**). In addition the spectrum displayed 18 carbon fatty chain consisting of signals at δ 1.27 m (28H), 1.63 m (2H) and 2.32 t (2H), a terminal methyl at δ 0.88 t and another methyl ester moiety at δ 3.68. All these signals allowed identification of compound **12** as methyl stearate. The ^1H NMR spectrum of ZRP-3 further showed signals similar to compound **12** except the methyl ester group at δ 3.68, indicating the free fatty acid. Thus the compound **13** was identified as stearic acid (Alexandri et al., 2017).



Methyl oleate



Methyl stearate



Stearic acid

Table 9 ^1H NMR spectral data (500 MHz, CDCl_3) of compound 11, 12 and 13 (ZRP-3)

	Compound 11	Compound 12	Compound 13
Position	δ_{H}	δ_{H}	δ_{H}
2	2.32 t (2H, $J = 7.4$ Hz)	2.32 t (2H, $J = 7.4$ Hz)	2.32 t (2H, $J = 7.4$ Hz)
3	1.63 2H, m	1.63 2H, m	1.63 2H, m
4-17	---	1.27 28H, m	1.27 28H, m
4-7, 12-17	1.27 20H, m	---	---
8, 11	2.03 4H, m	---	---
9, 10	5.36 2H, m	---	---
18-Me	0.88 t (3H, $J = 6.8$ Hz)	0.88 t (3H, $J = 6.8$ Hz)	0.88 t (3H, $J = 6.8$ Hz)
-OMe	3.68 3H, s	3.68 3H, s	---

2.8.1 NMR spectrum of compound 11, 12 and 13 as a mixture of as a mixture of methyl oleate, methyl stearate and stearic acid:

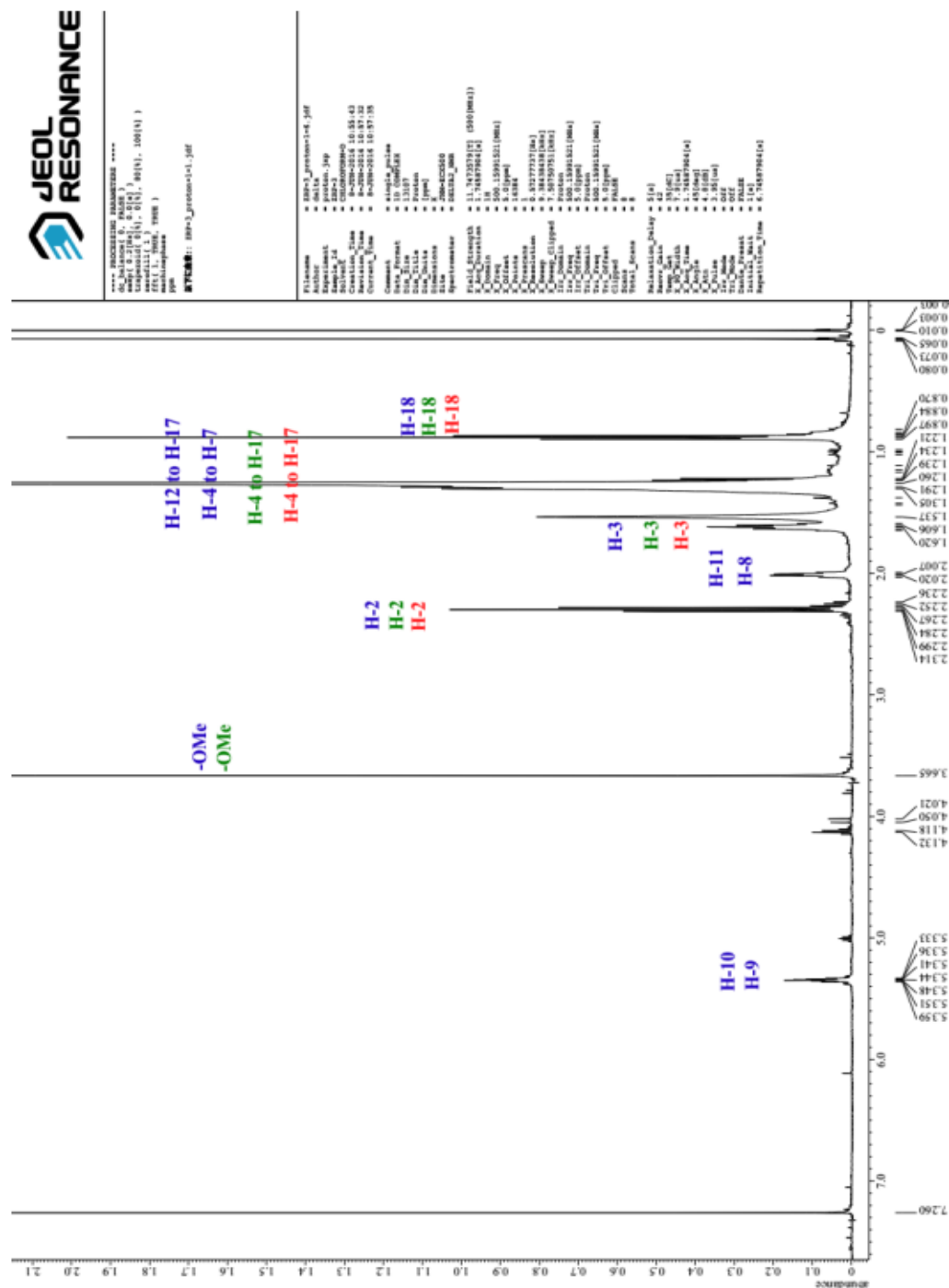


Figure 2.23 ^1H NMR (400 MHz, CDCl_3) spectrum values of compound 11, 12 and 13 (ZRP-3)