

Five new limonoids and their anti-TMV activities from
Munronia unifoliolata Oliv.

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[illegible][illegible]

Figure S 3. HMQC spectrum of munronoid K (1) in CDCl₃

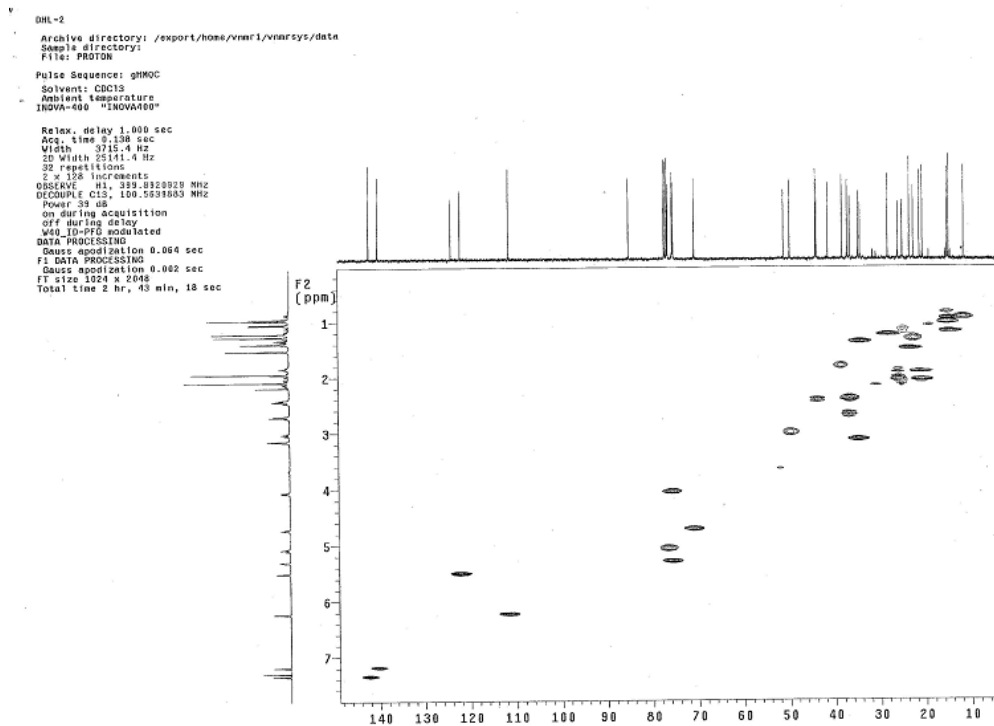


Figure S 4. HMBC spectrum of munronoid K (1) in CDCl₃

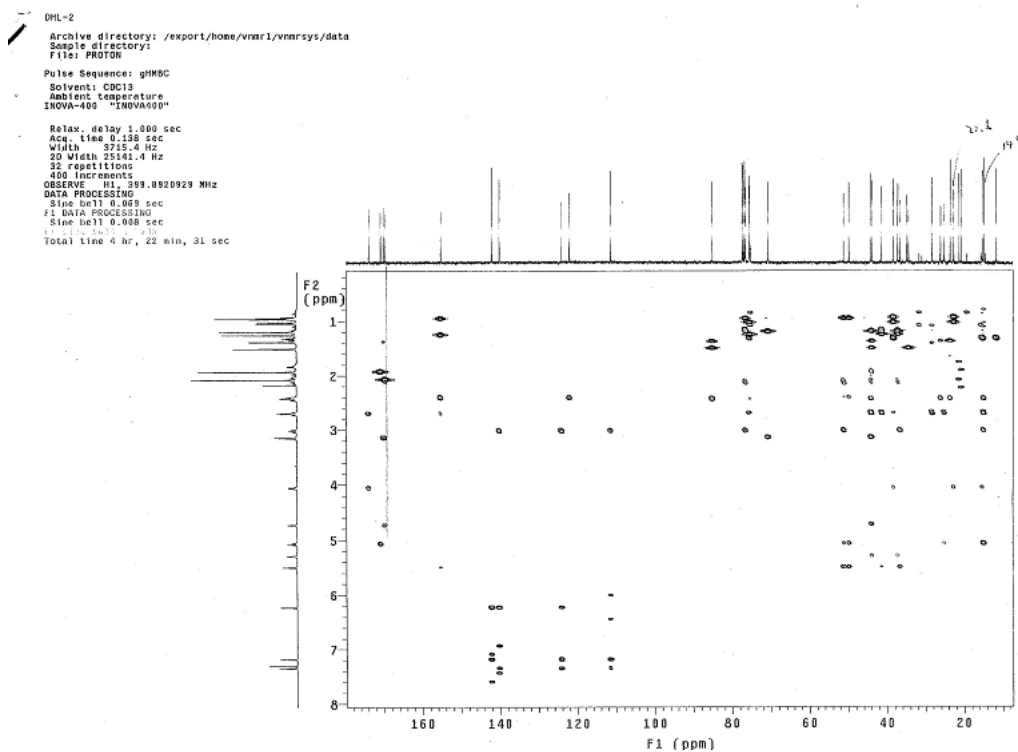


Figure S 5. ^1H - ^1H COSY spectrum of munronoid K (**1**) in CDCl_3

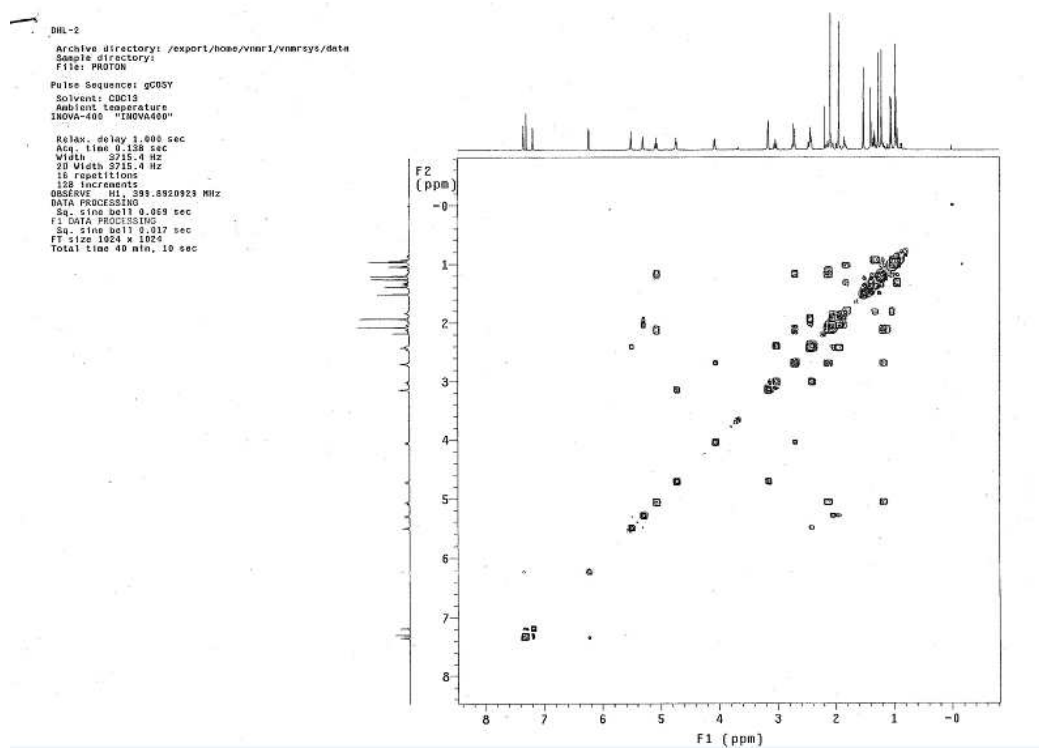


Figure S 6. ROESY spectrum of munronoid K (**1**) in CDCl_3

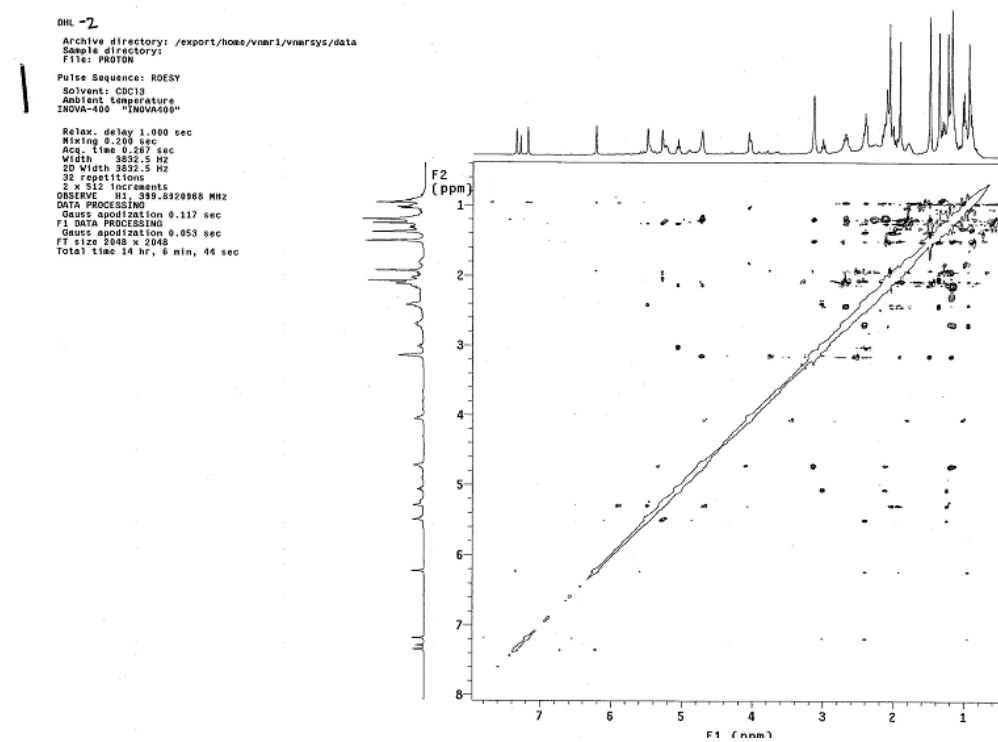


Figure S 7. IR spectrum of munronoid K (1)

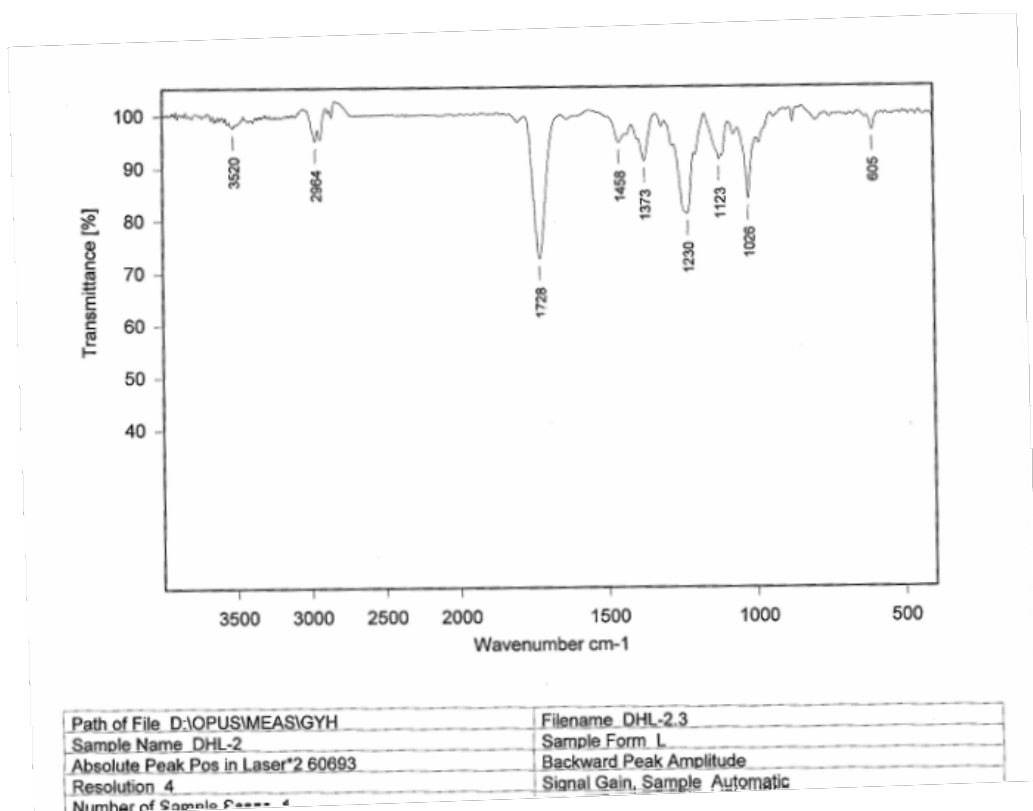


Figure S 8. EI-MS spectrum of munronoid K (1)

File : C:\HPCHEM\2\DATA\10051201.D
 Operator : wangdaoping
 Acquired : 12 May 2010 11:09 using AcqMethod WDP
 Instrument : GC/MS Ins
 Sample Name: DHL-2
 Misc Info : Ge Yong-hui
 Vial Number: 1

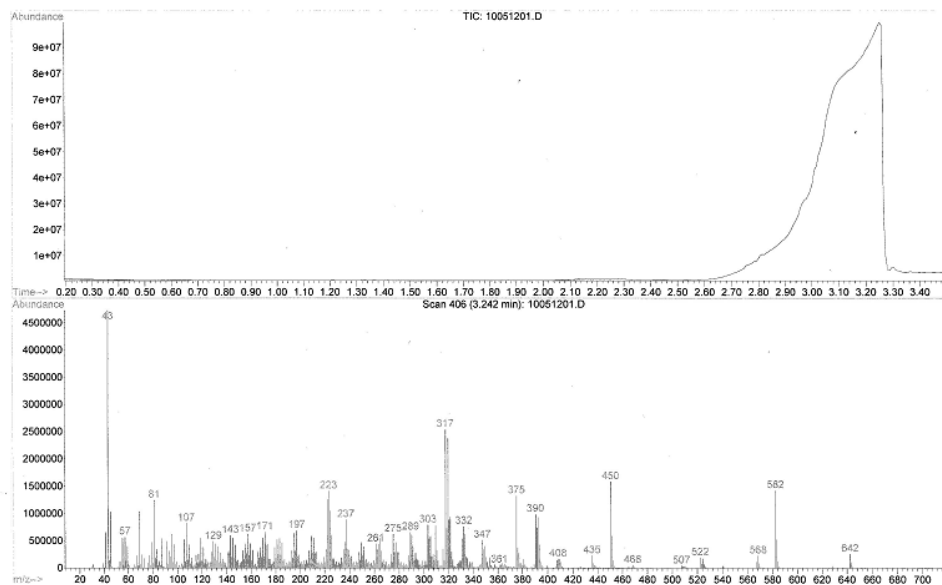


Figure S 9. ESI-MS spectrum of munronoid K (1)

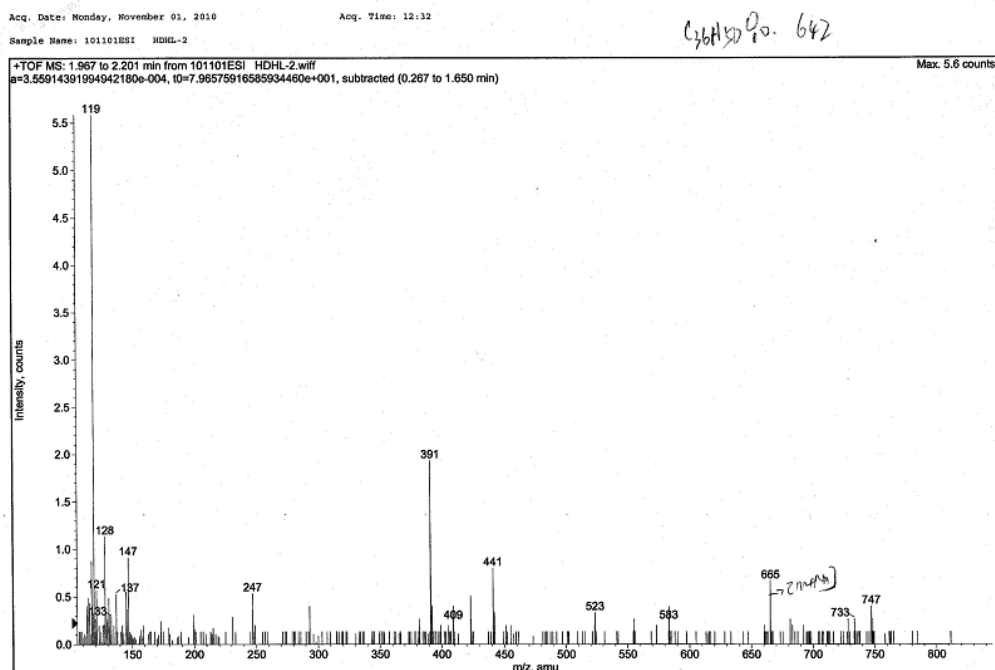
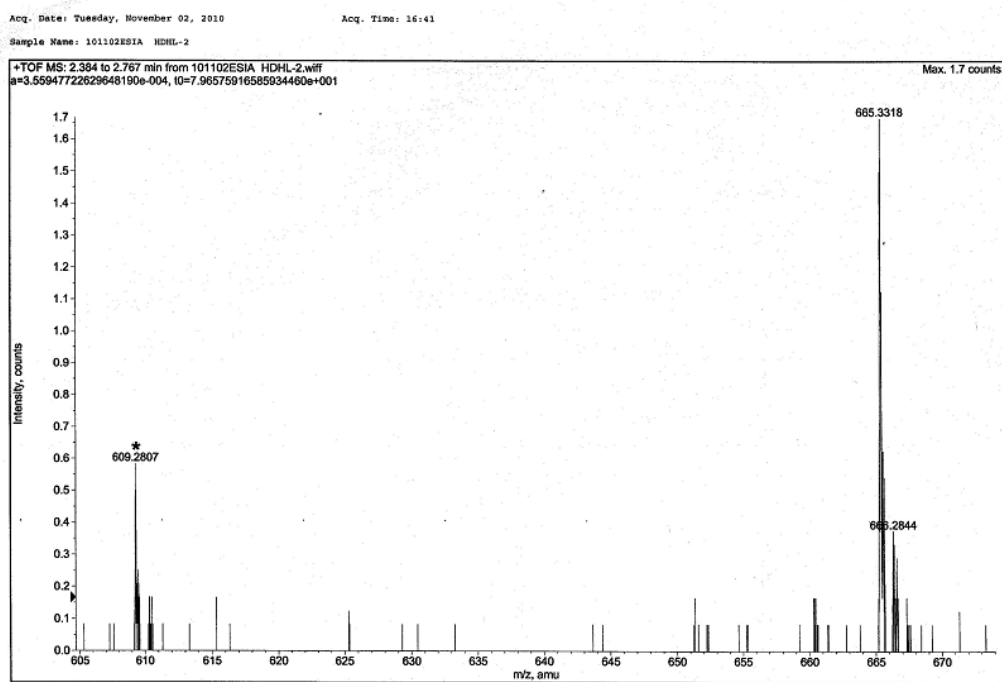


Figure S 10. HR-ESI-MS spectrum of munronoid K (1)



DHL-43

Archive directory: /export/home/vmr/chemsys/data
Sample directory: /
File: PROTON

Pulse Sequence: s2pul

Integration values (from left to right): 1.36, 1.00, 1.14, 1.14, 1.04, 1.08, 1.98, 0.96, 1.10, 1.12, 1.34, 8.54, 5.18, 3.24, 3.04, 8.11, 9.04.

Chemical shift values (ppm) listed on the right side of the spectrum:

- 3.152
- 3.012
- 2.989
- 2.955
- 2.925
- 2.764
- 2.752
- 2.527
- 2.486
- 2.331
- 2.304
- 2.150
- 2.174
- 2.146
- 2.118
- 2.107
- 2.066
- 2.031
- 2.024
- 1.953
- 1.836
- 1.807
- 1.784
- 1.513
- 1.404
- 1.384
- 1.175
- 1.158
- 1.154
- 1.114
- 1.050
- 1.009
- 0.894
- 0.873

[illegible]

Figure S 13. HMQC spectrum of munronoid L (2) in CDCl₃

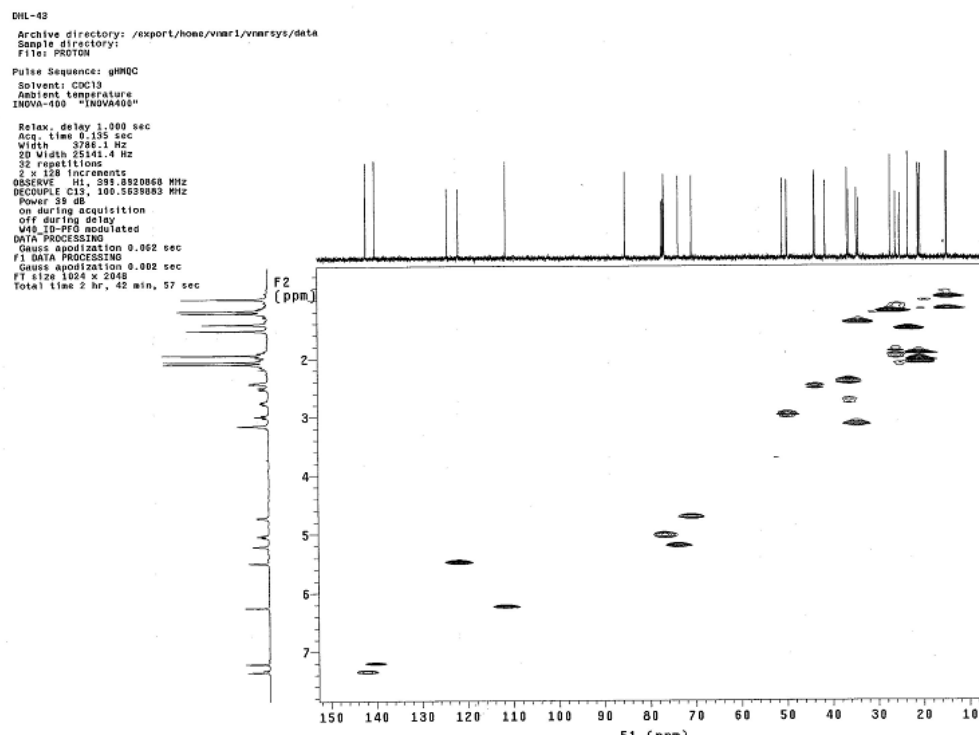


Figure S 14. HMBC spectrum of munronoid L (2) in CDCl₃

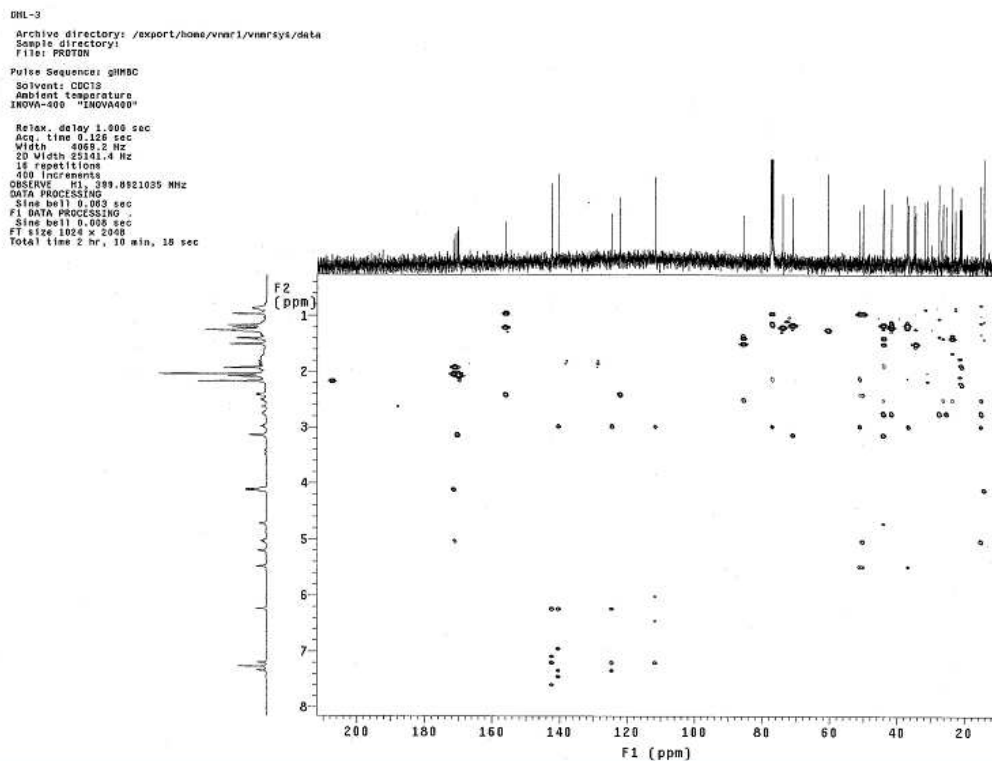


Figure S 15. ^1H - ^1H COSY spectrum of munronoid L (**2**) in CDCl_3

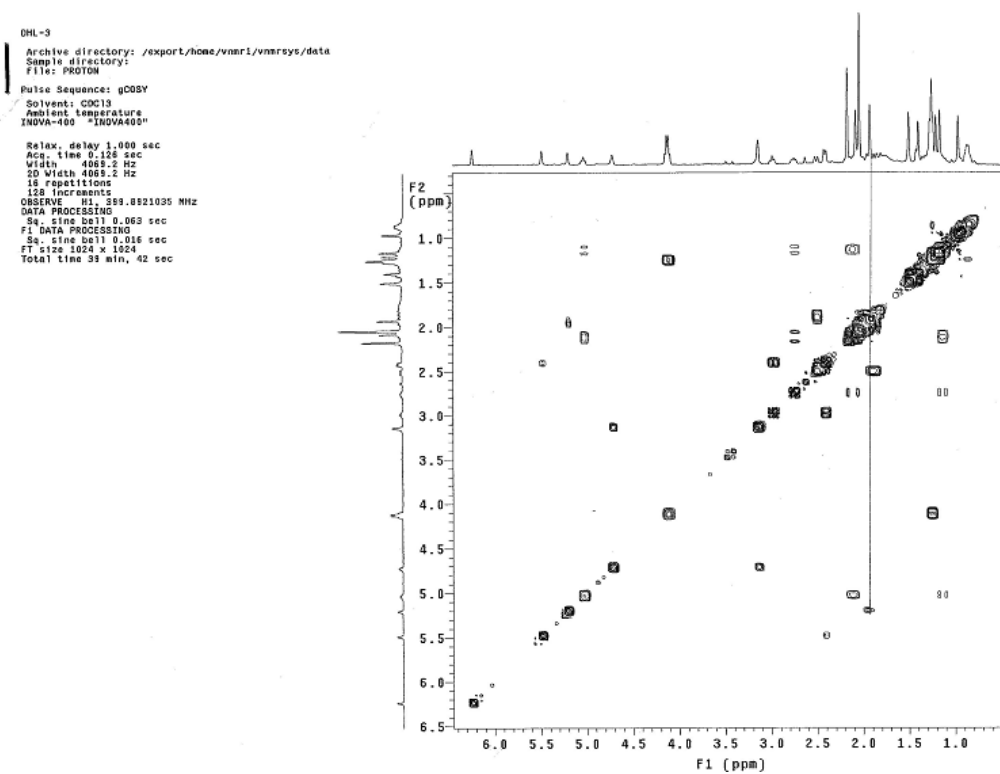


Figure S 16. ROESY spectrum of munronoid L (**2**) in CDCl_3

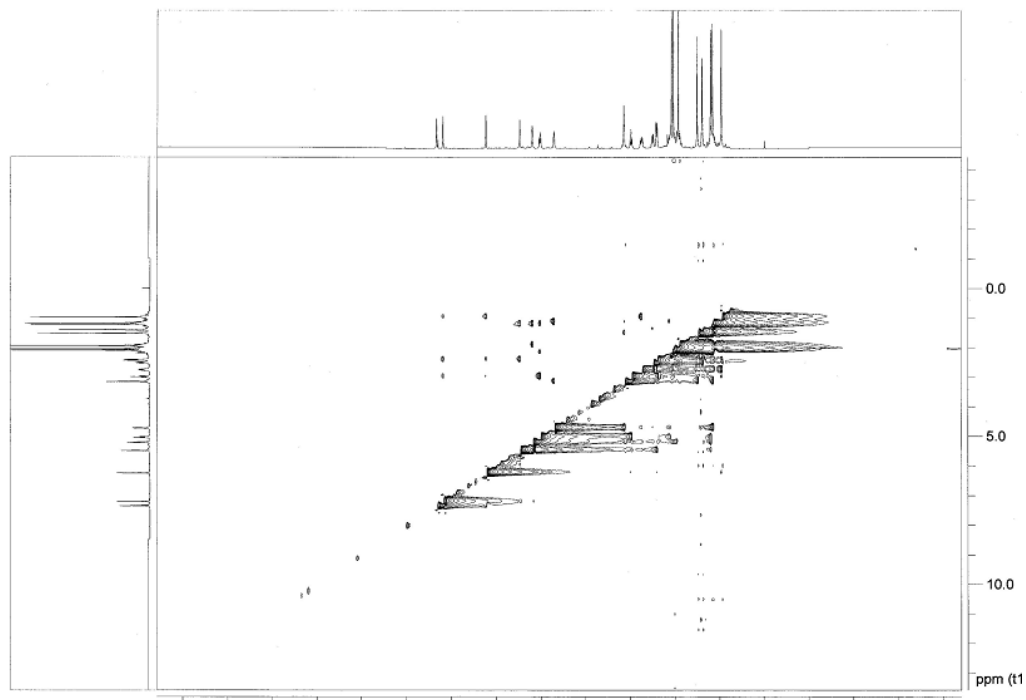
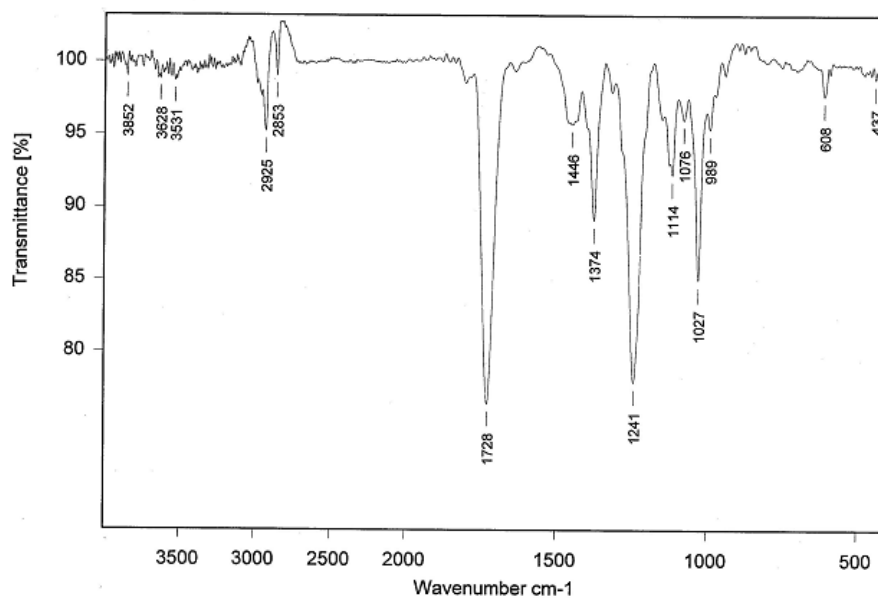


Figure S 17. IR spectrum of munronoid L (2)



Path of File D:\OPUS\MEAS\GYH	Filename DHL-3.0
Sample Name DHL-3	Sample Form L
Absolute Peak Pos in Laser*2 60693	Backward Peak Amplitude
Resolution 4	Signal Gain. Sample Automatic

Figure S 18. EI-MS spectrum of munronoid L (2)

File : C:\HPCHEM\2\DATA\10071407.D
 Operator : wangdaoping
 Acquired : 14 Jul 2010 11:03 using AcqMethod WDP
 Instrument : GC/MS Ins
 Sample Name: DHL-3
 Misc Info : Ge Yong-hui
 Vial Number: 1

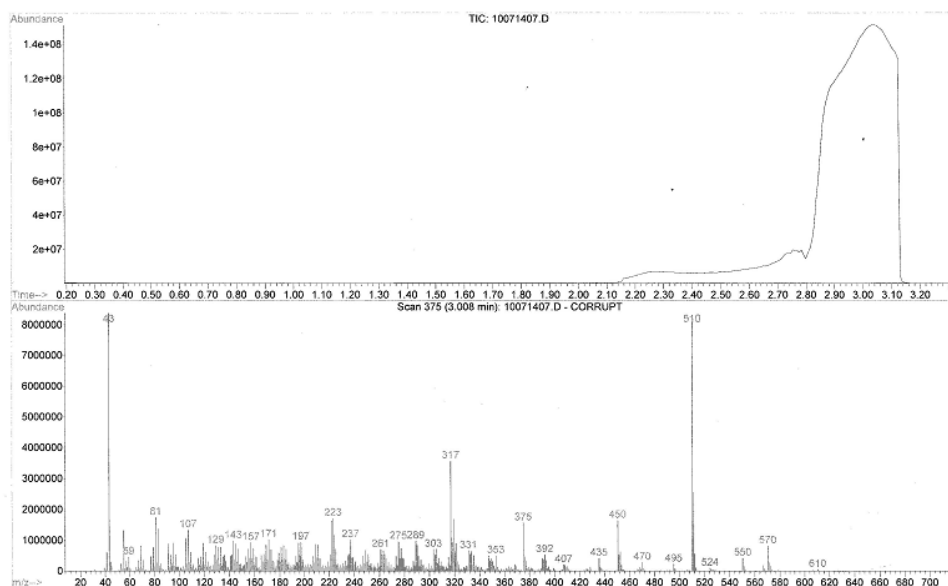


Figure S 19. ESI-MS spectrum of munronoid L (2)

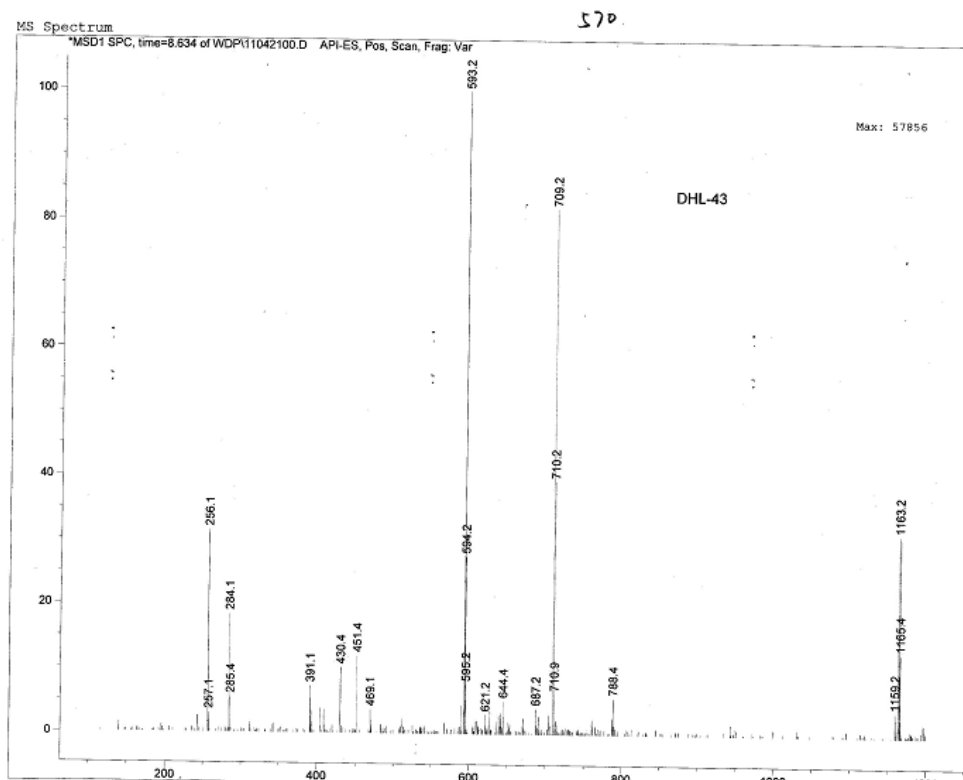
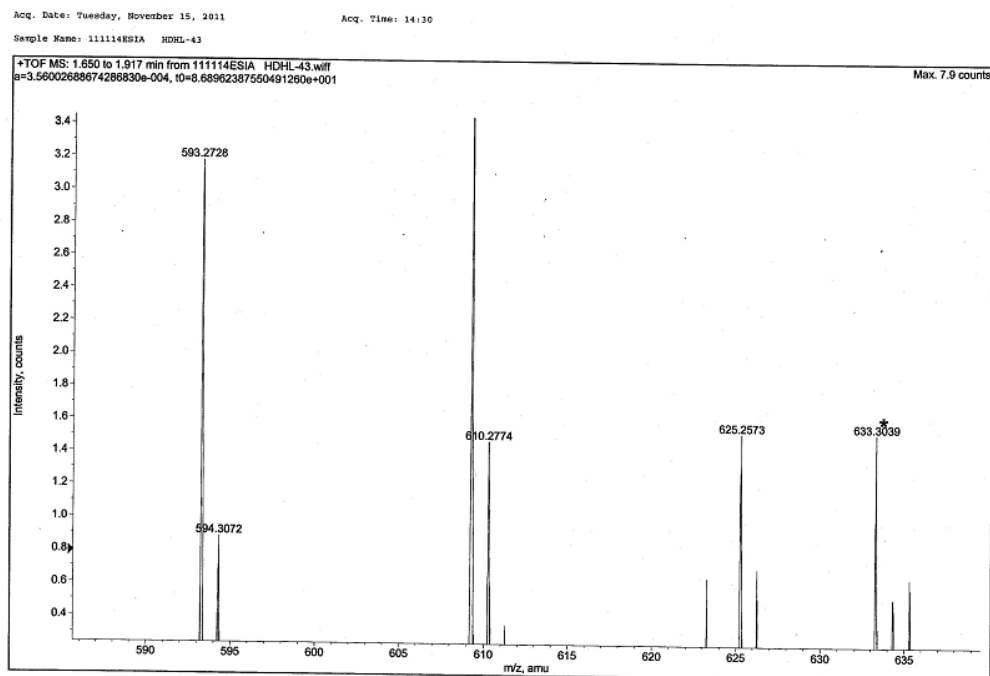


Figure S 20. HR-ESI-MS spectrum of munronoid L (2)



[illegible]

MS8 DML-8

Archive directory: /export/home/vnmr1/vnmr5ys/data
Sample directory:
File: CARBON

Pulse Sequence: s2pu1

Chemical shift values (ppm):

- 170.8516
- 170.1770
- 161.5060
- 155.6520
- 155.4880
- 142.004
- 137.671
- 137.420
- 134.305
- 131.511
- 131.772
- 131.644
- 111.568
- 85.447
- 77.000
- 77.000
- 77.000
- 76.876
- 75.864
- 75.850
- 75.162
- 70.380
- 69.331
- 51.130
- 49.881
- 48.376
- 44.120
- 44.054
- 41.883
- 41.794
- 41.650
- 41.618
- 35.612
- 34.802
- 34.518
- 30.313
- 28.660
- 27.582
- 24.442
- 24.032
- 23.700
- 23.700
- 21.327
- 19.882
- 17.235
- 15.289
- 14.856
- 14.464
- 10.859
- 11.309

Figure S 23. HMQC spectrum of munronoid M (3) in CDCl₃

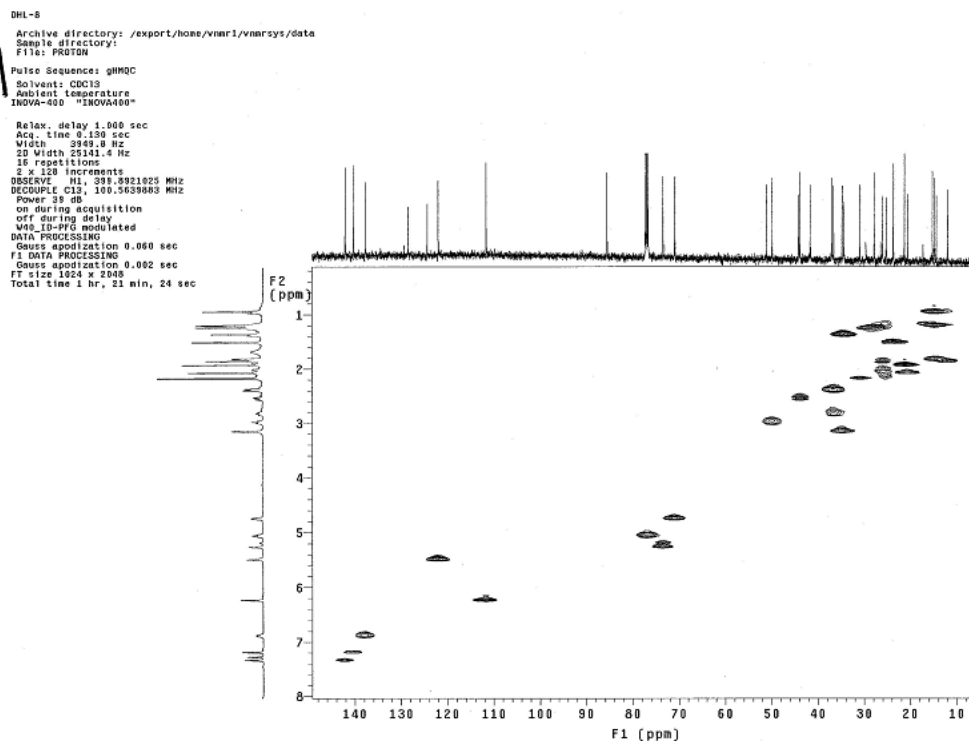


Figure S 24. HMBC spectrum of munronoid M (3) in CDCl₃

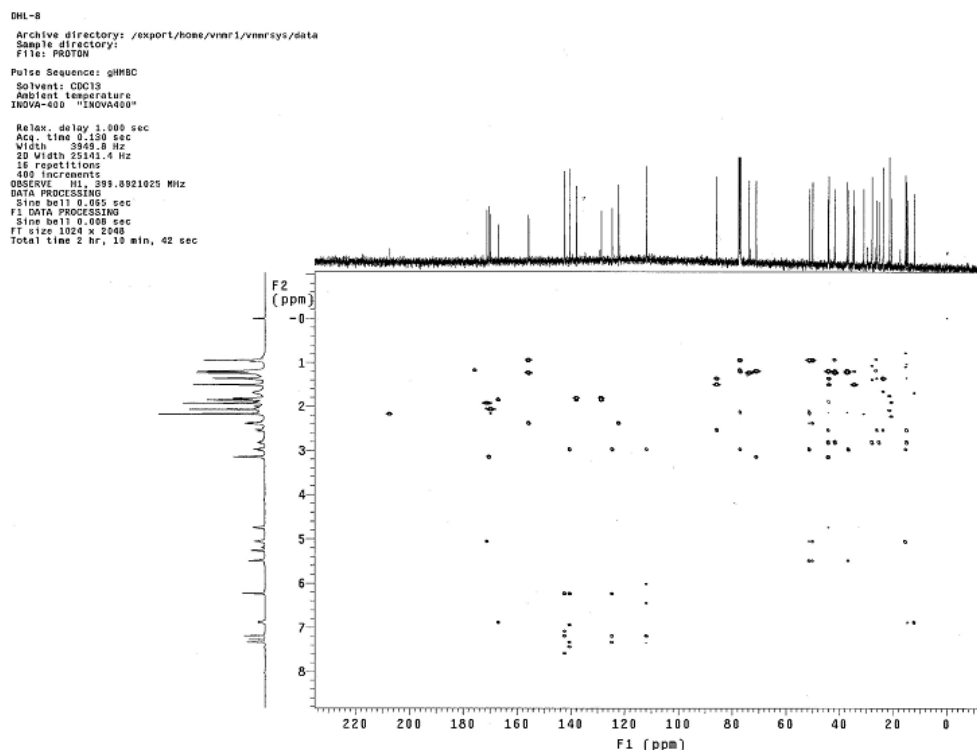


Figure S 25. ^1H - ^1H COSY spectrum of munronoid M (**3**) in CDCl_3

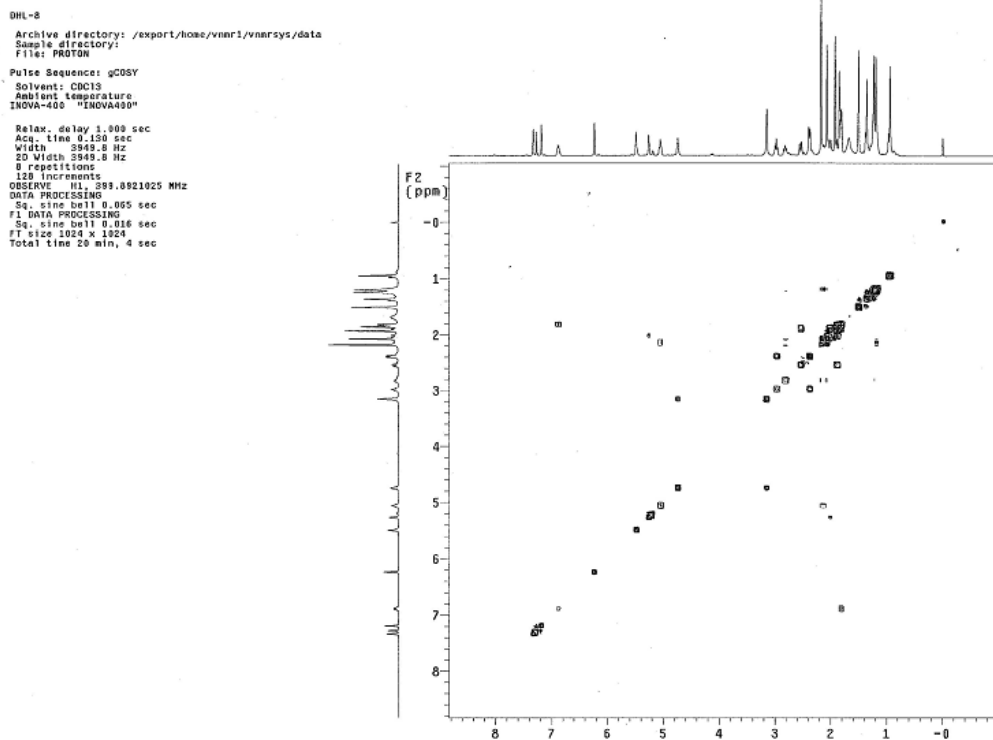


Figure S 26. ROESY spectrum of munronoid M (**3**) in CDCl_3

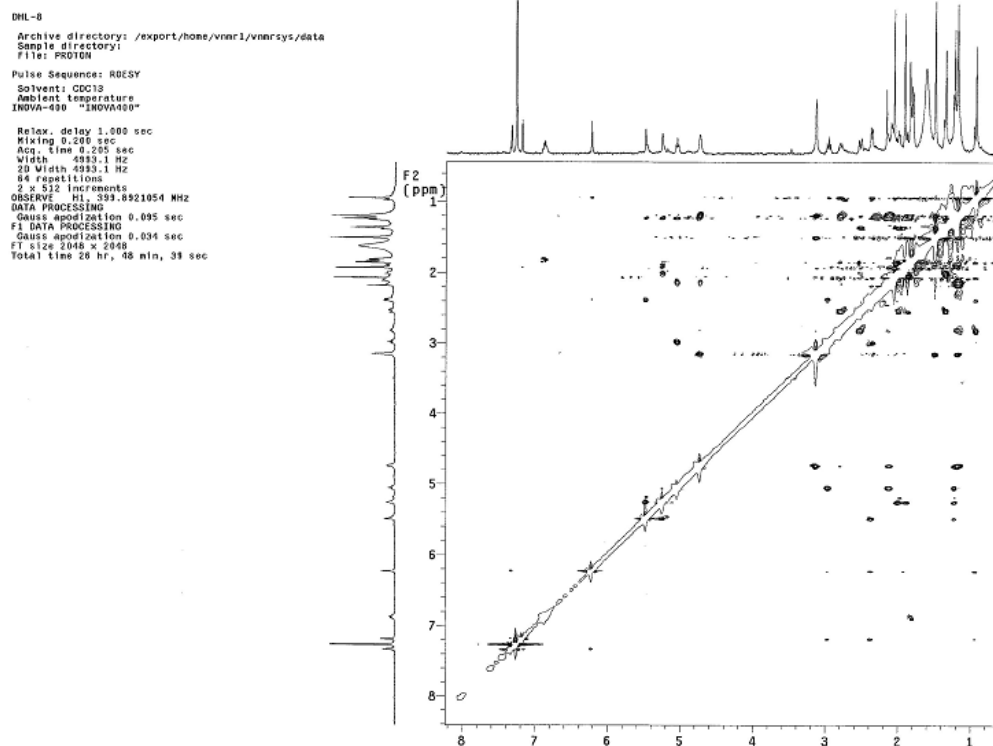
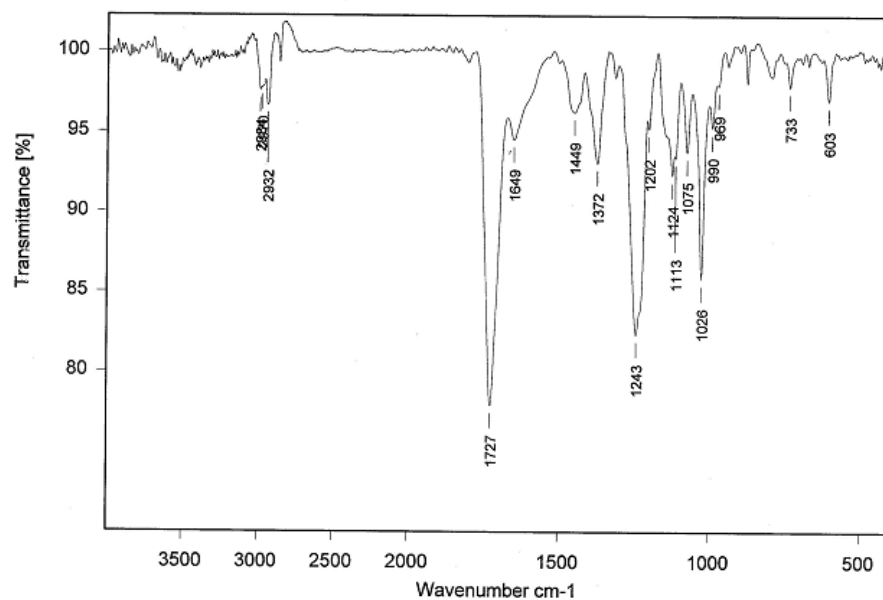


Figure S 27. IR spectrum of munronoid M (3)



Path of File D:\OPUS\MEAS\GYH	Filename DHL-8.0
Sample Name DHL-8	Sample Form L
Absolute Peak Pos in Laser*2 60693	Backward Peak Amplitude
Resolution 4	Signal Gain Sample Automatic

Figure S 28. EI-MS spectrum of munronoid M (3)

File : C:\HPCHEM\2\DATA\10071410.D
 Operator : wangdaoping
 Acquired : 14 Jul 2010 11:16 using AcqMethod WDP
 Instrument : GC/MS Ins
 Sample Name: DHL-8
 Misc Info : Ge Yong-hui
 Vial Number: 1

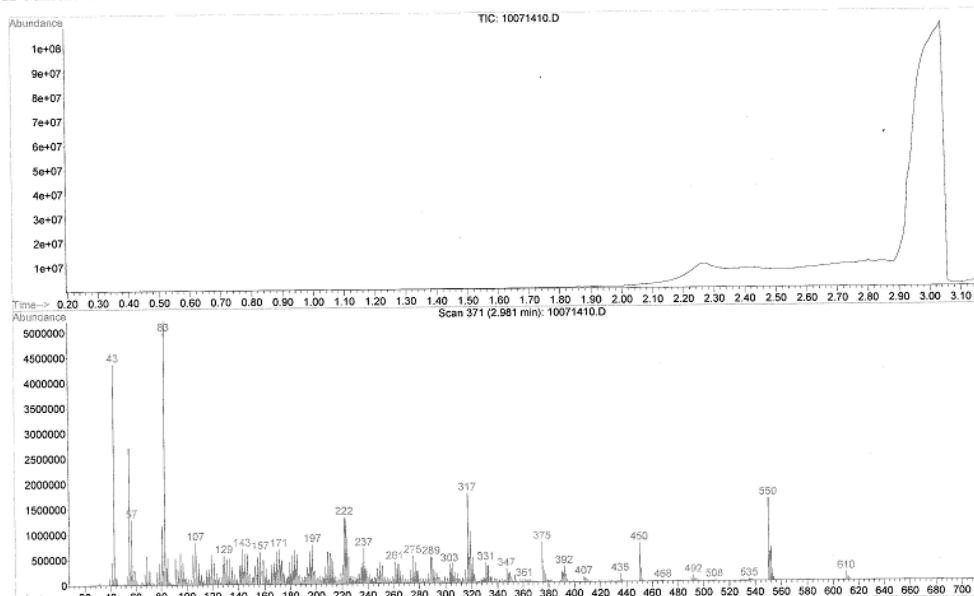


Figure S 29. ESI-MS spectrum of munronoid M (3)

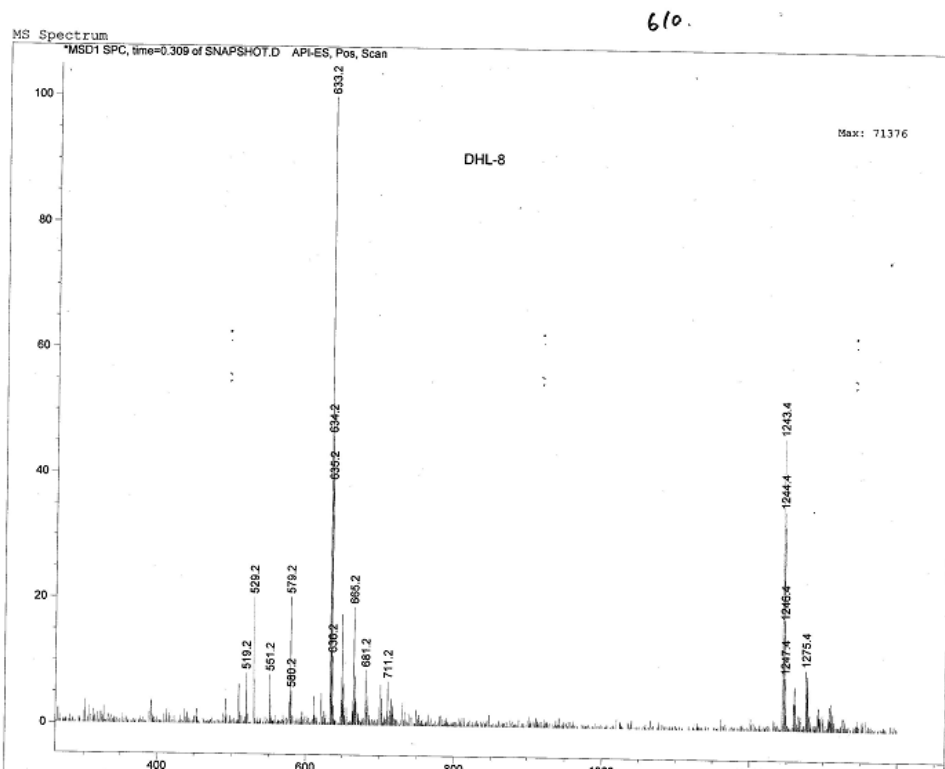
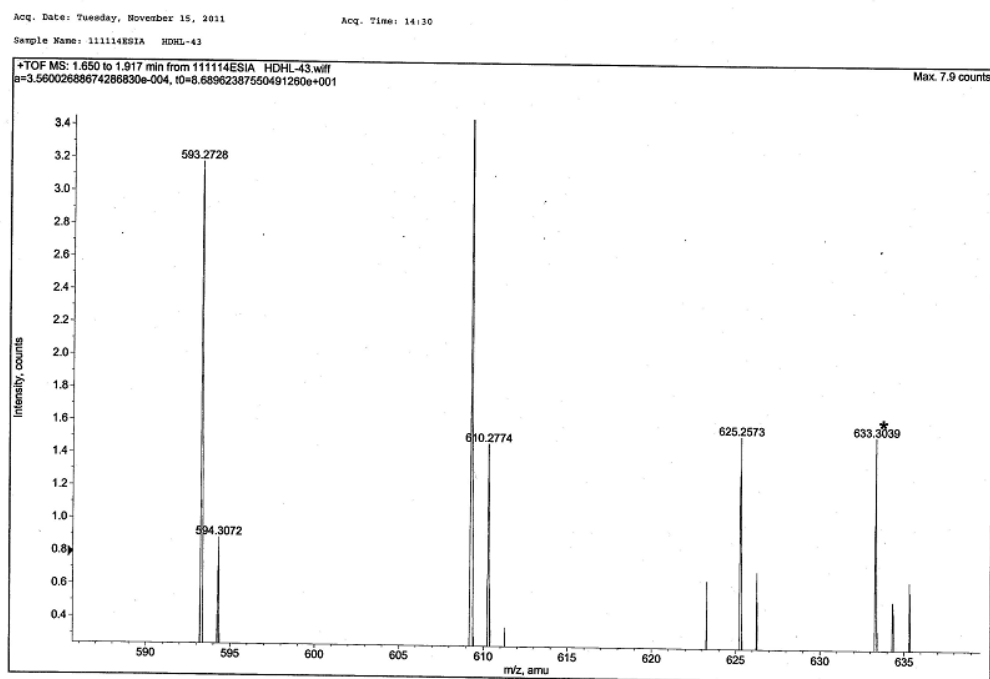


Figure S 30. HR-ESI-MS spectrum of munronoid M (3)



BHL-22

Archive directory: 2.28
Sample directory: 2.24
File: PROTON 2.21

Pulse Sequence: szpu1

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2.06
2.05
2.04
2.03
2.02
2.01
2.00
1.99
1.98
1.97
1.96
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1.03
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0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00

8 7 6 5 4 3 2 1 0 ppm

[illegible]

Figure S 33. HMQC spectrum of munronoid N (4) in CDCl₃

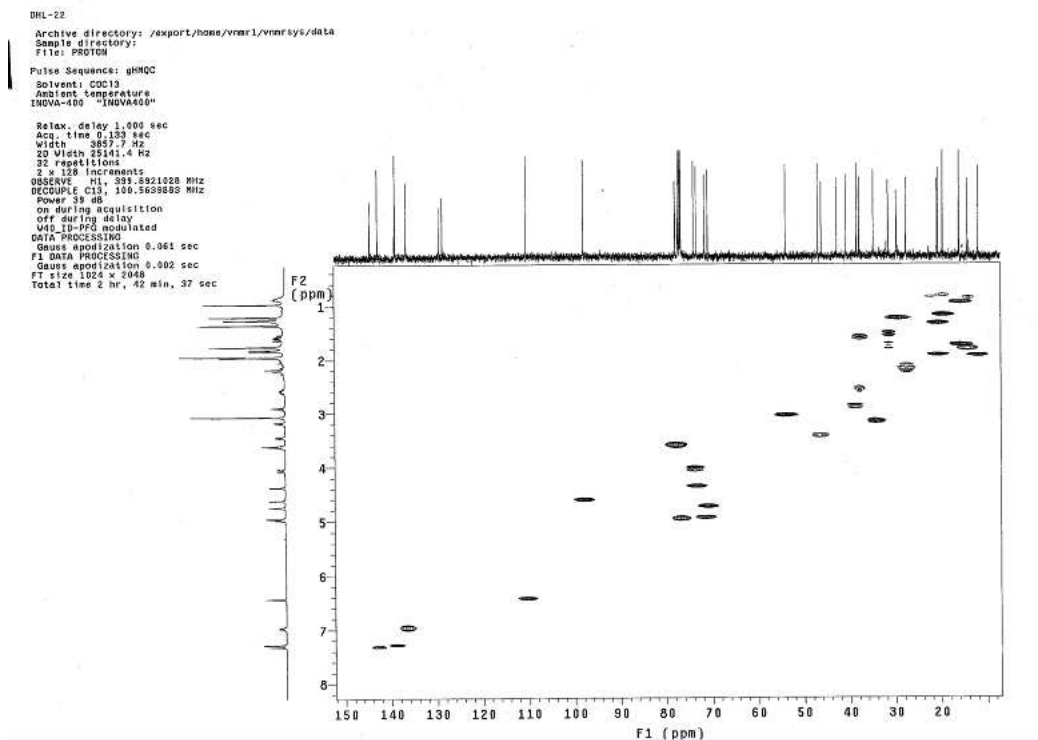


Figure S 34. HMBC spectrum of munronoid N (4) in CDCl₃

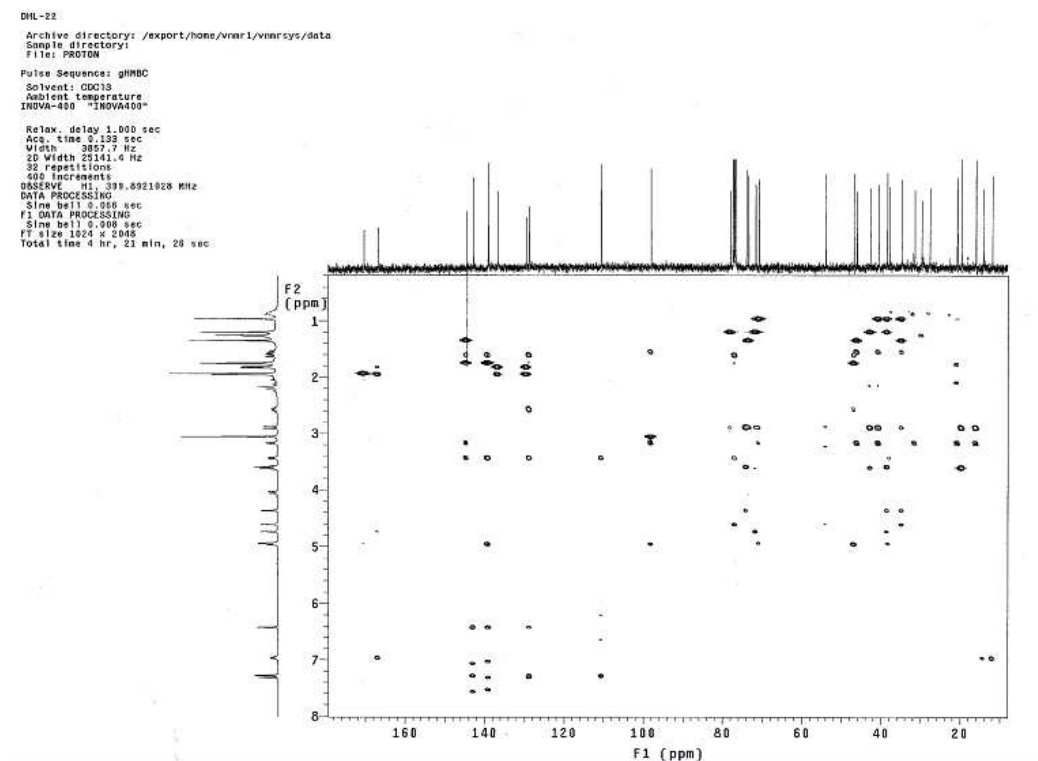


Figure S 35. ^1H - ^1H COSY spectrum of munronoid N (4) in CDCl_3

DHL-22
 Archive directory: /export/home/vmr1/vmr/sys/data
 Sample directory:
 File: PROTON
 Pulse Sequence: gCOSY
 Solvent: CDCl_3
 Ambient temperature
 INOVA-400 "INOVA400"
 Relax. delay 1.000 sec
 Acq. time 0.133 sec
 Width 3857.7 Hz
 2D Width 3857.7 Hz
 16 repetitions
 128 increments
 OBSERVE H1, 399.8921028 MHz
 DATA PROCESSING
 Sg. sine bell 0.066 sec
 F1 DATA PROCESSING
 Sg. sine bell 0.016 sec
 FT size 1024 x 1024
 Total time 59 min, 58 sec

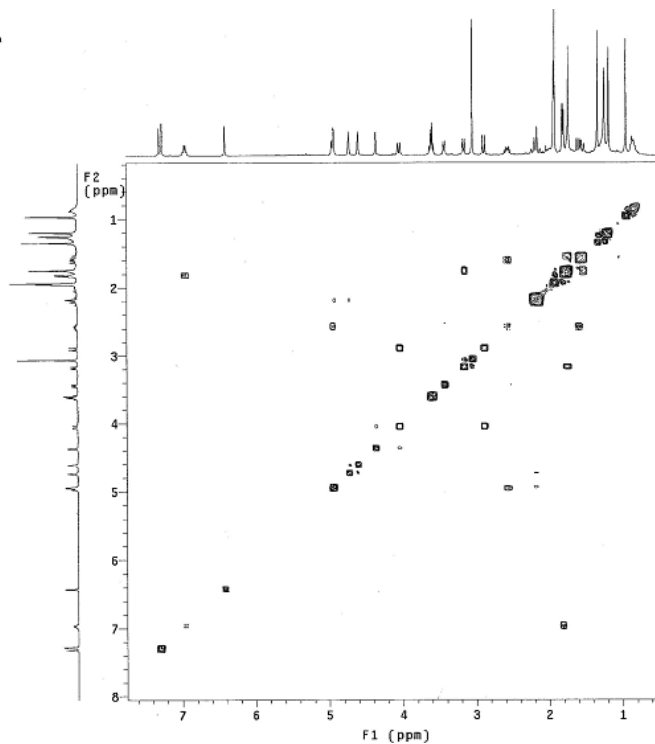


Figure S 36. ROESY spectrum of munronoid N (4) in CDCl_3

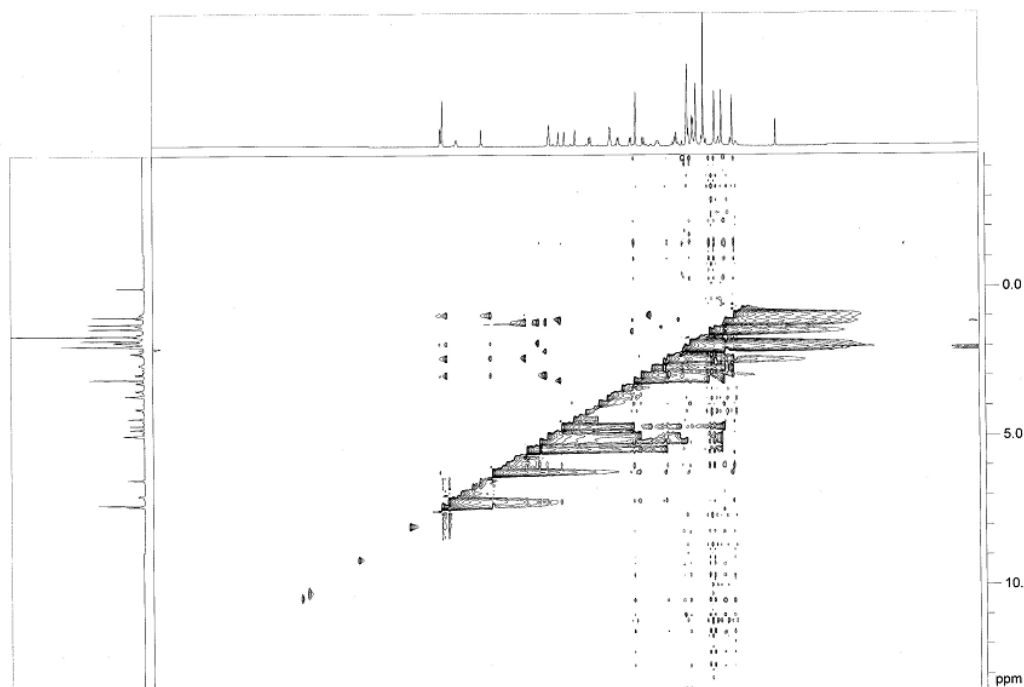
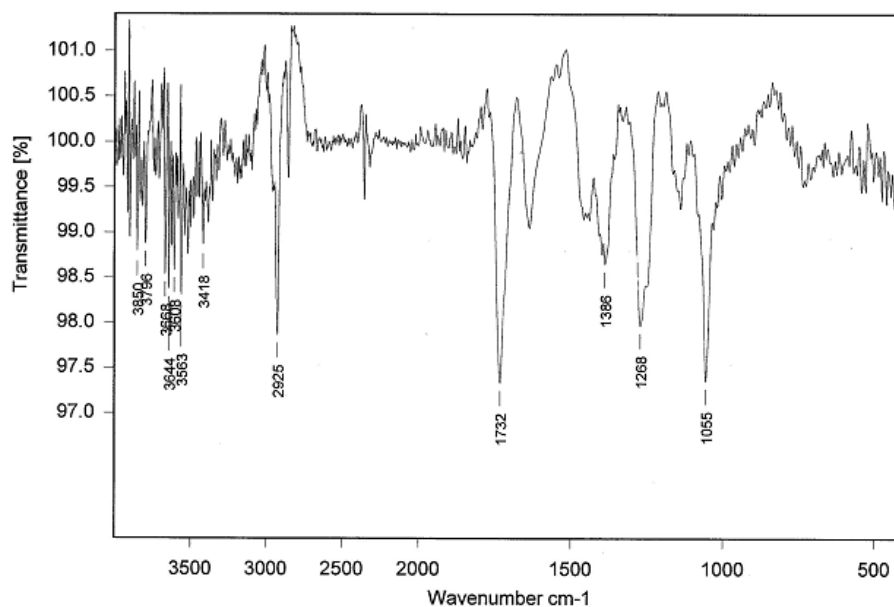


Figure S 37. IR spectrum of munronoid N (4)



Path of File D:\OPUS\MEAS\GYH	Filename DHL-7.0
Sample Name DHL-7	Sample Form L
Absolute Peak Pos in Laser*2 60693	Backward Peak Amplitude
Resolution 4	Signal Gain, Sample Automatic

Figure S 38. EI-MS spectrum of munronoid N (4)

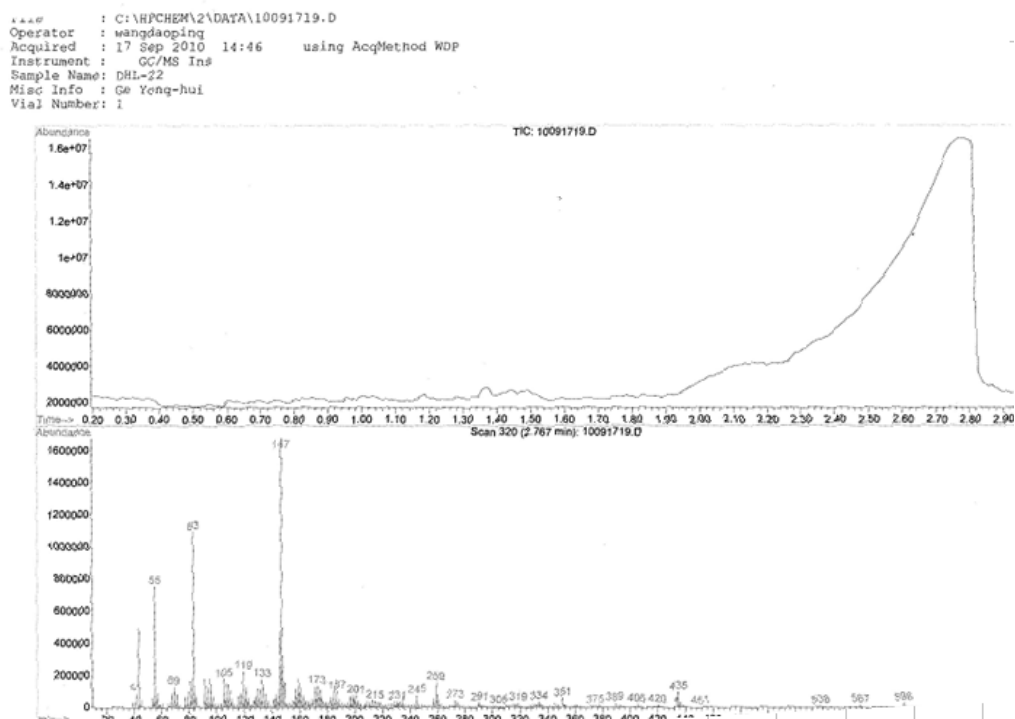


Figure S 39. ESI-MS spectrum of munronoid N (4)

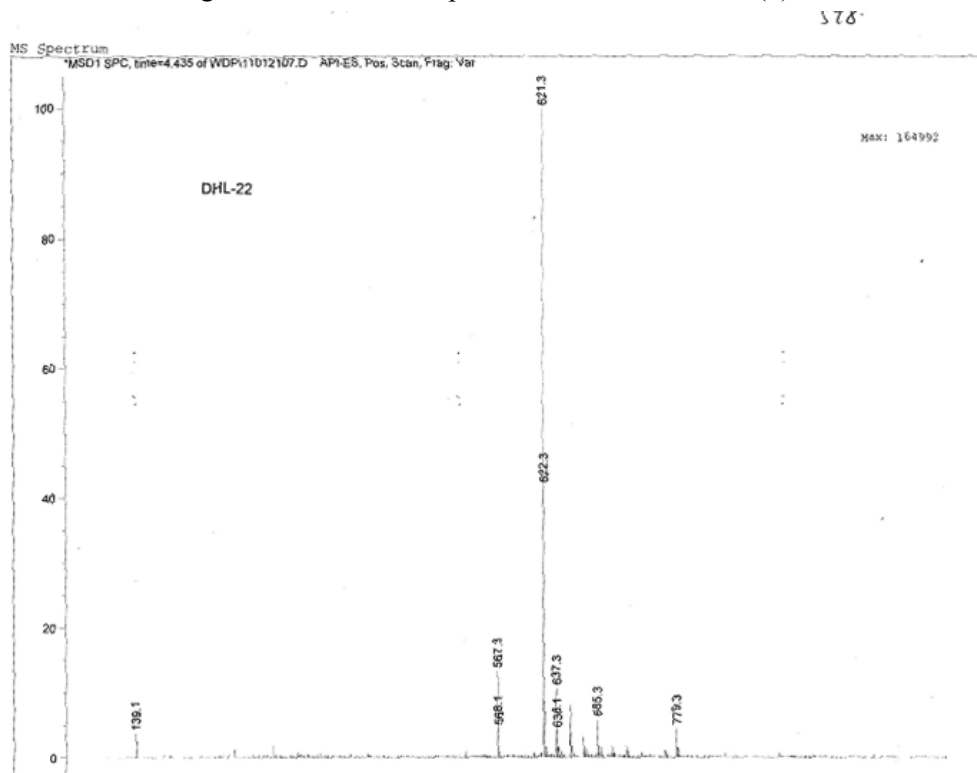
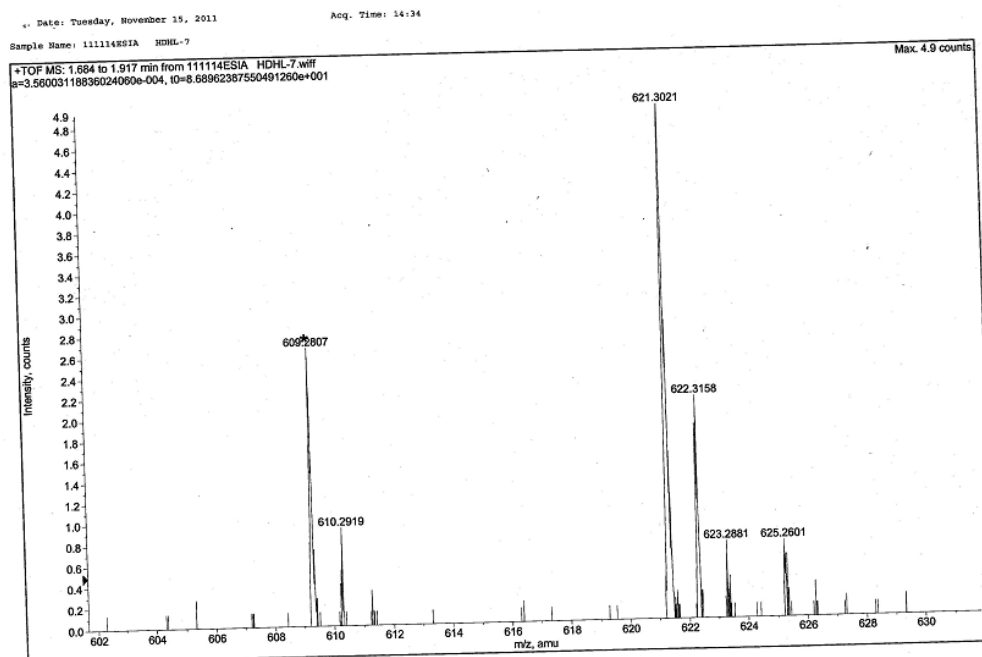


Figure S 40. HR-ESI-MS spectrum of munronoid K (1)



[illegible]

FYD-5

Archive directory: /export/home/vnmr1/vnmrSYS/data
Sample directory:
File: CDR60W

Pulse Sequence: s2pul

Chemical shifts (ppm):

- 173.558
- 173.558
- 173.425
- 169.323
- 166.659
- 148.367
- 148.041
- 136.206
- 131.688
- 126.367
- 121.463
- 88.555
- 77.500
- 77.500
- 77.500
- 77.500
- 71.078
- 70.378
- 69.015
- 59.724
- 50.167
- 50.167
- 50.033
- 46.157
- 45.886
- 38.918
- 34.887
- 32.718
- 30.119
- 29.781
- 22.318
- 20.579
- 20.346
- 13.282
- 0.023

Figure S 43. HMQC spectrum of munronoid O (**5**) in CDCl₃

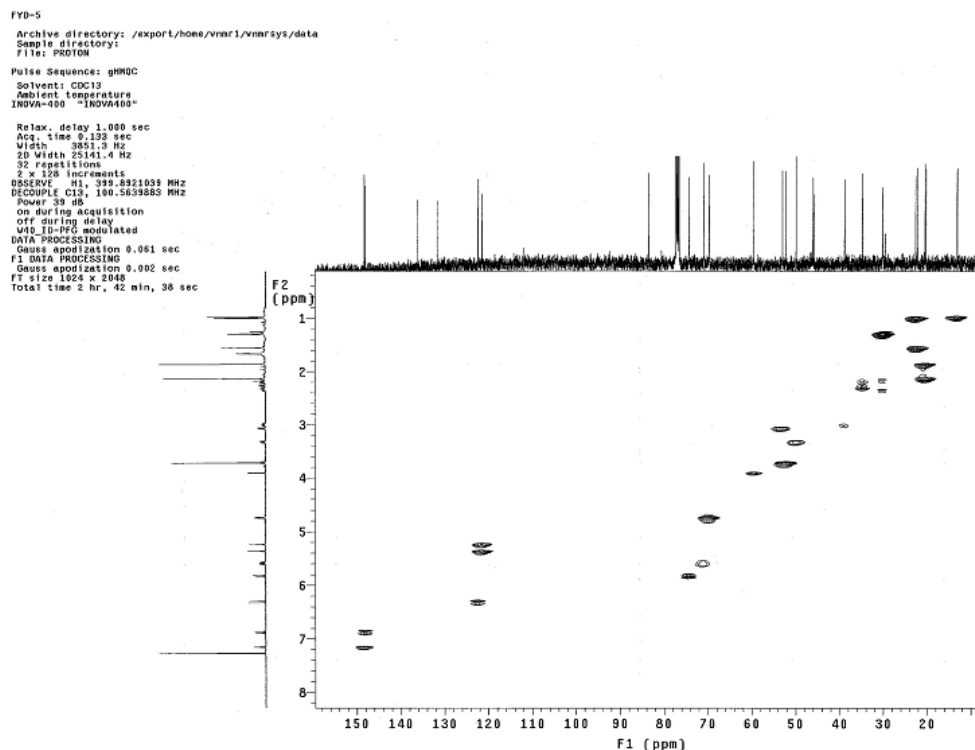


Figure S 44. HMBC spectrum of munronoid O (**5**) in CDCl₃

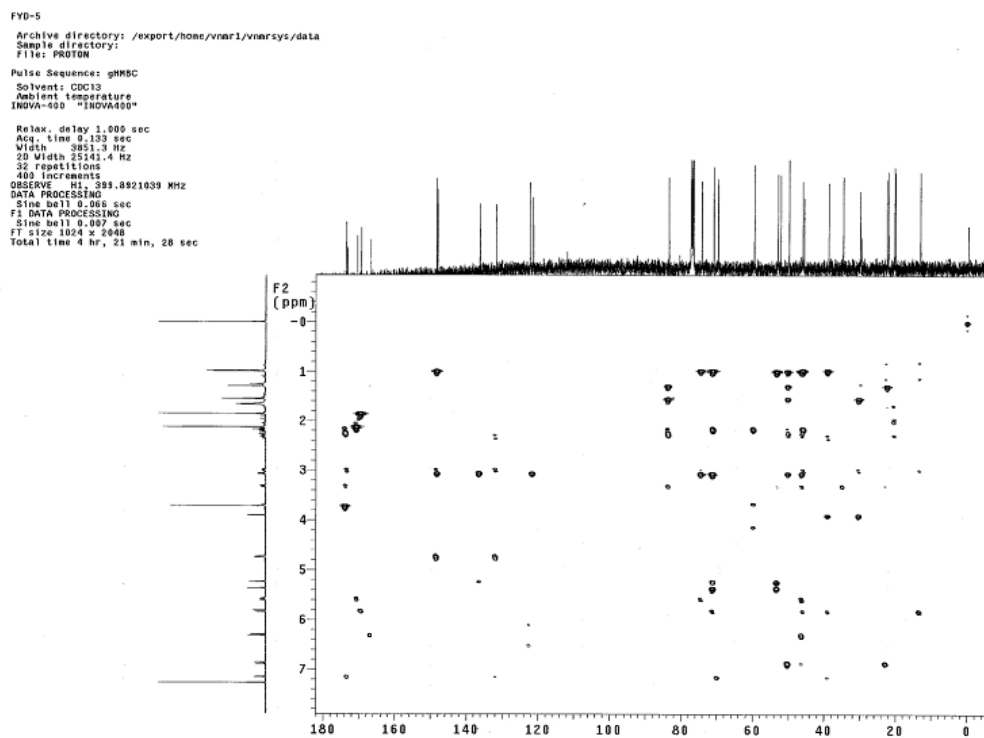


Figure S 45. ^1H - ^1H COSY spectrum of munronoid O (**5**) in CDCl_3

FYD-5

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON

Pulse Sequence: gCOSY

Solvent: CDCl_3

Ambient temperature

INNOVA-400 "INNOVA400"

Relax. delay 1.000 sec

Acq. time 0.133 sec

Width 3851.3 Hz

2D Width 3851.3 Hz

18 repetitions

128 increments

OBSERVE H1, 399.8921039 MHz

DATA PROCESSING

Sq. sine bell 0.066 sec

F1 DATA PROCESSING

Sq. sine bell 0.016 sec

FT size 1024 x 1024

Total time 38 min, 58 sec

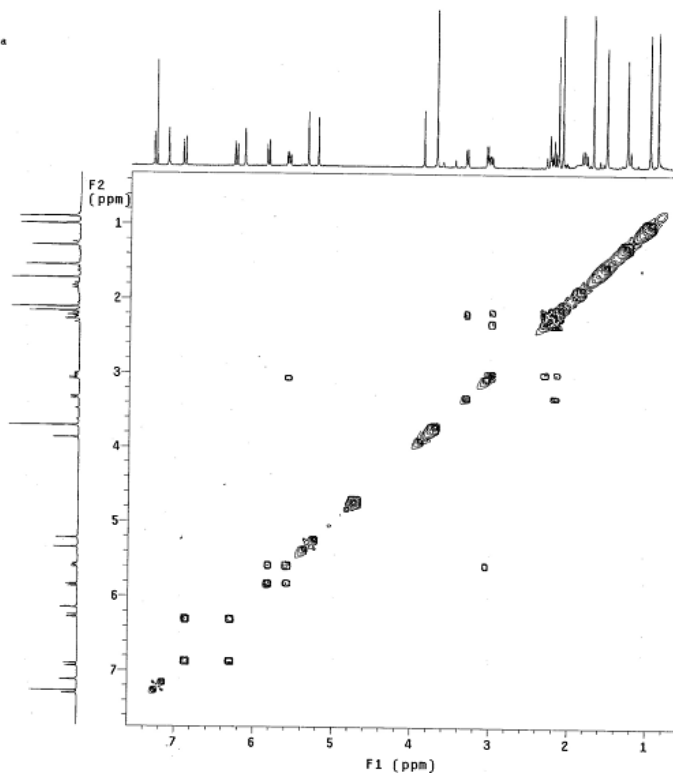


Figure S 46. ROESY spectrum of munronoid O (**5**) in CDCl_3

FYD-5

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON

Pulse Sequence: ROESY

Solvent: CDCl_3

Ambient temperature

INNOVA-400 "INNOVA400"

Relax. delay 1.000 sec

Mixing 0.200 sec

Acq. time 8.266 sec

Width 3851.3 Hz

2D Width 3851.3 Hz

32 repetitions

2 x 512 increments

OBSERVE H1, 399.8921039 MHz

DATA PROCESSING

Gauss apodization 0.110 sec

F1 DATA PROCESSING

Gauss apodization 0.040 sec

FT size 2048 x 2048

Total time 14 hr, 6 min, 38 sec

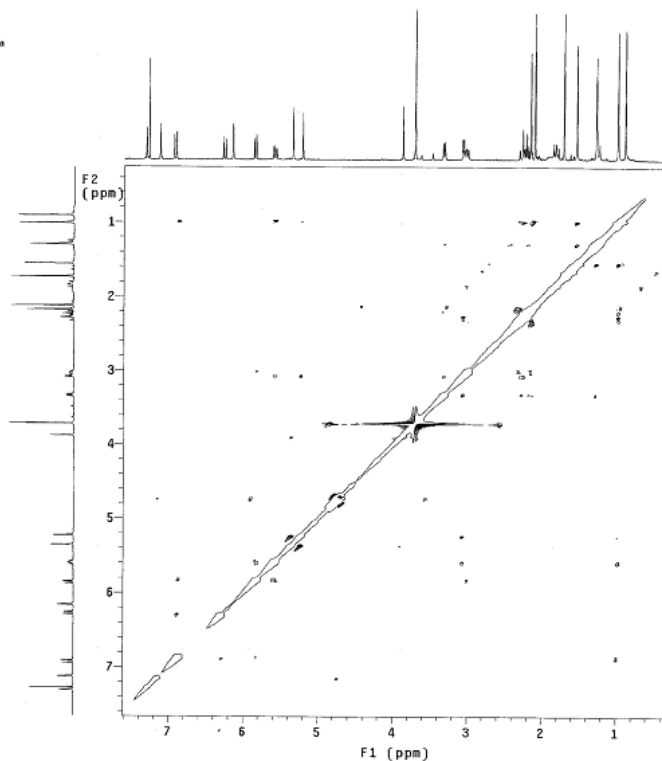
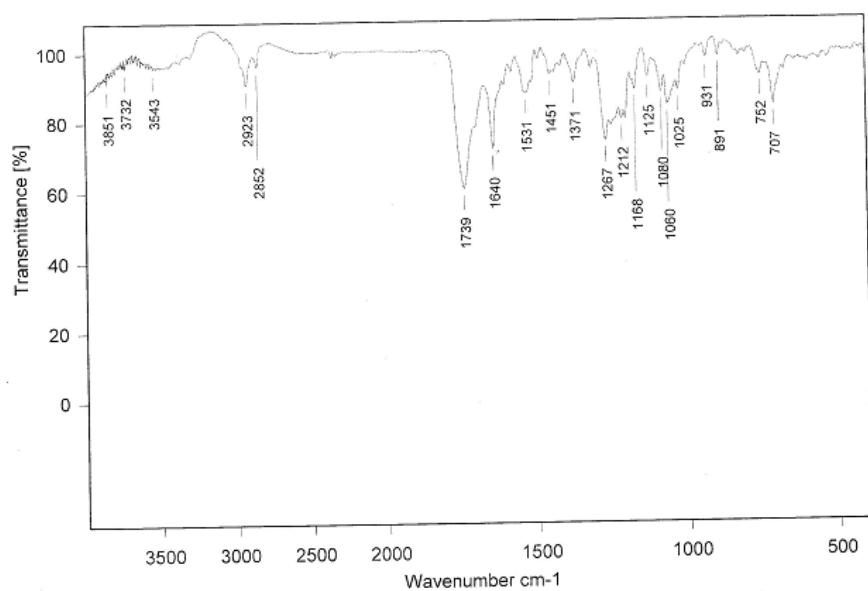


Figure S 47. IR spectrum of munronoid O (5)



Path of File D:\OPUS\wdp
Sample Name fyd-5
Absolute Peak Pos in Laser*2 60746
Resolution 4

Filename 111110.12
Sample Form L
Backward Peak Amplitude 2806
Signal Gain, Sample Automatic

Figure S 48. ESI-MS spectrum of munronoid O (5)

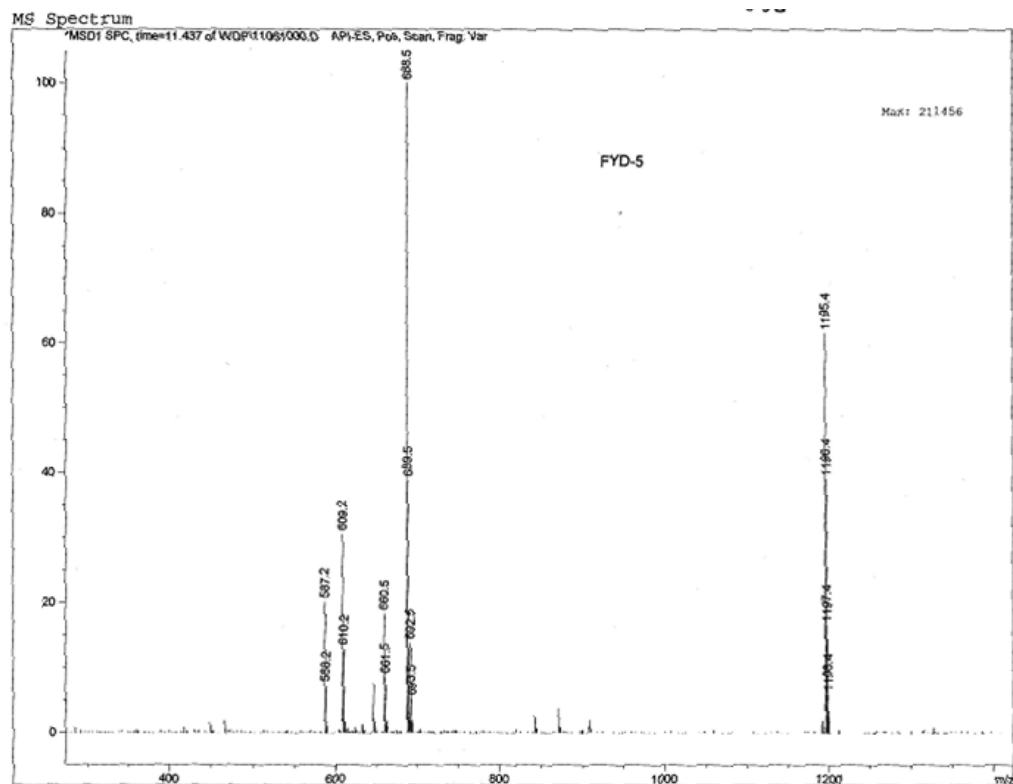


Figure S 49. HR-ESI-MS spectrum of munronoid O (5)

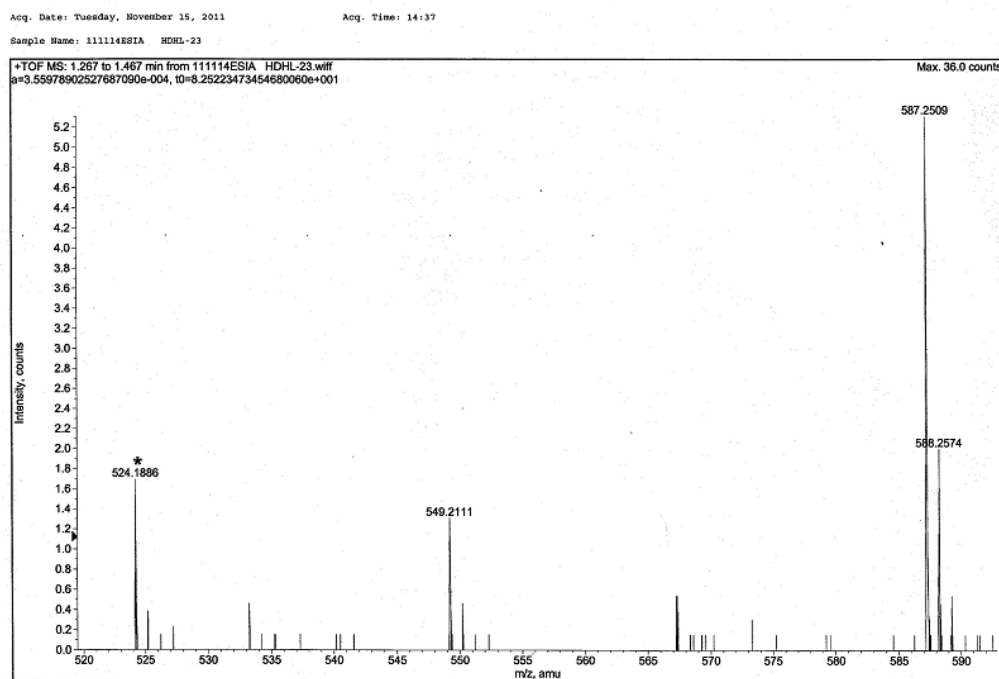


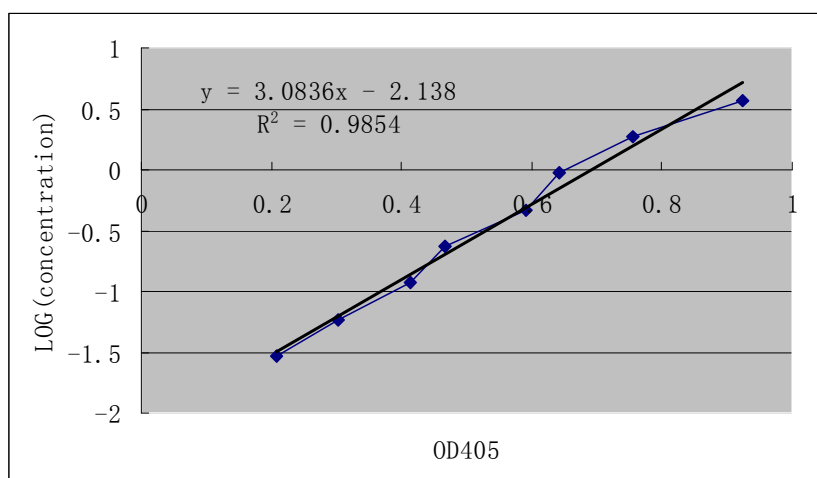
Figure S 50. ELISA Analysis of TMV

TMV concentration was calculated from a virus standard curve constructed using OD₄₀₅ values of TMV at concentrations of 3.75, 1.87, 0.93, 0.46, and 0.23 µg/mL.

Table 1 OD₄₀₅ values of TMV at concentrations of 3.75, 1.87, 0.93, 0.46, and 0.23 µg/mL.

TMV concentration	3.75	1.875	0.9375	0.4687	0.2344	0.1172	0.0586	0.0293
LOG TMV concentration	0.574031	0.273001	-0.02803	-0.32911	-0.63004	-0.93107	-1.2321	-1.53313
OD405	0.92452	0.75425	0.64125	0.5905	0.4675	0.41405	0.30321	0.20732

Table 2 Virus standard curve constructed using OD₄₀₅ values



X = OD₄₀₅ values; Y = LOG (concentrations of TMV)

Table 3 The value of OD₄₀₅ of limonoids 1 – 8 and control.

No.	1	2	3	4	5	6	7	8	blank	positive	negative
	0.486	0.505	0.574	0.602	0.499	0.584	0.689	0.533	0.165	0.545	0.683
	0.499	0.523	0.531	0.596	0.51	0.571	0.672	0.541	0.203	0.533	0.632
	0.478	0.552	0.546	0.588	0.501	0.595	0.663	0.538	0.199	0.529	0.663
	0.503	0.572	0.599	0.606	0.506	0.563	0.641	0.527	0.212	0.519	0.617
Average of OD ₄₀₅	0.4915	0.538	0.5625	0.598	0.504	0.57825	0.66625	0.53475	0.19475	0.5315	0.64875
Inhibition Rate (%)	67.2%	54.4%	45.7%	30.2%	64.2%	39.3%	-13.2%	55.4%		56.5%	

The inhibition rate of TMV = (1-TMV concentration of treatment / TMV concentration of negative control)×100%

Table 4 The value of OD₄₀₅ of different dosage of limonoids **1, 2, 5** and control.

No.	1					2				
Dosage	30µg/ml	25µg/ml	20µg/ml	10µg/ml	5µg/ml	30µg/ml	25µg/ml	20µg/ml	10µg/ml	5µg/ml
	0.492	0.506	0.554	0.601	0.642	0.528	0.545	0.579	0.618	0.632
	0.488	0.516	0.542	0.588	0.633	0.533	0.563	0.564	0.606	0.625
	0.499	0.521	0.539	0.611	0.65	0.519	0.552	0.572	0.621	0.619
	0.469	0.499	0.532	0.599	0.628	0.572	0.539	0.593	0.615	0.643
Average of OD ₄₀₅	0.487	0.5105	0.54175	0.59975	0.63825	0.538	0.54975	0.577	0.615	0.62975
Inhibition Rate (%)	67.8	62.0	52.6	28.5	6.0	53.8	49.8	39.1	20.3	11.5
No.	5									
Dosage	30µg/ml	25µg/ml	20µg/ml	10µg/ml	5µg/ml	blank	positive	negative		
	0.489	0.531	0.553	0.588	0.633	0.197	0.539	0.633		
	0.506	0.525	0.548	0.593	0.629	0.194	0.537	0.669		
	0.523	0.519	0.56	0.602	0.643	0.205	0.541	0.658		
	0.499	0.541	0.541	0.579	0.636	0.221	0.51	0.636		
Average of OD ₄₀₅	0.50425	0.529	0.5505	0.5905	0.63525	0.20425	0.53175	0.649		
Inhibition Rate (%)	63.7	56.7	49.5	33.0	8.0		56.5			

Table 5 The value of OD₄₀₅ of different dosage of limonoids **8** and control.

No.	8					N				
Dosage	30µg/ml	25µg/ml	20µg/ml	10µg/ml	5µg/ml	30µg/ml	25µg/ml	20µg/ml	10µg/ml	5µg/ml
	0.533	0.558	0.588	0.602	0.649	0.539	0.543	0.565	0.599	0.636
	0.541	0.563	0.573	0.611	0.632	0.537	0.552	0.57	0.601	0.629
	0.538	0.571	0.592	0.623	0.624	0.541	0.538	0.583	0.587	0.645
	0.527	0.557	0.569	0.619	0.643	0.51	0.54	0.578	0.612	0.629
Average of OD ₄₀₅	0.53475	0.56225	0.5805	0.61375	0.637	0.53175	0.54325	0.574	0.59975	0.63475
Inhibition Rate (%)	55.5	45.9	38.5	22.1	8.1	56.5	52.8	41.2	29.5	9.6
No.	blank	positive	negative							
	0.197	0.539	0.633							
	0.194	0.537	0.669							
	0.205	0.541	0.658							
	0.221	0.51	0.636							
Average of OD ₄₀₅	0.20425	0.53175	0.649							
	0.9574	0.5650								
Inhibition Rate (%)		56.5								

Table 6 Inhibition curve constructed using Inhibition Rate (%) and dosage of compounds

