

General Procedure for Feeding Experiments with Isotopically Labeled Compounds.

Phoma sp. was inoculated to the medium (100 mL, pH 8.5) in 500 mL Erlenmeyer flask containing polypeptone 10 g/L, sucrose 20 g/L, K₂HPO₄ 5 g/L. After inoculation, the organism was grown at 25°C and 180 rpm. On the fifth day after inoculation, the aqueous solution (8.8 mL) of 400 mg of isotopically labeled compounds (sodium [1-¹³C]- (99 atom % ¹³C), [1, 2-¹³C₂]acetates (99 atom % ¹³C), Cambridge Isotope Lab) filtered through sterilized microfilter (0.2 μm) was equally distributed into four 500 mL flasks. After further incubation for 12 days, mycelium was collected by filtration, disrupted with Polytron homogenizer, and extracted with acetone. This extract was concentrated *in vacuo* and the resultant aqueous layer was extracted with EtOAc. Drying over Na₂SO₄, the EtOAc extract was concentrated *in vacuo* to afford the oily residue which was chromatographed with SiO₂ column (100 % hexane) to collect fractions containing phomactatriene. These fractions were concentrated and the residue was separated by HPLC with the GL Science reversed phase column (Inertsil ODS-2, 5 μm, φ4 x 250 mm; flow rate 1 mL/min; UV 205 nm) with 100 % CH₃CN to give phomactatriene. The yield of phomactatriene was 0.5 to 1 mg in each experiment.

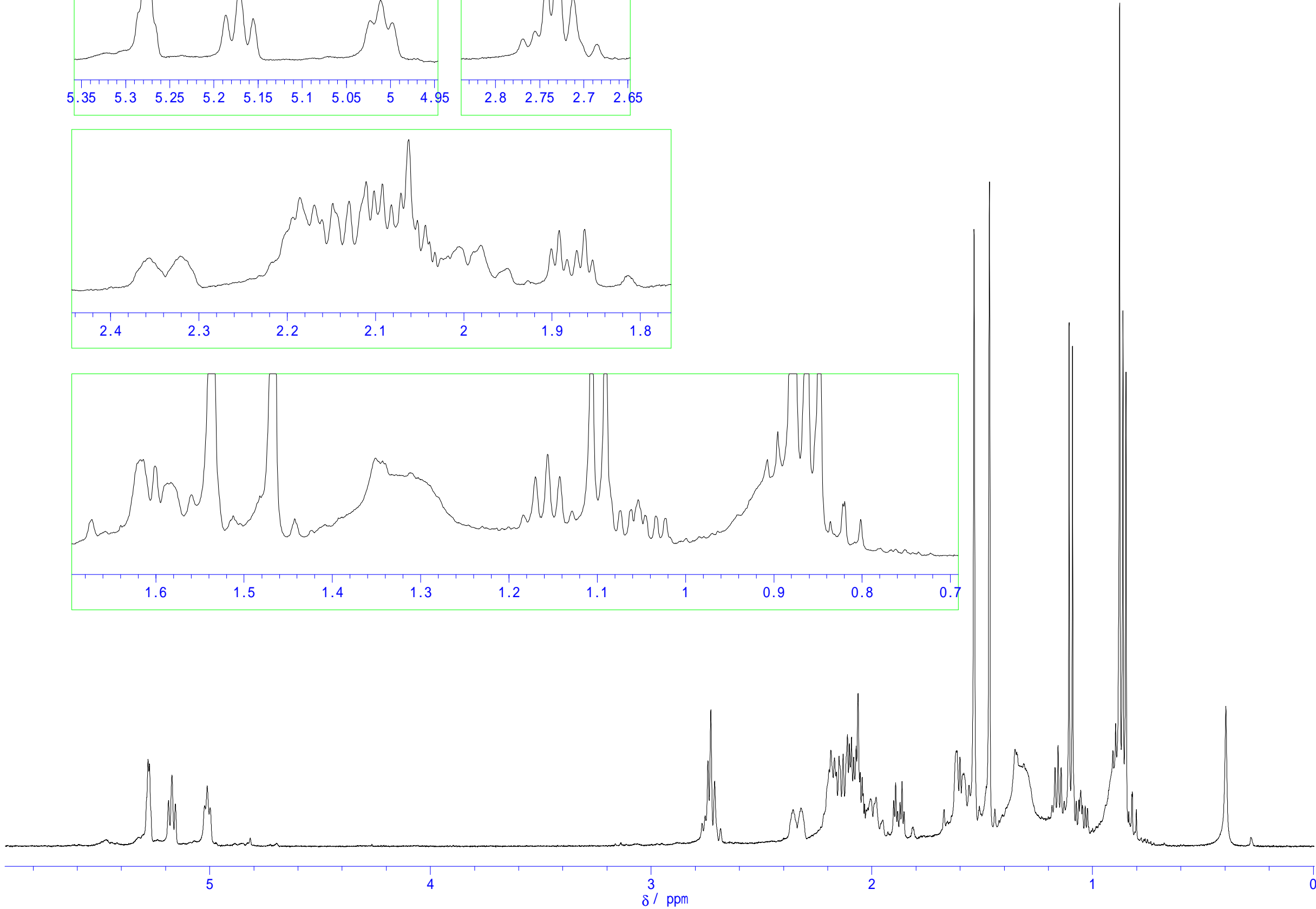
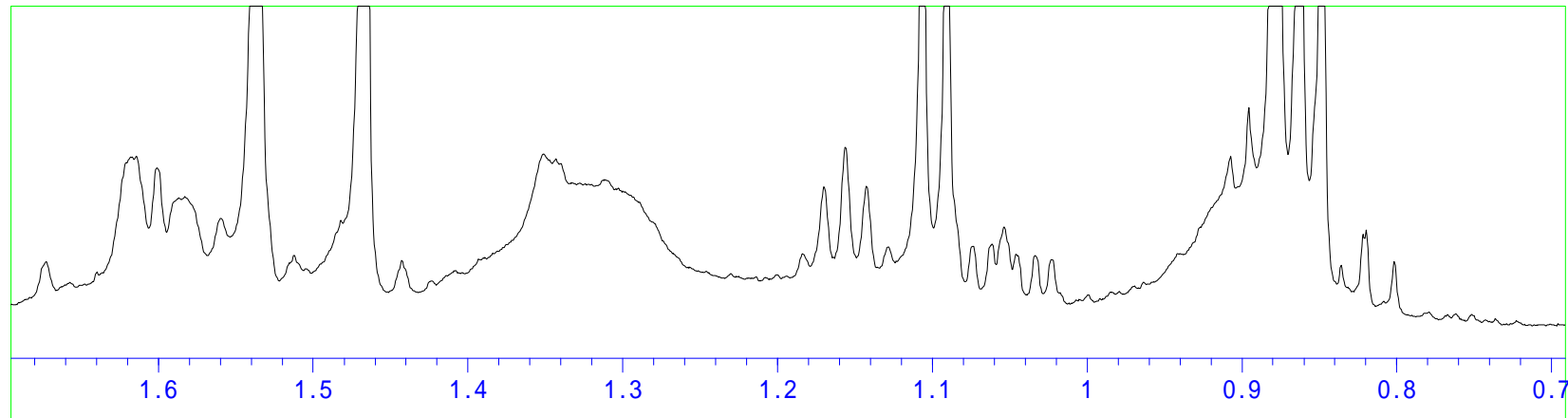
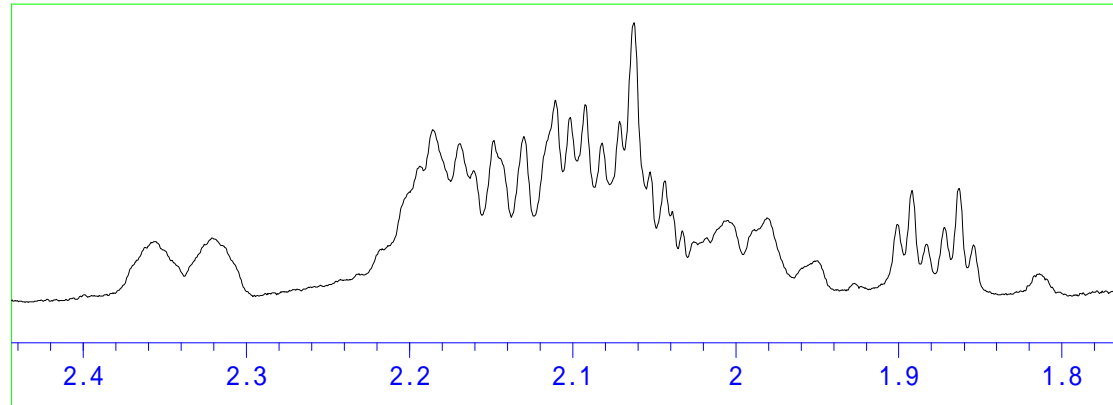
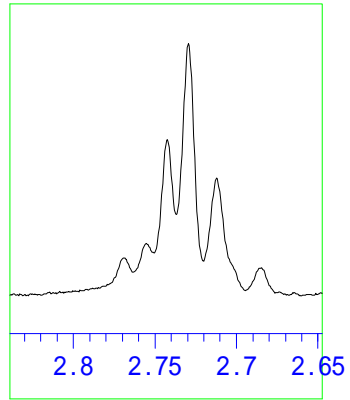
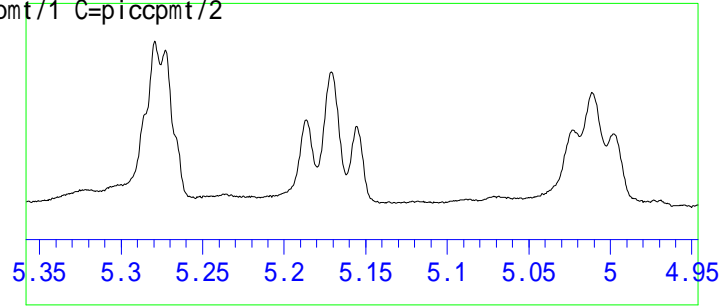
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=128.0ppm
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2

¹H-NMR spectrum of phomactatriene (C6D6, 500 MHz)

File F:\XWIN_NMR\PICCPMT\1\PDAT
Date
Comment
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=1
Bruker AMX-500/ GC-MS & NMR
Grad. Sch. Agric., Hokkaido
H=piccpmt/1 C=piccpmt/2

ObsNuc ?
ExMode
ObsFreq 500.12 MHz
ObsSet 0.0 kHz
ObsFine 9994.533 Hz
Point 32768
Frequency(Span) 8064.516 Hz
Scan 0
AcqTime 4.0632 s
PD 0.0 s
Pulse1 10.0 μs
IrrNuc
ProbeHead
Instrument
Pulse Program
Gradient Program
Temperature 0.0
Solvent
Reference 13.9576 pp
Broad.Factor 0.25 Hz
Window Exponential
RGain 0

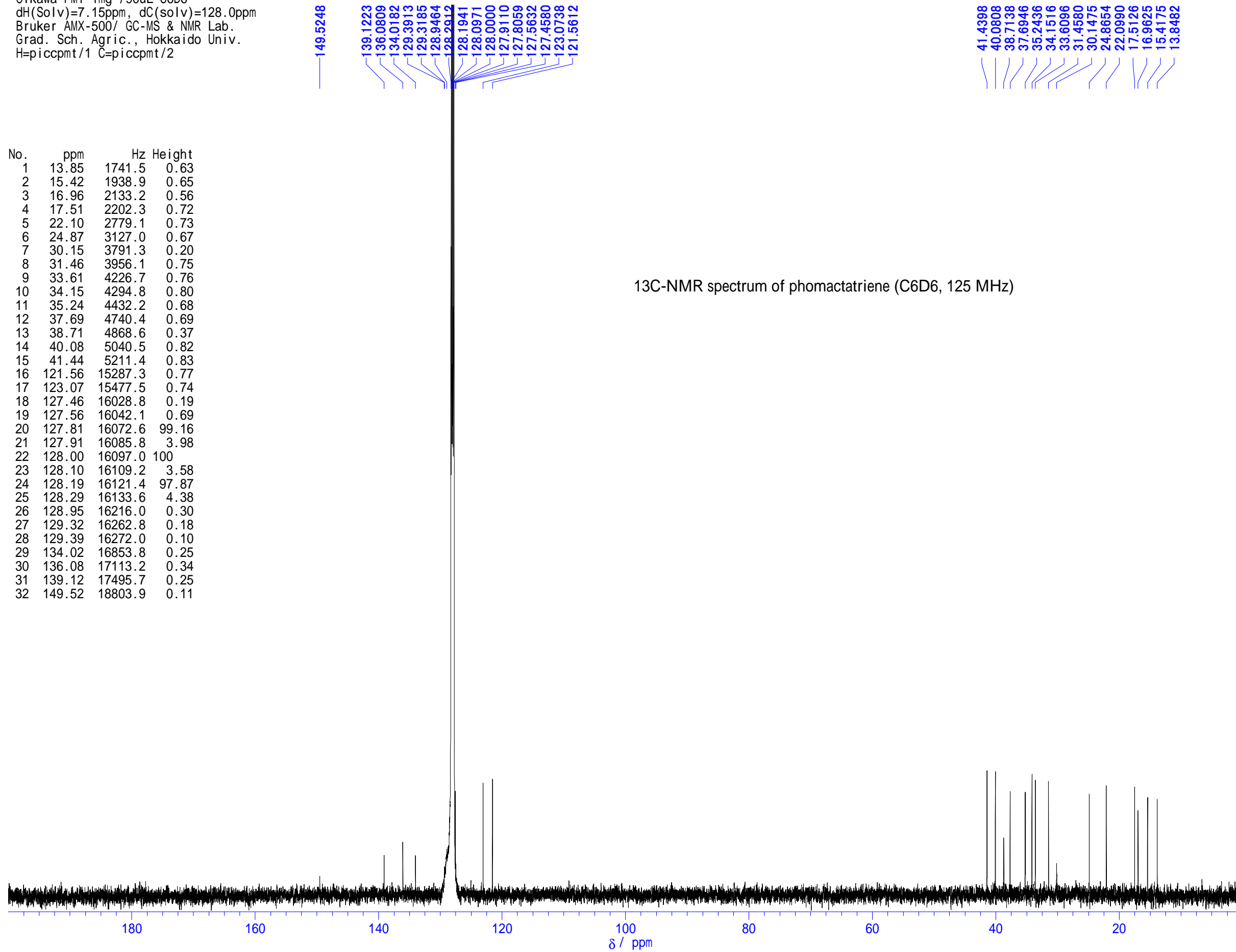
Operator _____



Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=128.0ppm
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2

No.	ppm	Hz	Height
1	13.85	1741.5	0.63
2	15.42	1938.9	0.65
3	16.96	2133.2	0.56
4	17.51	2202.3	0.72
5	22.10	2779.1	0.73
6	24.87	3127.0	0.67
7	30.15	3791.3	0.20
8	31.46	3956.1	0.75
9	33.61	4226.7	0.76
10	34.15	4294.8	0.80
11	35.24	4432.2	0.68
12	37.69	4740.4	0.69
13	38.71	4868.6	0.37
14	40.08	5040.5	0.82
15	41.44	5211.4	0.83
16	121.56	15287.3	0.77
17	123.07	15477.5	0.74
18	127.46	16028.8	0.19
19	127.56	16042.1	0.69
20	127.81	16072.6	99.16
21	127.91	16085.8	3.98
22	128.00	16097.0	100
23	128.10	16109.2	3.58
24	128.19	16121.4	97.87
25	128.29	16133.6	4.38
26	128.95	16216.0	0.30
27	129.32	16262.8	0.18
28	129.39	16272.0	0.10
29	134.02	16853.8	0.25
30	136.08	17113.2	0.34
31	139.12	17495.7	0.25
32	149.52	18803.9	0.11

¹³C-NMR spectrum of phomactatriene (C6D6, 125 MHz)



41.4398
40.0808
38.7138
37.6946
35.2436
34.1516
33.6096
31.4580
30.1475
24.8654
22.0990
17.5126
16.9625
15.4175
13.8482

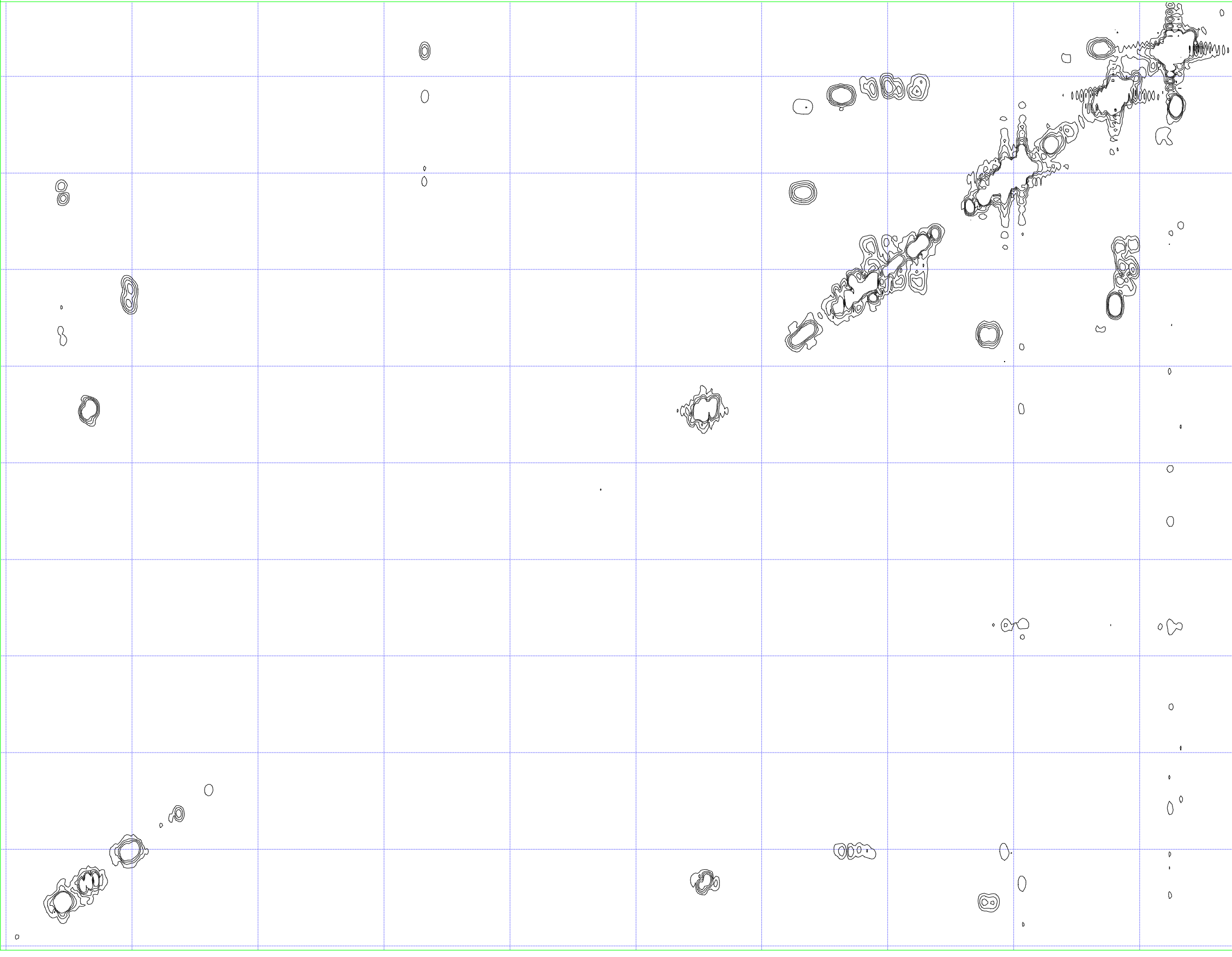
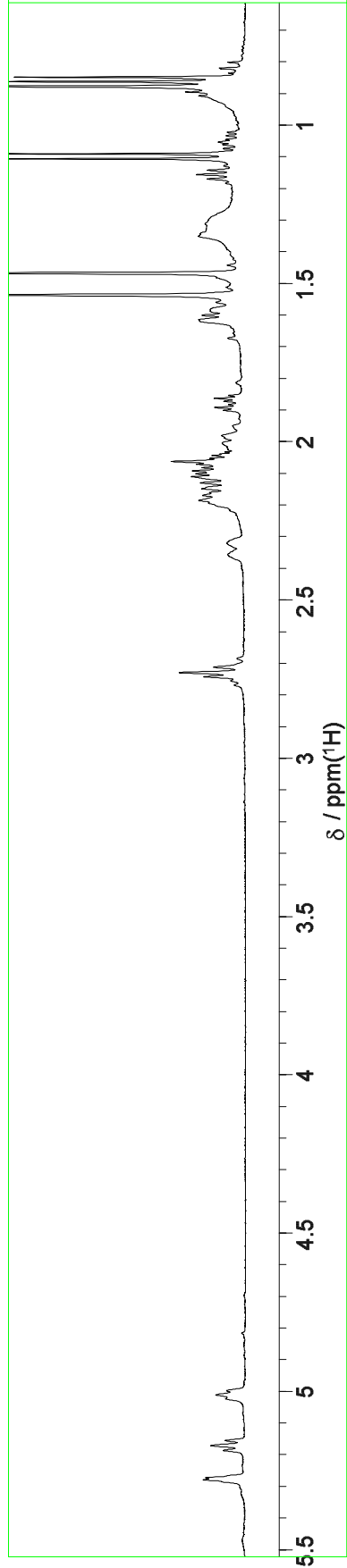
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dH(Solv)=7.15ppm, dC(solv)=1
Bruker AMX-500/ GC-MS & NMR
Grad. Sch. Agric., Hokkaido
H=piccpmt/1 C=piccpmt/2

ObsNuc ?
ExMode
ObsFreq 125.76 MHz
ObsSet 0.0 kHz
ObsFine 10000.78 Hz
Point 32768
Frequency(Span) 33333.33 Hz
Scan 0
AcqTime 0.983 s
PD 0.0 s
Pulse1 10.0 μs
IrrNuc
ProbeHead
Instrument
Pulse Program
Gradient Program
Temperature 0.0
Solvent
Reference 241.812 ppm
Broad.Factor 0.25 Hz
Window Exponential
RGain 0
Operator _____

Exp 1.2

1H-1H COSY spectrum of phomactatriene (C6D6, 500 MHz)

5.5 5 4.5 4 3.5 3 2.5 2 1.5 1 ppm(1H)



Comment
File piccpmt/3
COSY
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=128.0p
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2

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PULPROG cosygs
TD 1024
NS 1
DS 2
D[0] 0.000003 sec
D[1] 1.999999 sec
HL1 5 dB
IN[0] 0.000218 sec
P[1] 12.000000 usec
SW 9.171891 ppm
SWH 4587.155963 Hz
FIDRES 0.223232 Hz
AQ 0.111616 sec
RG 4096
NUCLEUS 1H
SF01 500.131982 MHz
BF1 500.130000 MHz
O1 1982.147592 MHz
SF02 0.000000 MHz
BF2 500.130000 MHz
O2 1982.147592 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-GR
TE 300 K
GRDPROG 2sine
CNST[21] 10 %
CNST[22] -10 %
D[16] 0.000100 sec
P[16] 1000.000000 usec
L[21] 100

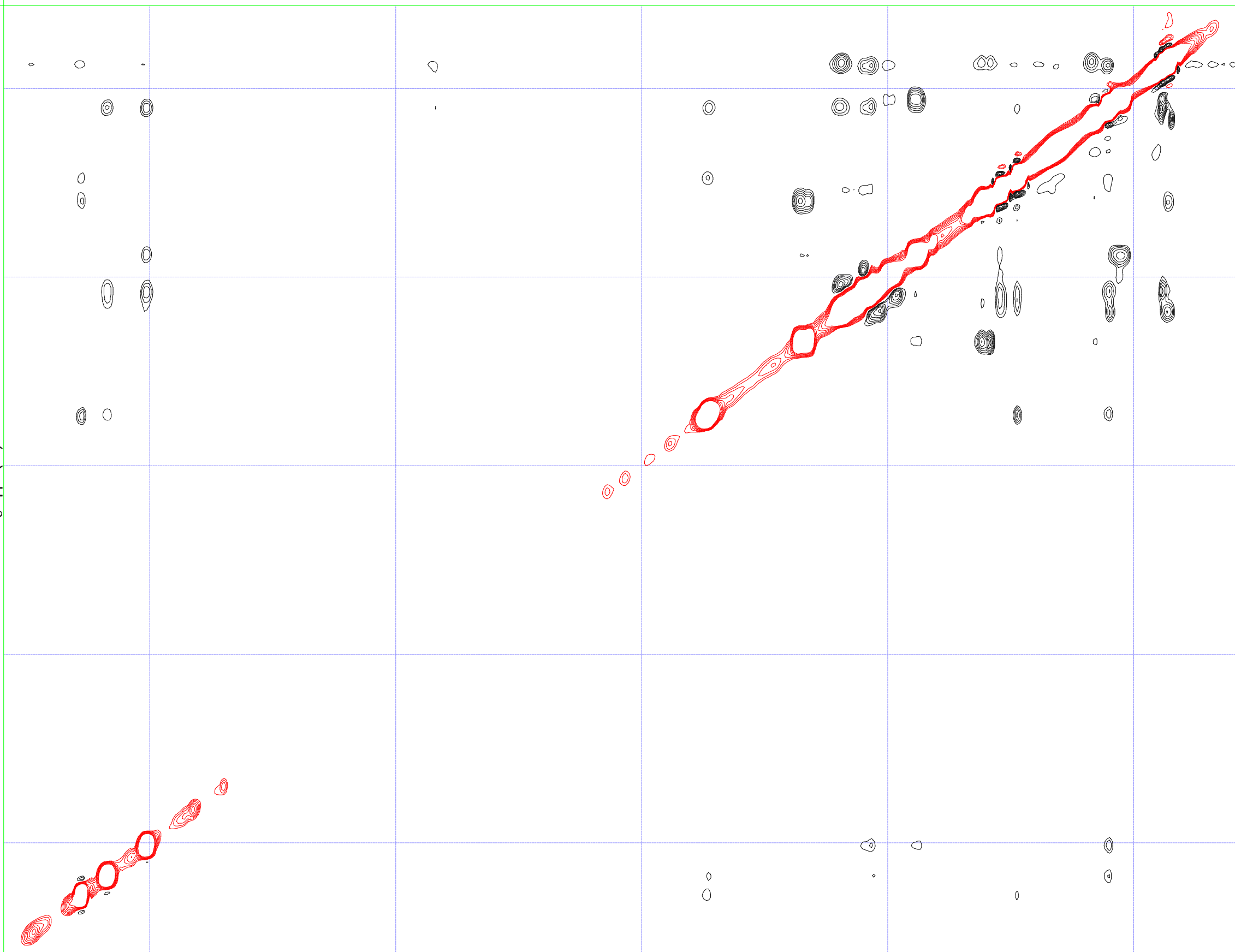
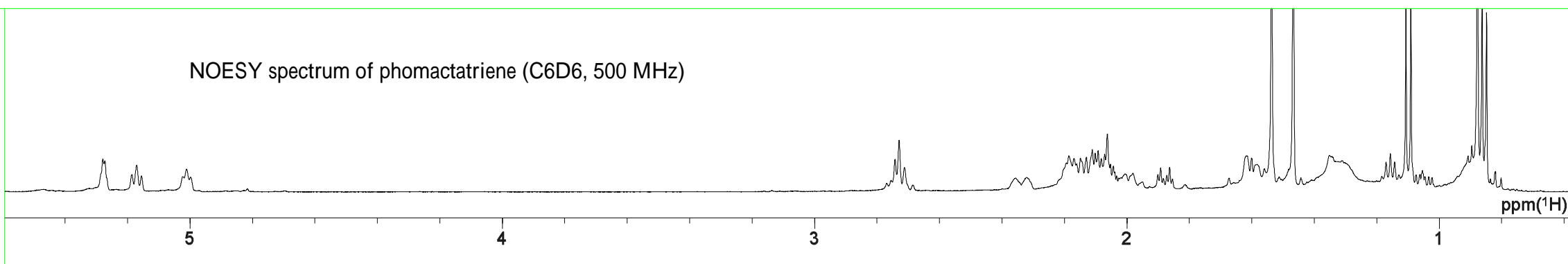
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TD 256
ND0 1
SW 9.171891 ppm
SWH 4587.155963 Hz
FIDRES 0.055808 Hz

\$ F2-Processing Parameters
SI 1024
SF 500.130062538213
OFFSET 8.424184
HZpPT 4.479648 Hz
MC2 qf
WDW SINE
SSB 0.000000

\$ F1-Processing Parameters
SI 512
SF 500.130062538213
OFFSET 8.424184
REVERSE yes
HZpPT 8.959297 Hz
MC2 qf
WDW SINE
SSB 0.000000
expt = ca. 9 min

NOESY spectrum of phomactatriene (C6D6, 500 MHz)

Exp 1.2



Comment
 File piccpmt/4
 noesy
 Oikawa PMT 1mg /90uL C6D6
 dH(Solv)=7.15ppm, dC(solv)=128.0p
 Bruker AMX-500/ GC-MS & NMR Lab.
 Grad. Sch. Agric., Hokkaido Univ.
 H=piccpmt/1 C=piccpmt/2

\$ F2-Acquisition Parameters

Date Mon Jun 17 16:59:41 2002
 PULPROG noesygs.jp
 TD 1024
 NS 16
 DS 4
 D[0] 0.000003 sec
 D[1] 2.002048 sec
 D[9] 1.000000 sec
 HL1 5 dB
 IN[0] 0.000109 sec
 P[1] 12.000000 usec
 V9 2.000000 %
 SW 9.171891 ppm
 SWH 4587.155963 Hz
 FIDRES 0.223232 Hz
 AQ 0.111616 sec
 RG 2048
 NUCLEUS 1H
 SF01 500.131982 MHz
 BF1 500.130000 MHz
 O1 1982.147592 MHz
 SF02 0.000000 MHz
 BF2 500.130000 MHz
 O2 1982.147592 MHz
 LOKNUC 2H
 SOLVENT C6D6
 PROBHD 2.5 mm DUL 13C-1H-D Z-GR
 TE 300 K
 GRDPROG 1sine
 CNST[21] 30 %
 D[16] 0.000100 sec
 P[16] 1000.000000 usec
 L[21] 100

\$ F1-Acquisition Parameters

TD 256
 ND0 2
 SW 9.171891 ppm
 SWH 4587.155963 Hz
 FIDRES 0.055808 Hz

\$ F2-Processing Parameters

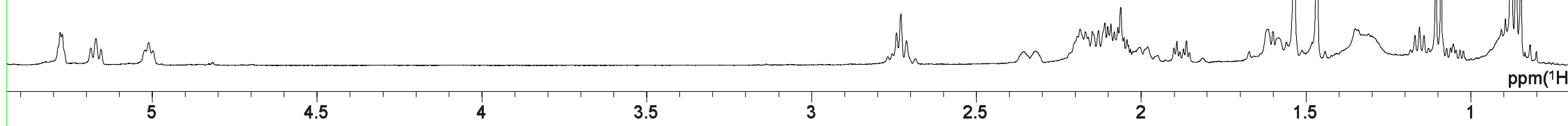
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 SF 500.130062538213
 OFFSET 8.424184
 HZpPT 8.959297 Hz
 MC2 qf
 WDW QSINE
 SSB 2.000000
 PH_mod pk
 PHC0 35.422300 degree
 PHC1 25.800010 degree

\$ F1-Processing Parameters

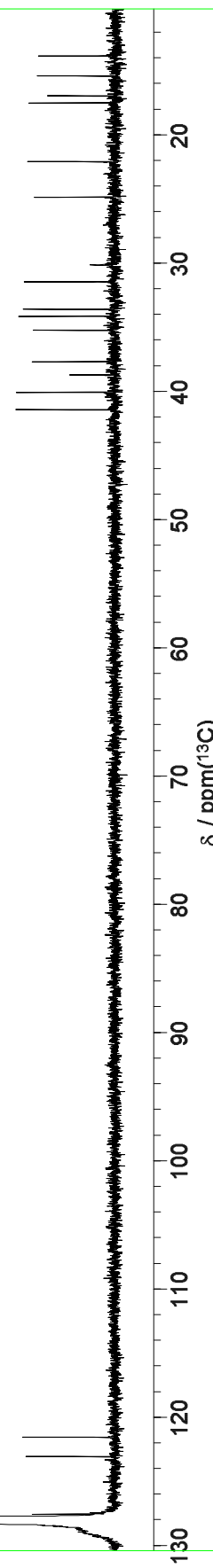
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 OFFSET 8.424184
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 HZpPT 8.959297 Hz
 MC2 TPP1
 WDW QSINE
 SSB 2.000000
 PH_mod pk
 PHC0 15.443750 degree
 PHC1 -29.200000 degree
 expt = ca. 4 hr

Exp 1.2

HMQC spectrum of phomactatriene (C6D6, 500 MHz)



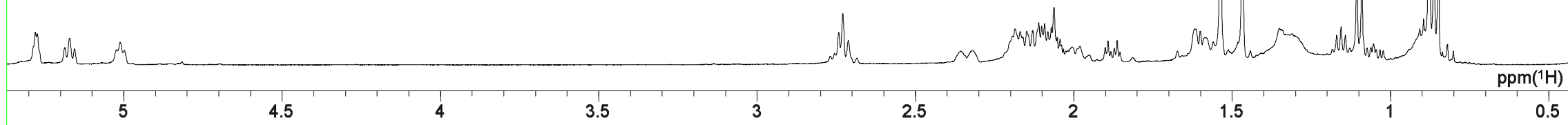
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hsqc
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=128.0p
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2



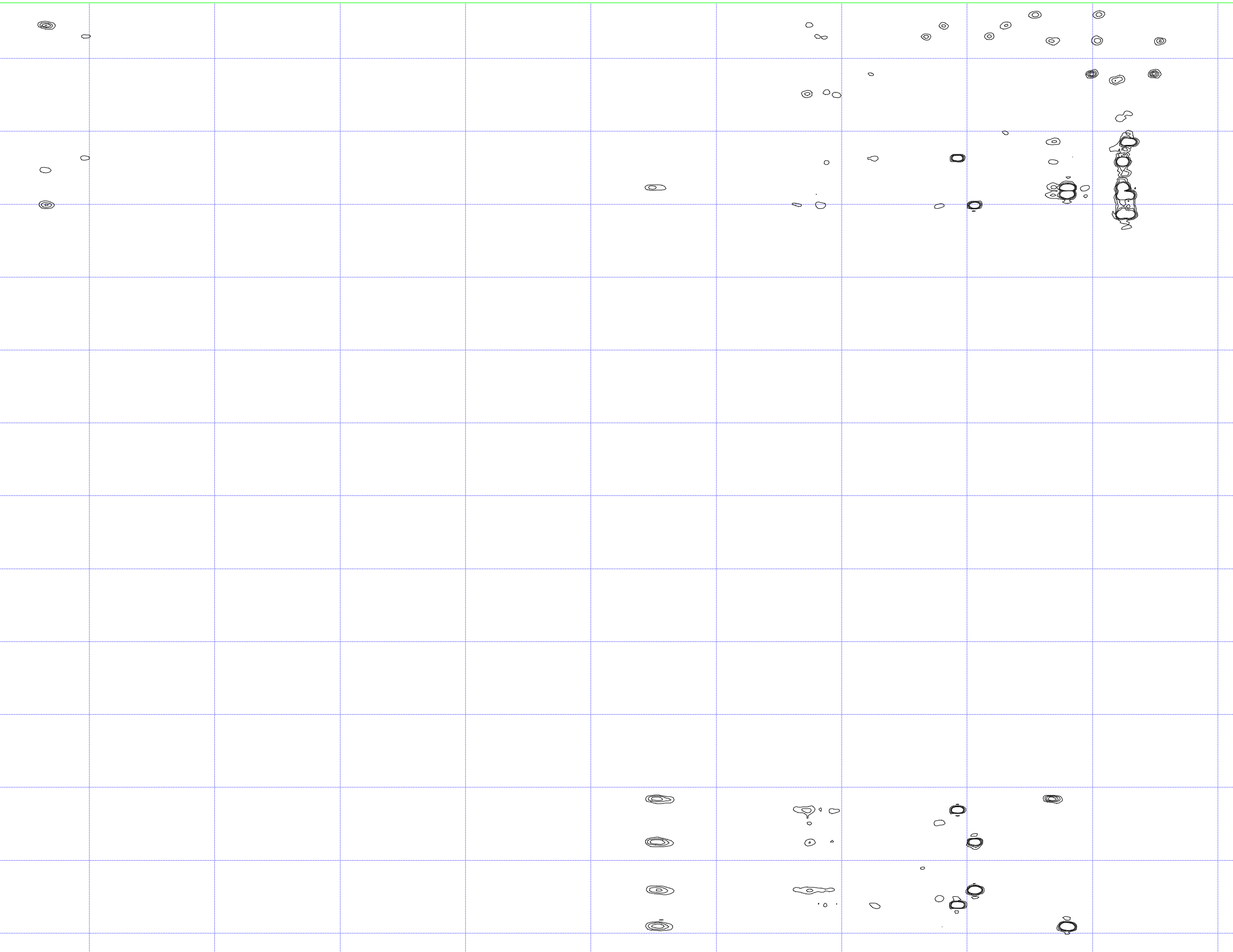
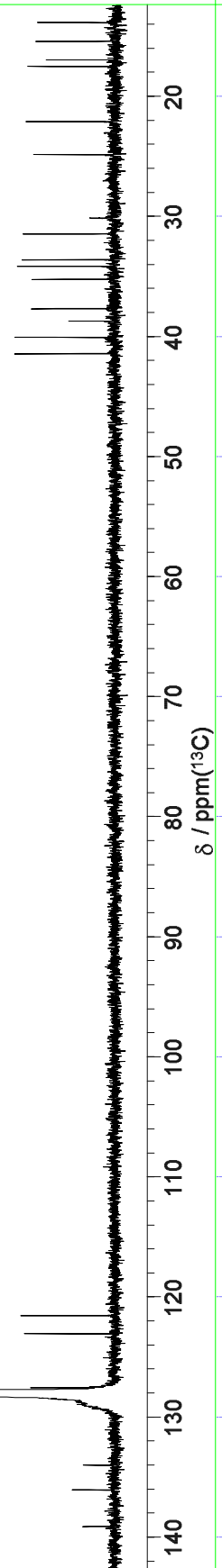
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PULPROG invigstp.m
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NS 96
DS 2
D[0] 0.00003 sec
D[1] 2.181247 sec
D[4] 0.001800 sec
DL[0] 19 dB
DL[5] 41 dB
HL1 5 dB
IN[0] 0.000013 sec
P[1] 12.000000 usec
P[3] 5.800000 usec
P[31] 0.000000 usec
SW 9.171891 ppm
SWH 4587.155963 Hz
FIDRES 0.223232 Hz
AQ 0.111616 sec
RG 32768
NUCLEUS 1H
SF01 500.131982 MHz
BF1 500.130000 MHz
O1 1982.147592 MHz
SF02 125.768146 MHz
BF2 125.757739 MHz
O2 10407.375084 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-GR
TE 300 K
GRDPROG 3sine
CNST[21] 40 %
CNST[22] 15 %
CNST[23] 10 %
D[16] 0.000000 sec
P[16] 1000.000000 usec
L[21] 100
\$ F1-Acquisition Parameters
TD 256
ND0 4
SW 159.022778 ppm
SWH 20000.000000 Hz
FIDRES 0.012800 Hz
\$ F2-Processing Parameters
SI 1024
SF 500.130062538213
OFFSET 8.424184
HZpPT 4.479648 Hz
MC2 qf
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 -100.325000 degree
PHC1 -25.600000 degree
\$ F1-Processing Parameters
SI 512
SF 125.757762832763
OFFSET 162.0858
REVERSE no
HZpPT 39.062500 Hz
MC2 TPPI
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 289.530900 degree
PHC1 -253.800000 degree
expt = ca. 16 hr

Exp 1.2

HMBC spectrum of phomactatriene (C6D6, 500 MHz)

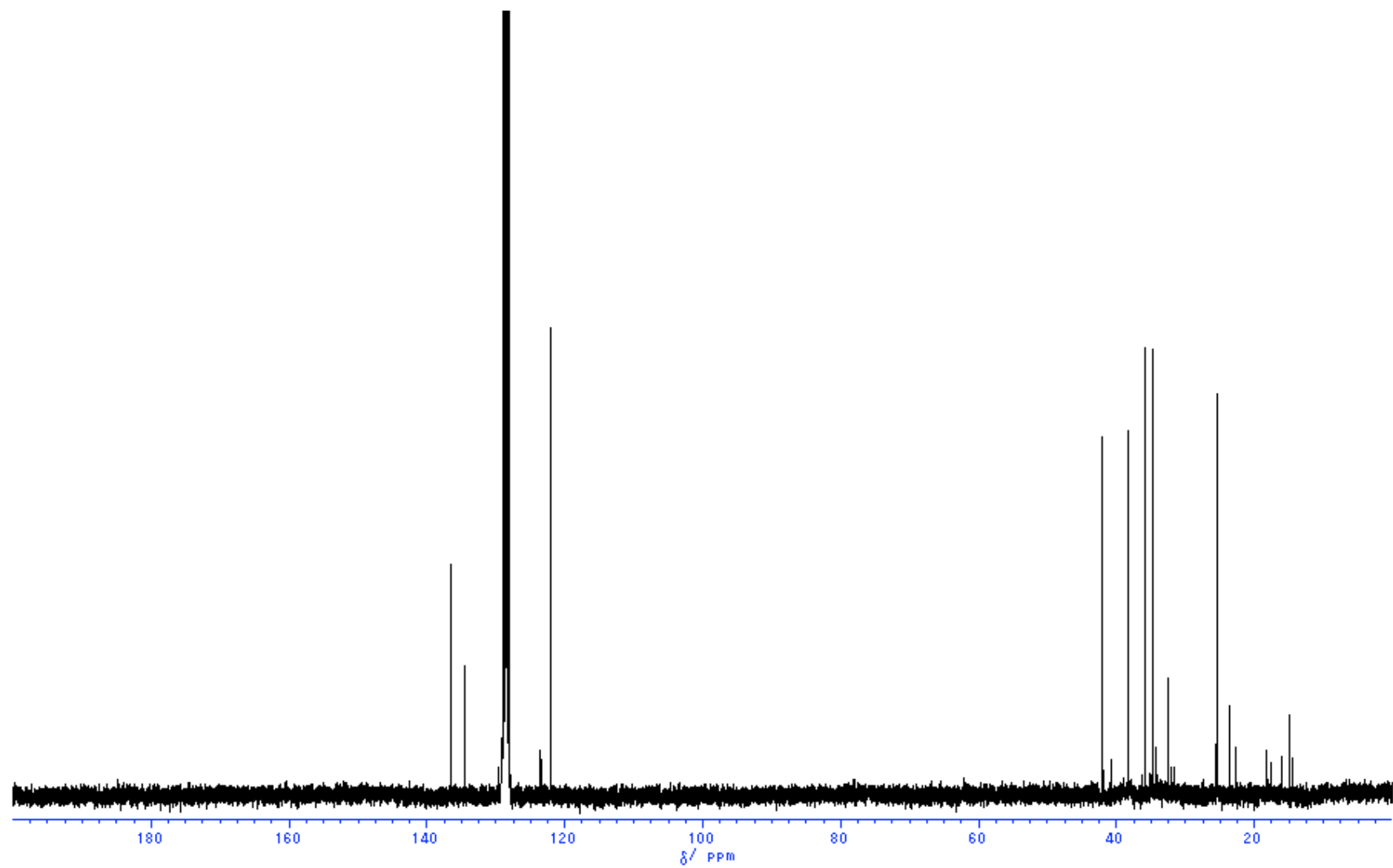


Comment File piccpmt/6
HMBC
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=128.0p
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2

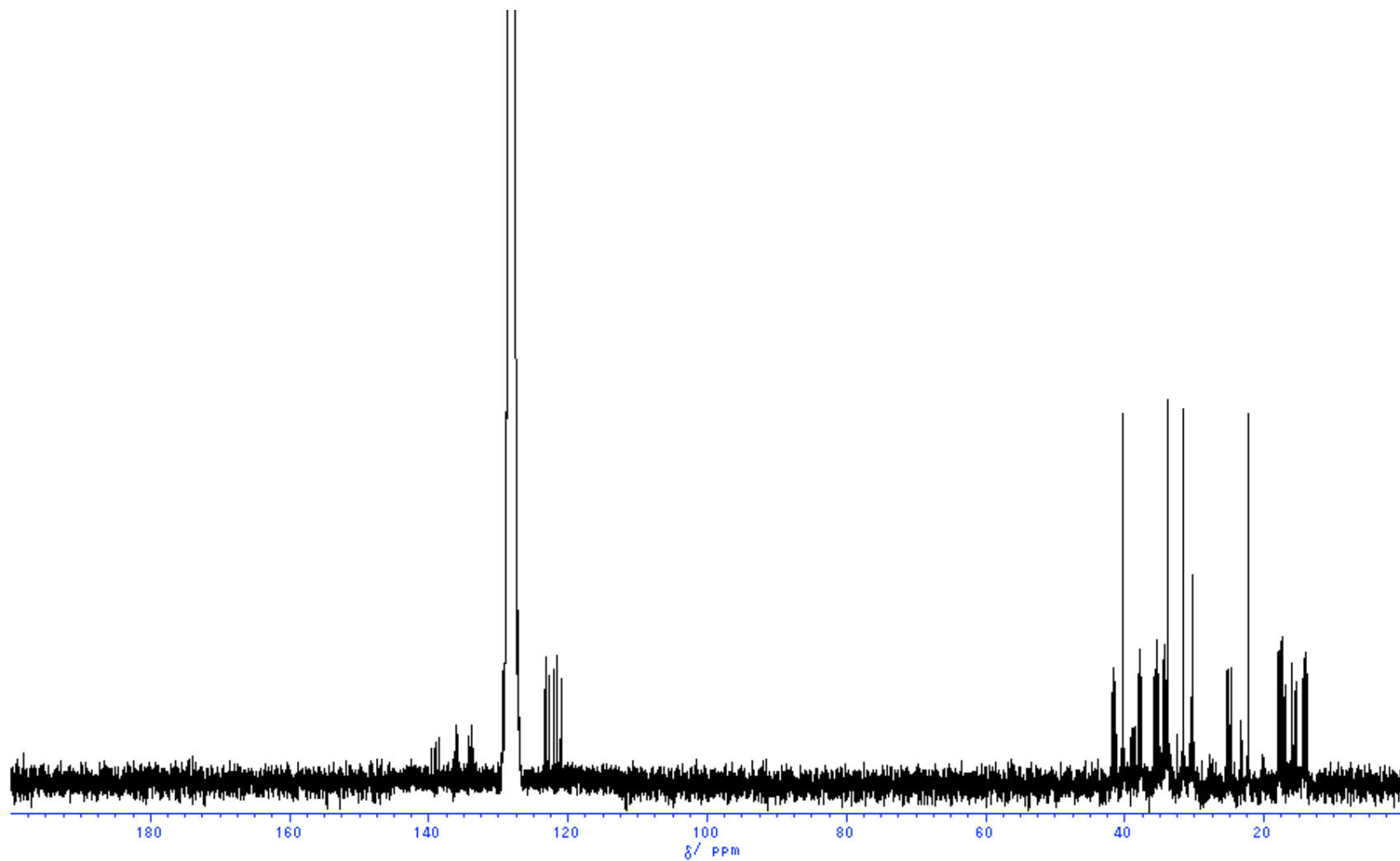


\$ F2-Acquisition Parameters
Date Tue Jun 18 02:02:20 2002
PULPROG inv4gs1prnd.jp
TD 1024
NS 192
DS 2
D[0] 0.000003 sec
D[1] 2.000000 sec
D[2] 0.003450 sec
D[6] 0.070000 sec
DL[0] 19 dB
HL1 5 dB
IN[0] 0.000025 sec
P[1] 12.000000 usec
P[3] 5.800000 usec
SW 9.171891 ppm
SWH 4587.155963 Hz
FIDRES 0.223232 Hz
AQ 0.111616 sec
RG 32768
NUCLEUS 1H
SF01 500.131982 MHz
BF1 500.130000 MHz
O1 1982.147592 MHz
SF02 125.768146 MHz
BF2 125.757739 MHz
O2 10407.375084 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-GR
TE 300 K
GRDPROG 3sine
CNST[21] 10 %
CNST[22] 10 %
CNST[23] 5 %
D[16] 0.000000 sec
P[16] 1000.000000 usec
L[21] 100
\$ F1-Acquisition Parameters
TD 256
NDO 2
SW 159.022778 ppm
SWH 20000.000000 Hz
FIDRES 0.012800 Hz
\$ F2-Processing Parameters
SI 1024
SF 500.130062538213
OFFSET 8.424184
HZpPT 4.479648 Hz
MC2 qf
WDW GM
GB 0.300000
LB -10.000000
\$ F1-Processing Parameters
SI 512
SF 125.757762832763
OFFSET 162.0858
REVERSE no
HZpPT 39.062500 Hz
MC2 qf
WDW QSINE
SSB 6.000000
expt = ca. 29 hr

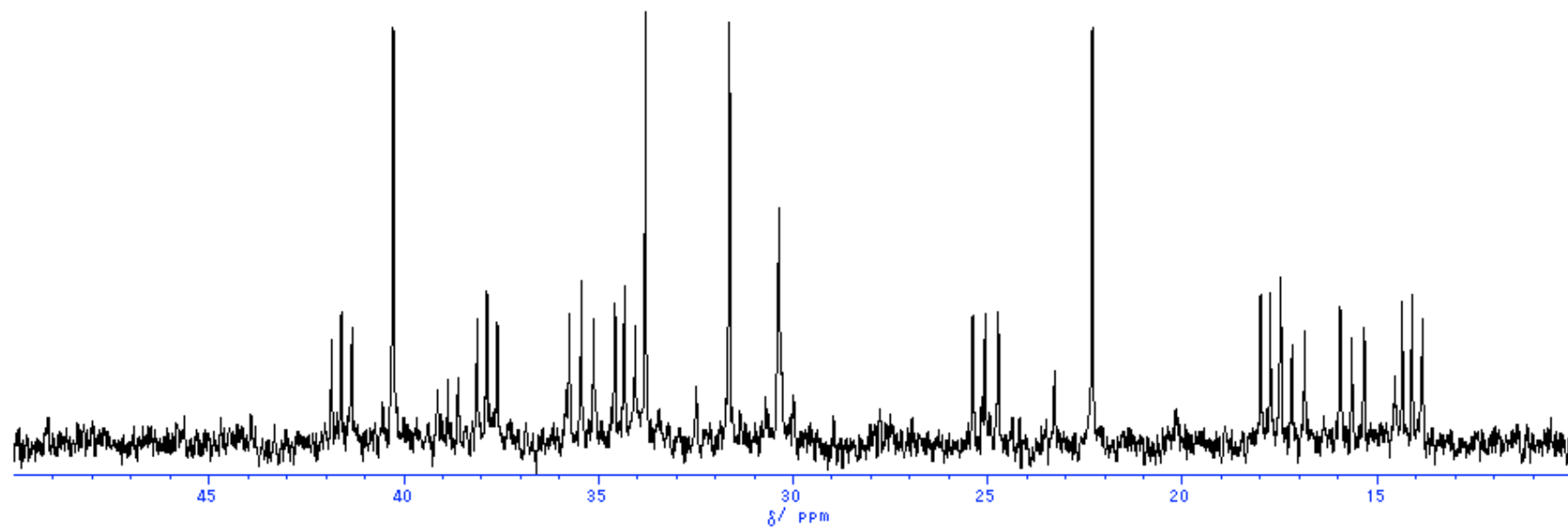
¹³C-NMR spectrum of phomactatriene incorporated with [1-¹³C]-acetate (C₆D₆, 125 MHz)



¹³C-NMR spectrum of phomactatriene incorporated with [1,2-¹³C₂]-acetate (C₆D₆, 125 MHz)



^{13}C -NMR spectrum of phomactatriene incorporated with
[1,2- $^{13}\text{C}_2$]-acetate (C_6D_6 , 125 MHz)



^{13}C -NMR spectrum of phomactatriene incorporated with
[1,2- $^{13}\text{C}_2$]-acetate (C_6D_6 , 125 MHz)

