

Supporting Information

Anti-tuberculosis Cycloartane Triterpenoids from *Radermachera boniana*

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General

Optical rotation was recorded on a Polax-2L polarimeter in CHCl_3 . Melting points were recorded on a Buchi B-545 instrument and IR spectra were measured on a Nicolet Impact-410 FT-IR spectrometer. High resolution mass spectra were measured on a VARIAN 910 spectrometer, while the ^{13}C NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 125.76 MHz. ^1H and 2D NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 500.13 MHz. ^1H chemical shifts were referenced to CHCl_3 and CD_3OD at 7.27 ppm and 3.33 ppm, respectively, while the ^{13}C chemical shifts were referenced to the central peak of CDCl_3 at 77.0 ppm and 49.0 ppm for CD_3OD . For HMBC experiments the delay ($1/2J$) was 70 ms and for the NOESY experiments the mixing time was 150 ms.

Plant material

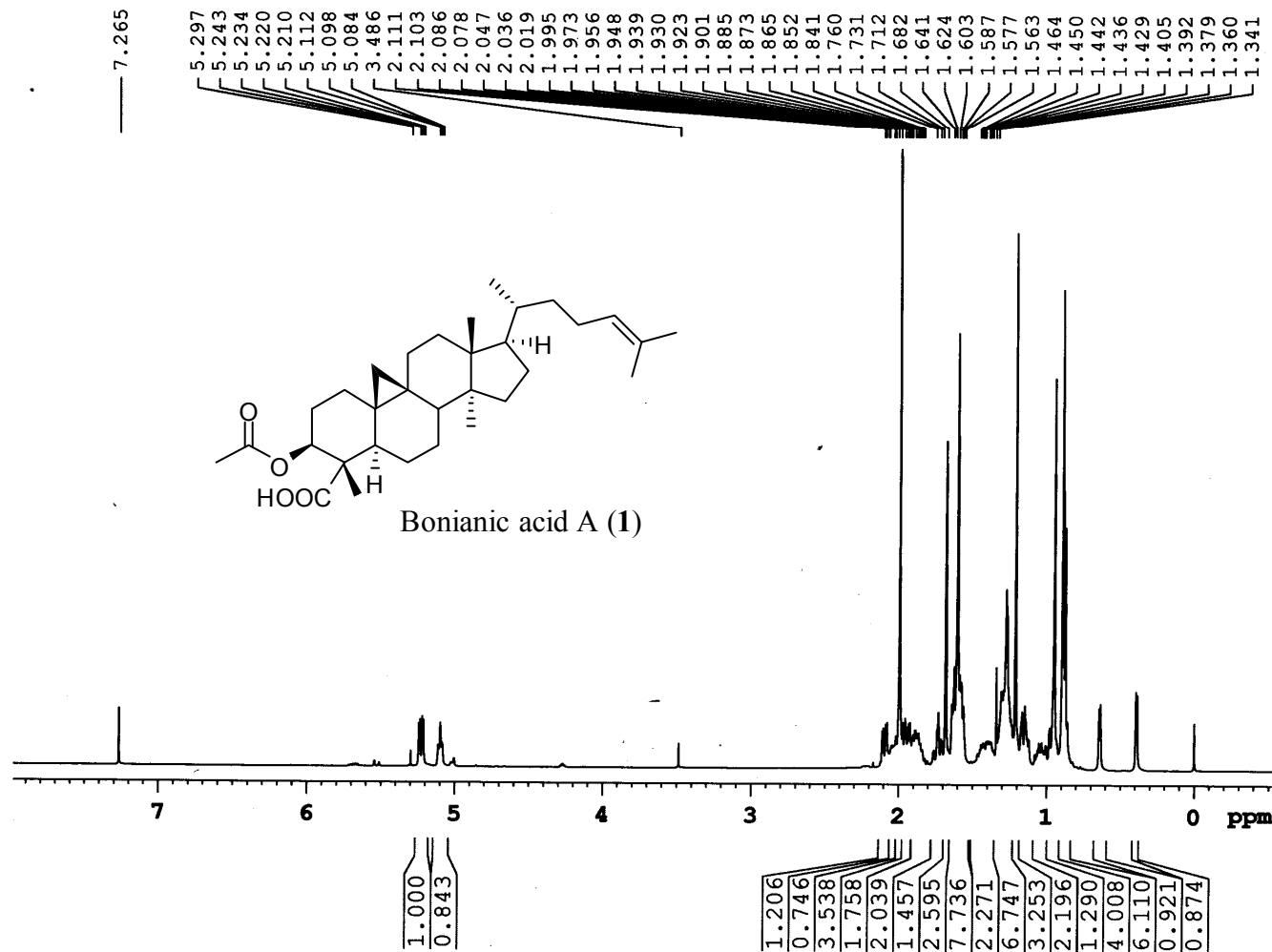
Leaf and twig sample SVA2933 of *R. boniana* was collected in Cuc Phuong National Park in April 2007, from the same location, $20^\circ 17.901''$ N, $105^\circ 39.310''$ E) where the original primary active sample (SV2933) was collected in 2002. A voucher herbarium specimen of the recollected sample (*Mai Van Xinh 1226*) and that of the primary sample (*Ma Van Xinh 595*) have been deposited at each of the following institutions: Cuc Phuong National Park Herbarium (CPNP) in Nho Quan, Ninh Binh, Vietnam; Herbarium of the Department of Botany (HN) of the Vietnam Academy of Science and Technology, Hanoi, Vietnam; and at the J.D. Searle Herbarium of the Field Museum (F), Chicago, USA.

Bioassays

The virulent H_{37}Rv strain of *M. tuberculosis* (ATCC 27294, American Type Culture Collection, Rockville, MD) was used for the anti-TB bioassay.^{14a,b} The test materials were dissolved in DMSO at 10 mg/mL and tested in a series of 2-fold dilutions with the highest concentration of 100 $\mu\text{g/mL}$ (and 1% v/v DMSO). Samples were incubated for 7 days with *M. tuberculosis* in a 96-well plates, and then cell growth was determined using the Alamar Blue dye with fluorometric detection. The MIC was defined as the lowest concentration resulting in ninety percent or greater inhibition of fluorescence compared to bacteria-only controls. Rifampin was used as a positive control, exhibiting a MIC of 0.12 $\mu\text{g/mL}$, while DMSO at a final concentration of 1% v/v was the negative control. Cytotoxicity for green monkey kidney (VERO) cells was

determined following 72 hrs exposure.^{14b} Viability was assessed on the basis of cellular conversion of MTS into a soluble formazan product using the Promega CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay.

¹H NMR spectrum (500.13 MHz, CDCl₃) of bonianic acid A (1)



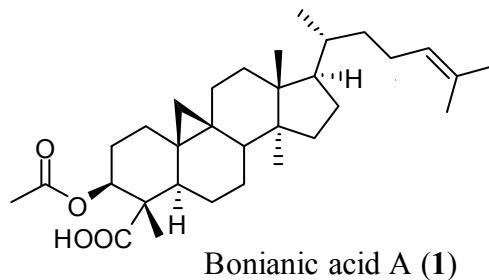
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 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 16
 DS 0
 SWH 10000.000 Hz
 FIDRES 0.152588 Hz
 AQ 3.2769001 sec
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 DE 6.00 usec
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 MCWRK 0.01500000 sec

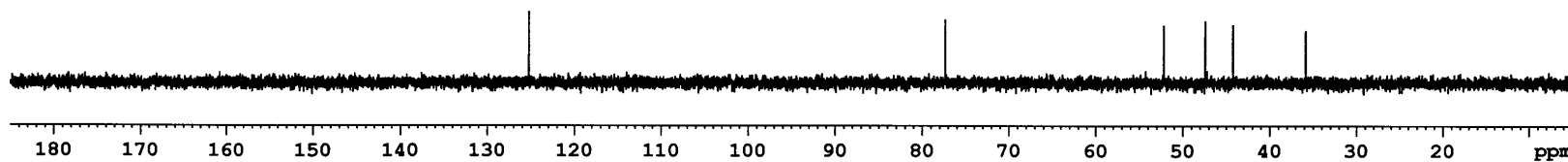
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F2 - Processing parameters
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 GB 0
 PC 1.00

¹³C NMR and DEPT spectra (125.76 MHz, CDCl₃) of bonianic acid A (1)



DEPT90

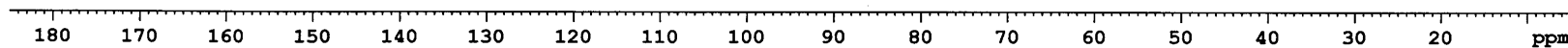


DEPT135

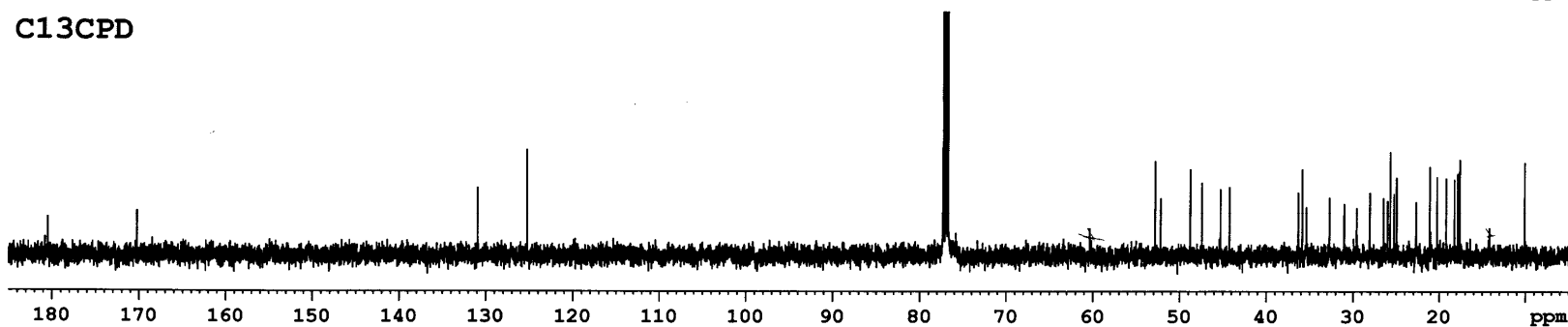
CH&CH₃



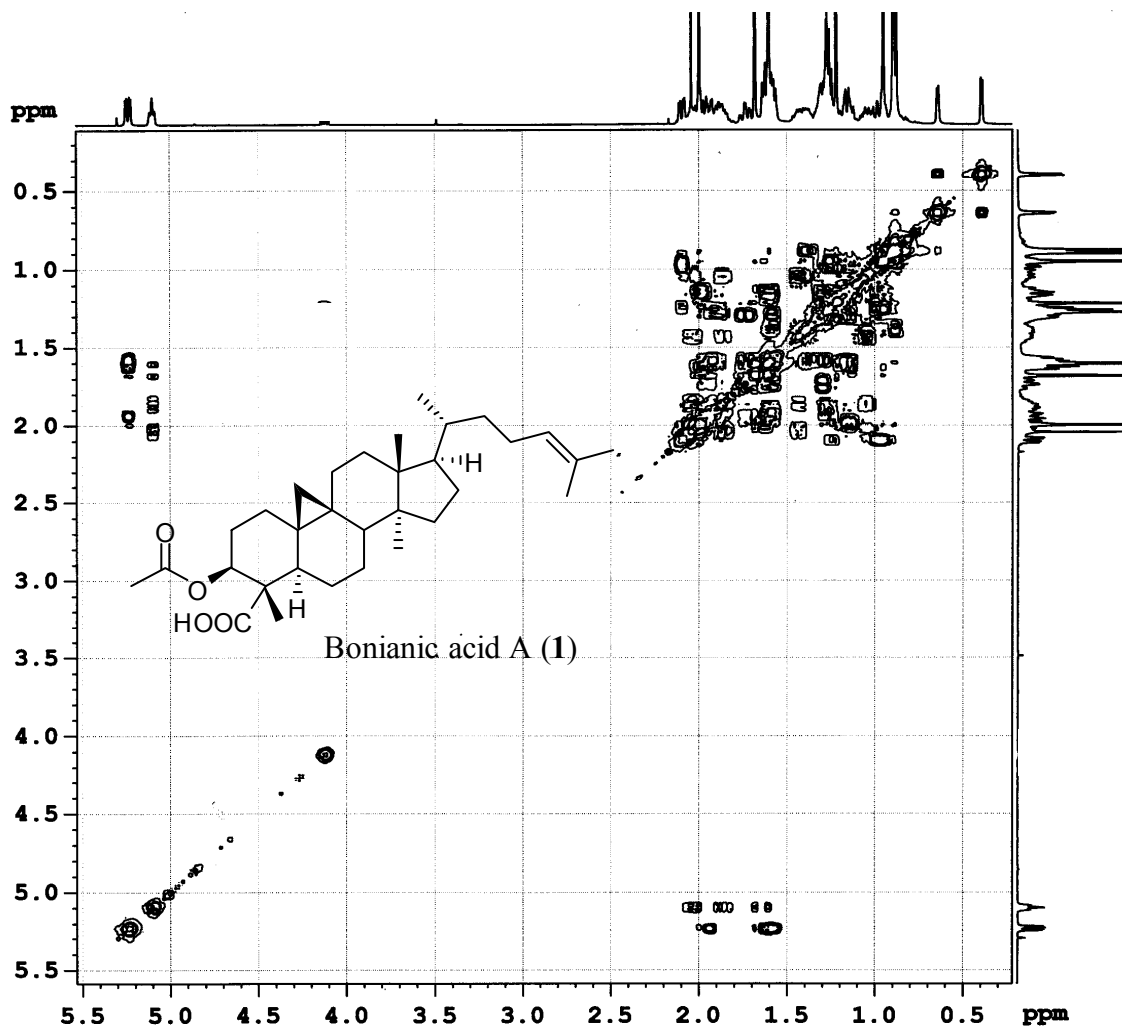
CH₂



C13CPD



^1H - ^1H COSY spectrum (500.13 MHz, CDCl_3) of bonianic acid A (1)



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 EXPNO 7
 PROCNO 1

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 PULPROG cosygpgf
 TD 2048
 SOLVENT CDCl_3
 NS 4
 DS 8
 SWH 5000.000 Hz
 FIDRES 2.441406 Hz
 AQ 0.2049500 se
 RG 71.8
 DW 100.000 us
 DE 6.00 us
 TE 300.6 K
 d0 0.00000300 se
 D1 1.48689198 se
 d13 0.00000400 se
 D16 0.00015000 se
 INO 0.00020000 se
 MCREST 0.00000000 se
 MCWRK 1.48689198 se

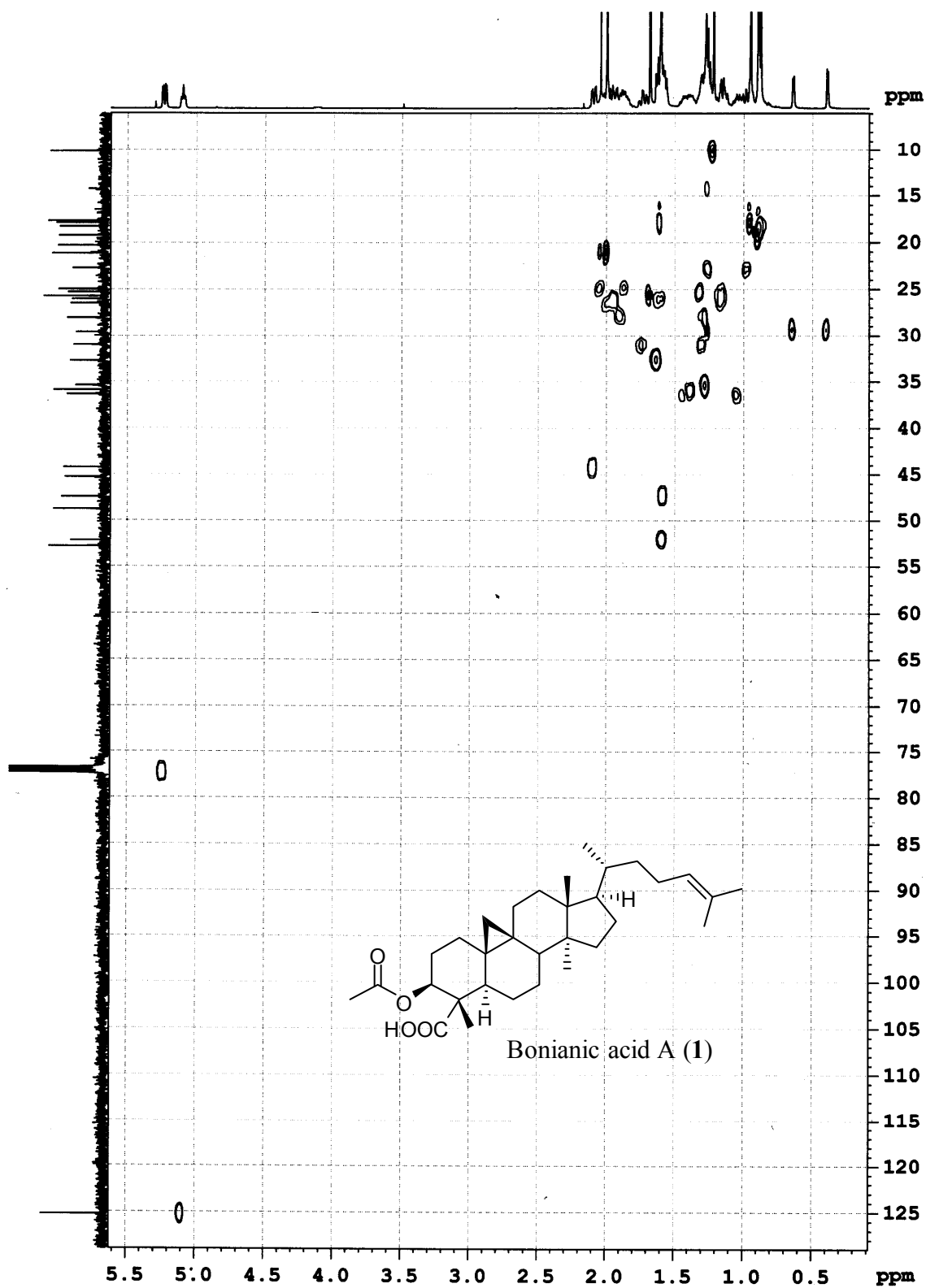
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 P1 10.50 us
 PL1 -3.00 dE
 SF01 500.1322506 MH

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 GPNAM2 SINE.100
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 GPY1 0.00 %
 GPY2 0.00 %
 GPZ1 10.00 %
 GPZ2 10.00 %
 P16 1000.00 us

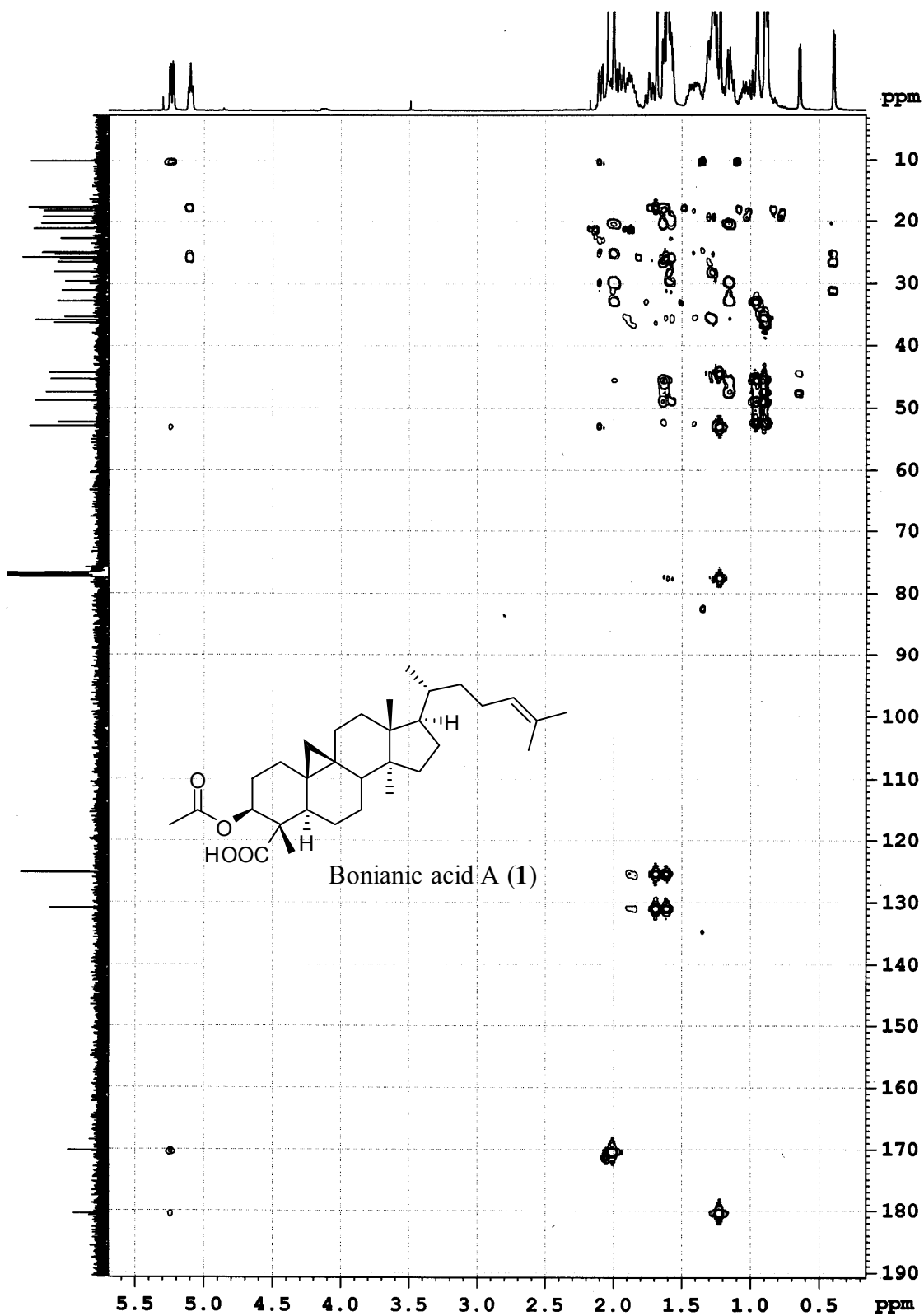
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 FMODE QF

F2 - Processing parameters
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 SSB 0
 LB 0.00 Hz
 GB 0
 PC 1.00

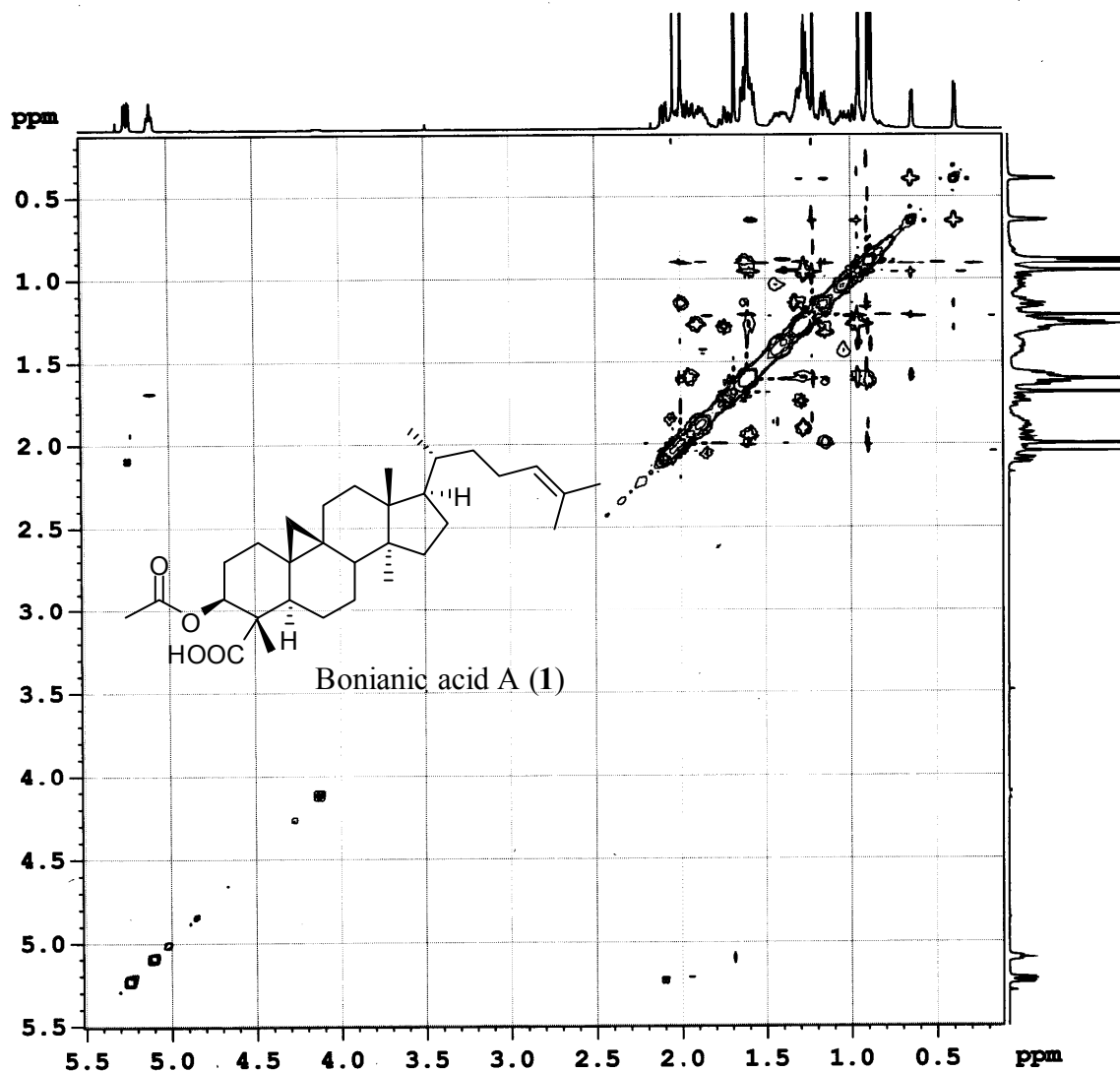
HSQC spectrum (^1H : 500.13 MHz, ^{13}C : 125.76 MHz, CDCl_3)
of bonianic acid A (1)



HMBC spectrum (^1H : 500.13 MHz, ^{13}C : 125.76 MHz, CDCl_3)
of bonianic acid A (1)



NOESY spectrum (500.13 MHz, CDCl₃) of bonianic acid A (1)



Current Data Parameters
 NAME 2L.HUNG_RaderF11973
 EXPNO 6
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20070721
 Time_ 23.13
 INSTRUM av500
 PROBHD 5 mm Multinucl
 PULPROG noesyph
 TD 2048
 SOLVENT CDCl3
 NS 48
 DS 4
 SWH 4006.410 Hz
 FIDRES 1.956255 Hz
 AQ 0.2557652 sec
 RG 181
 DW 124.800 usec
 DE 6.00 usec
 TE 300.0 K
 d0 0.0001160 sec
 D1 2.0000000 sec
 D8 0.34999999 sec
 INQ 0.00024994 sec
 MCREST 0.00000000 sec
 MCWRK 1.00000000 sec
 STICNT 128

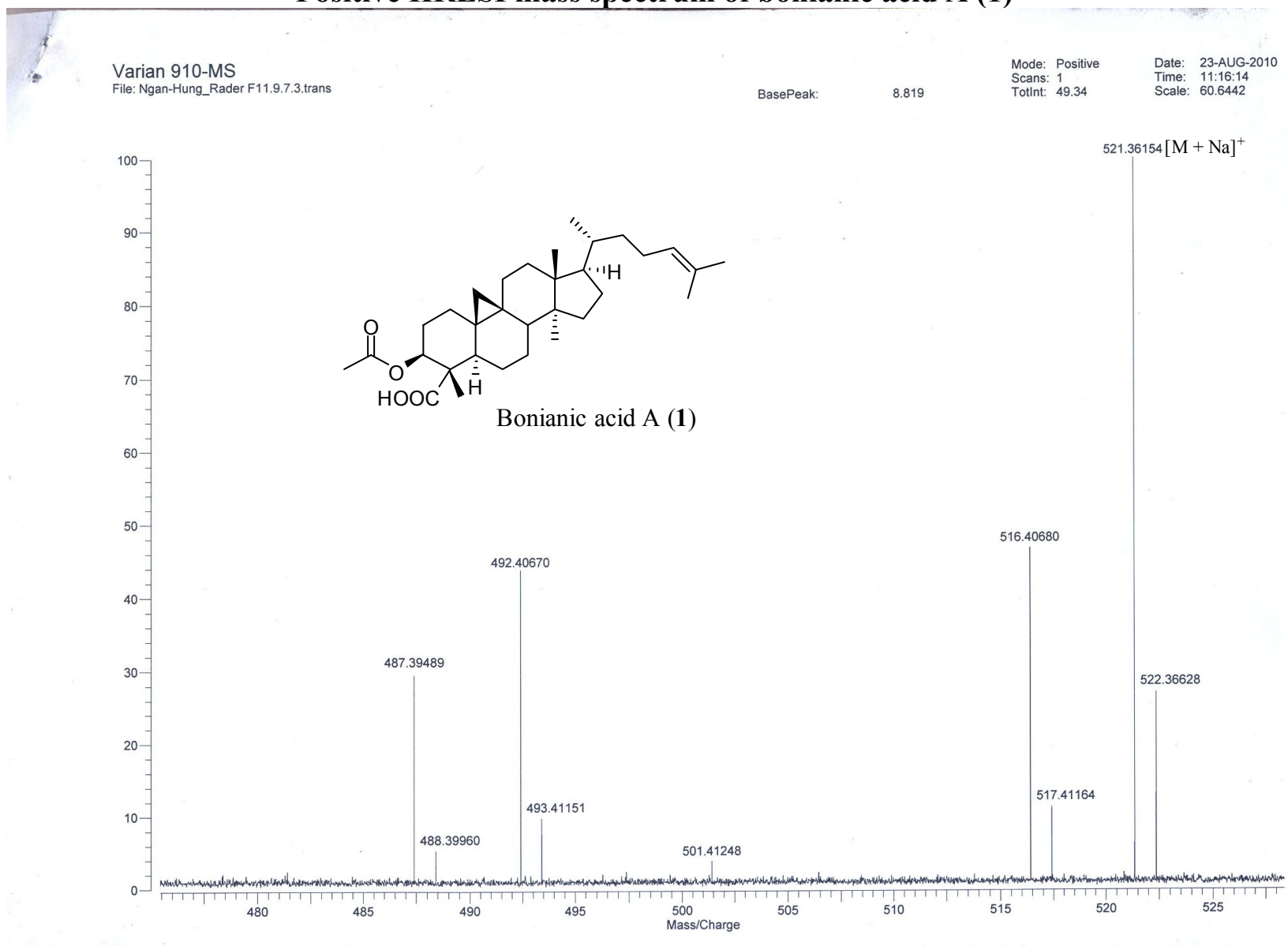
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 P1 10.50 usec
 PL1 -3.00 dB
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 FIDRES 15.628907 Hz
 SW 8.000 ppm
 FhMODE States-TPPI

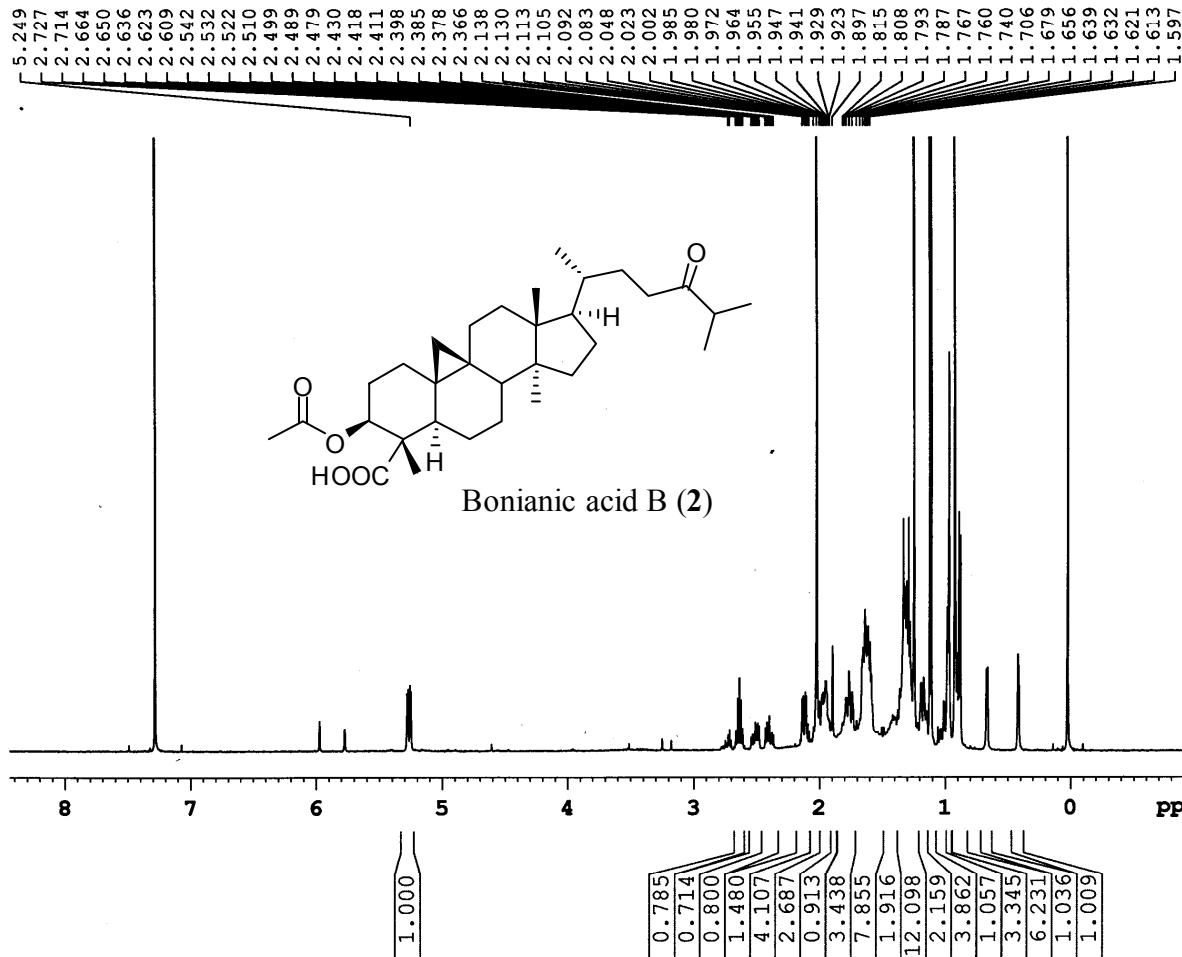
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 SF 500.1300103 MHz
 WDW QSINE
 SSB 2
 LB 0.00 Hz
 GB 0
 PC 1.00

F1 - Processing parameters
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 MC2 States-TPPI
 SF 500.1305148 MHz
 WDW QSINE
 SSB 2
 LB 0.00 Hz
 GB 0

Positive HRESI mass spectrum of bonianic acid A (1)



¹H NMR spectrum (500.13 MHz, CDCl₃) of bonianic acid B (2)



Current Data Parameters
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 EXPNO 1
 PROCNO 1

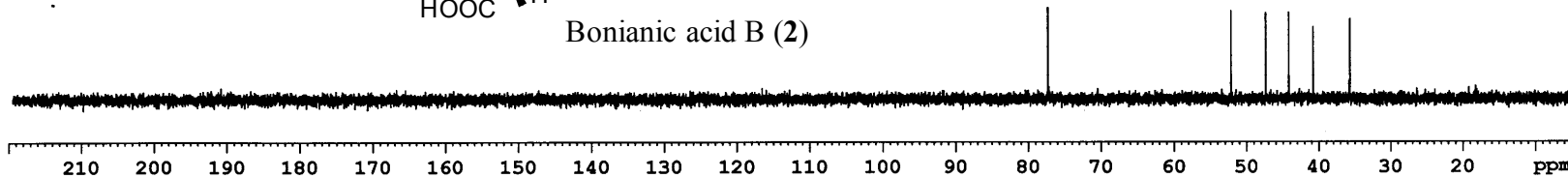
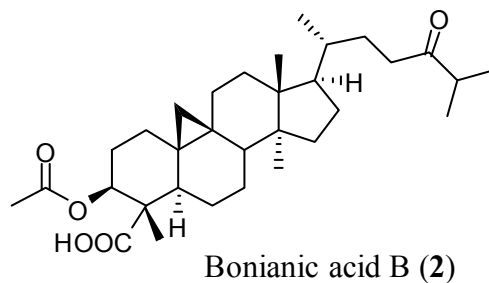
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 SOLVENT CDCl3
 NS 16
 DS 0
 SWH 10000.000 Hz
 FIDRES 0.152588 Hz
 AQ 3.2769001 sec
 RG 256
 DW 50.000 usec
 DE 6.00 usec
 TE 300.8 K
 D1 1.00000000 sec
 MCREST 0.00000000 sec
 MCWRK 0.01500000 sec

----- CHANNEL f1 -----
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 P1 10.50 usec
 PL1 -3.00 dB
 SFO1 500.1335009 MHz

F2 - Processing parameters
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 SF 500.1300011 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

¹³C NMR and DEPT spectra (125.76 MHz, CDCl₃) of bonianic acid B (2)

DEPT90

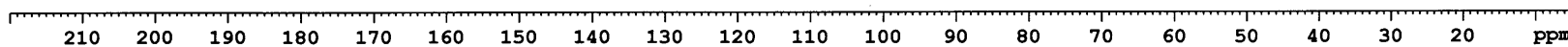


DEPT135

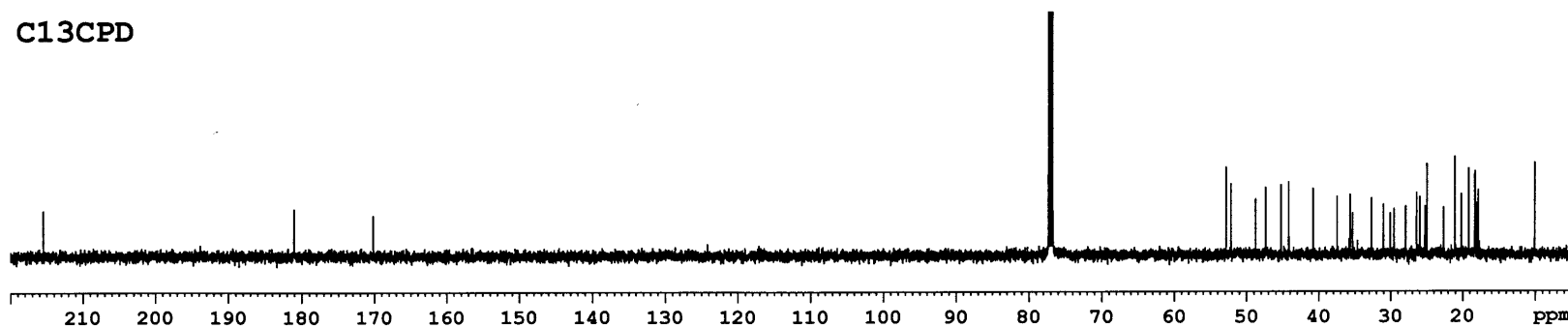
CH&CH₃



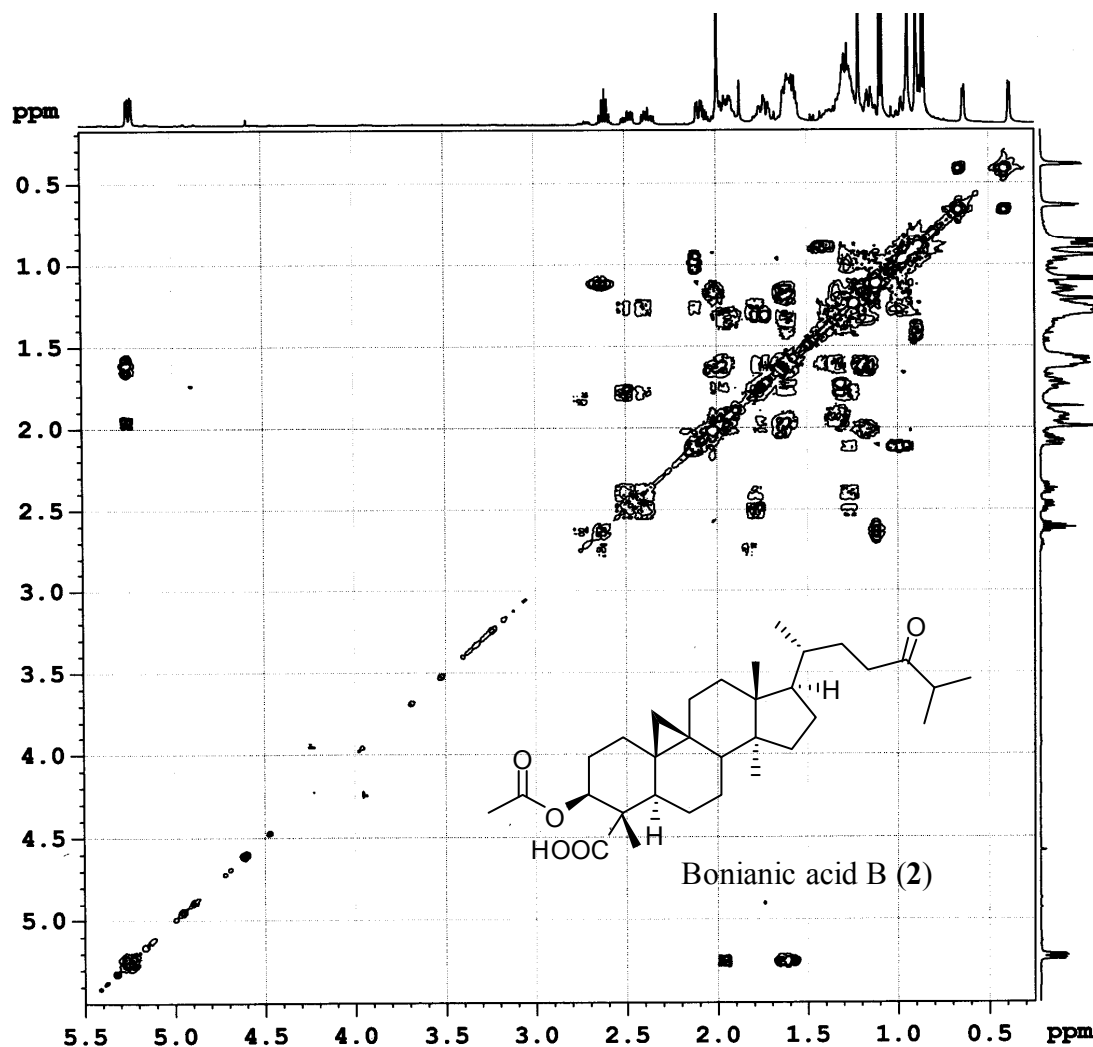
CH₂



C13CPD



^1H - ^1H COSY spectrum (500.13 MHz, CDCl_3) of bonianic acid B (2)



Current Data Parameters
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EXPNO 5
PROCNO 1

F2 - Acquisition Parameter
Date_ 20071018
Time 9.30
INSTRUM av500
PROBHD 5 mm Multinucl
PULPROG cosygpqf
TD 2048
SOLVENT CDCl_3
NS 4
DS 8
SWH 5000.000 Hz
FIDRES 2.441406 Hz
AQ 0.2049500 se
RG 114
DW 100.000 us
DE 6.00 us
TE 300.0 K
d0 0.00000300 se
D1 1.48689198 se
d13 0.00000400 se
D16 0.00015000 se
IN0 0.00020000 se
MCREST 0.00000000 se
MCWRK 1.48689198 se

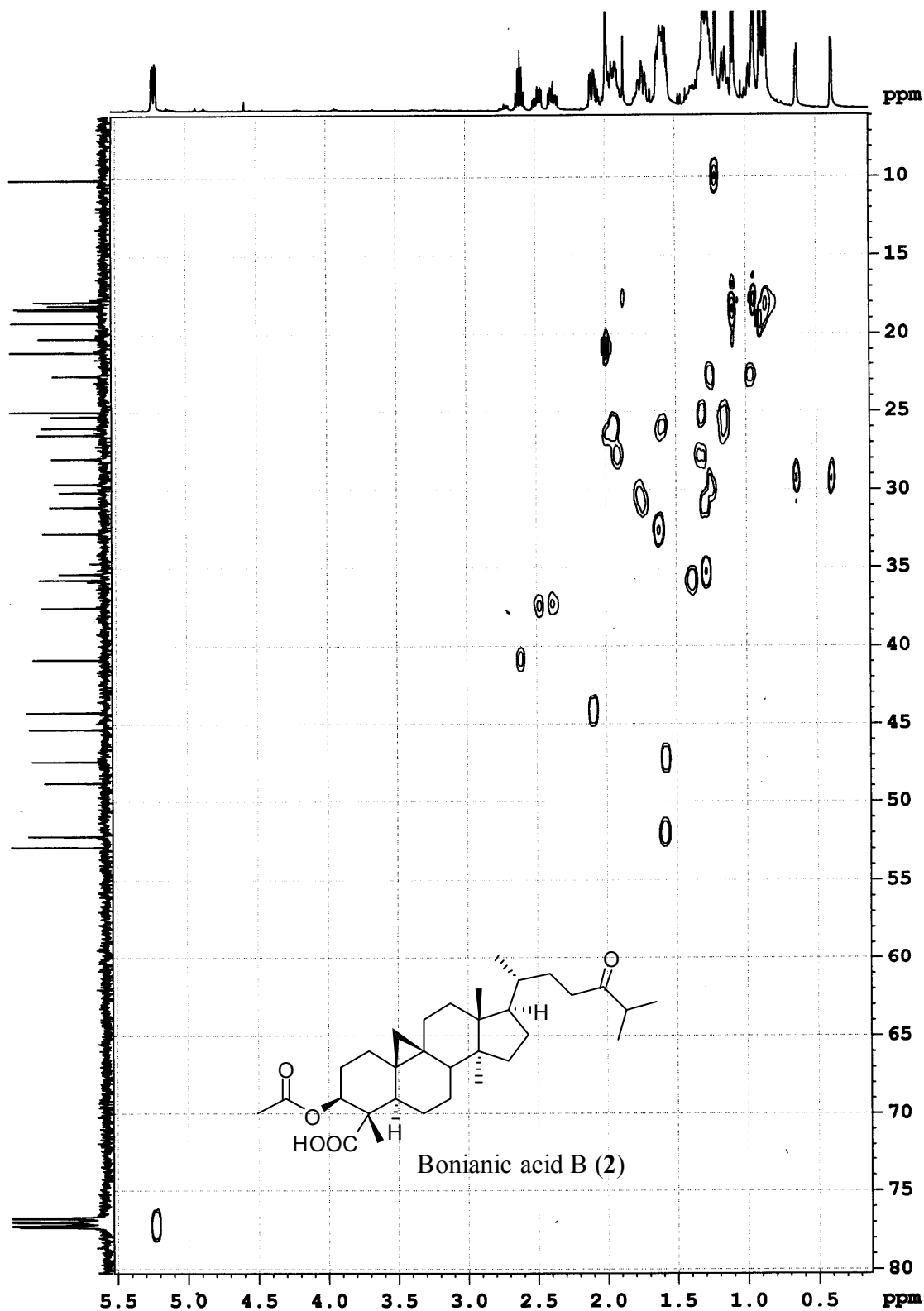
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P1 10.50 us
PL1 -3.00 dB
SFO1 500.1322506 MHz

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GPY1 0.00 %
GPY2 0.00 %
GPZ1 10.00 %
GPZ2 10.00 %
P16 1000.00 us

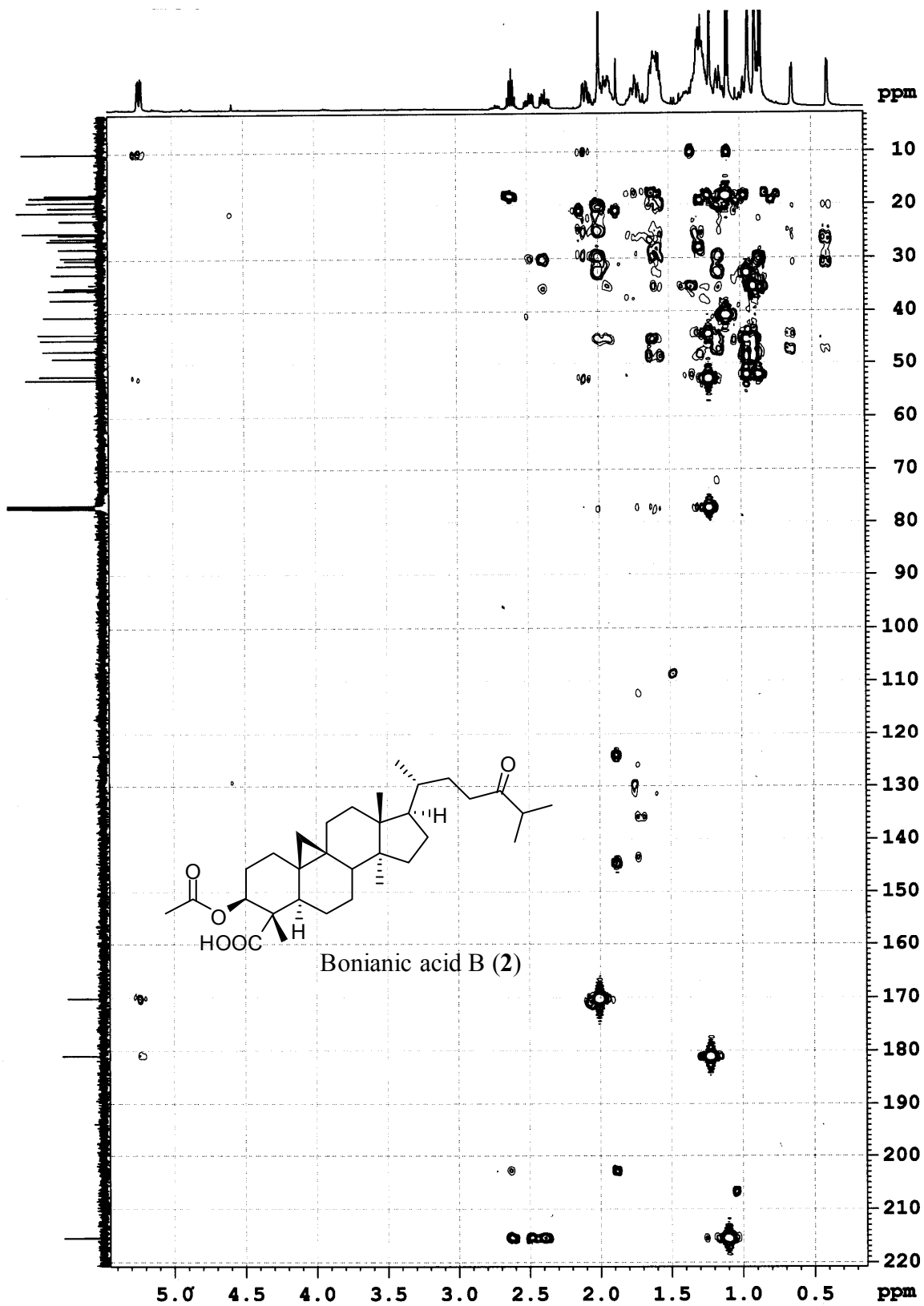
F1 - Acquisition parameter
ND0 1
TD 256
SFO1 500.1323 MHz
FIDRES 19.531250 Hz
SW 9.997 MHz
FnMODE QF

F2 - Processing parameters
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SF 500.1300000 MHz
WDW SINE
SSB 0
LB 0.00 Hz
GB 0
PC 1.40

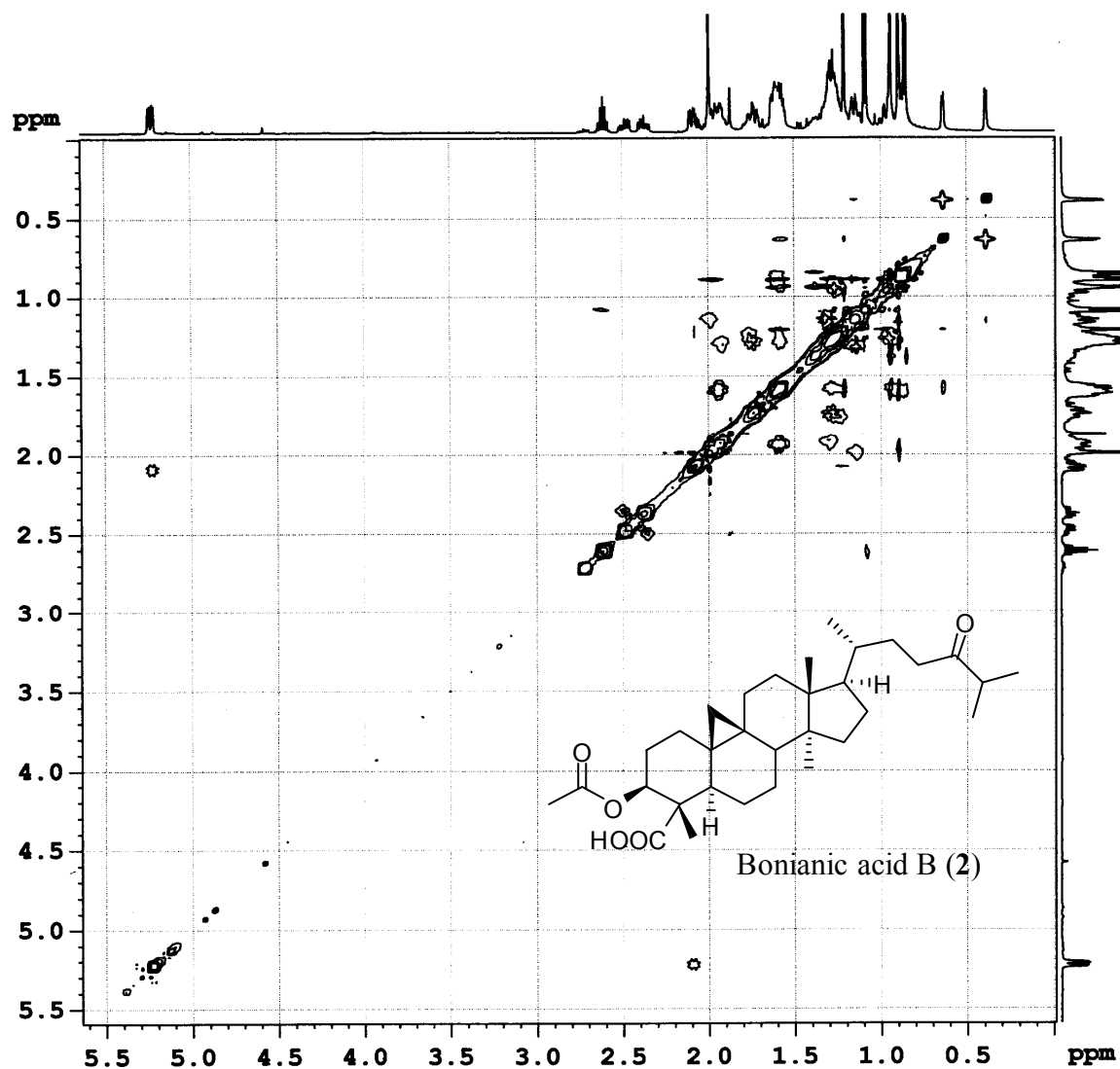
HSQC spectrum (^1H : 500.13 MHz, ^{13}C : 125.76 MHz, CDCl_3)
of bonianic acid B (2)



HMBC spectrum (^1H : 500.13 MHz, ^{13}C : 125.76 MHz, CDCl_3)
of bonianic acid B (2)



NOESY spectrum (500.13 MHz, CDCl₃) of bonianic acid B (2)



Current Data Parameters
 NAME 2LUONGHUNG.F11974
 EXNO 8
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20071018
 Time_ 12.00
 INSTRUM av500
 PROBHD 5 mm Multinucl
 PULPROG noesyph
 TD 2048
 SOLVENT CDCl3
 NS 16
 DS 4
 SWH 4496.403 Hz
 FIDRES 2.195509 Hz
 AQ 0.2278988 sec
 RG 35.9
 DW 111.200 usec
 DE 6.00 usec
 TE 300.0 K
 d0 0.00009771 sec
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 D8 0.80000001 sec
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 MCWRK 0.75000000 sec
 ST1CNT 128

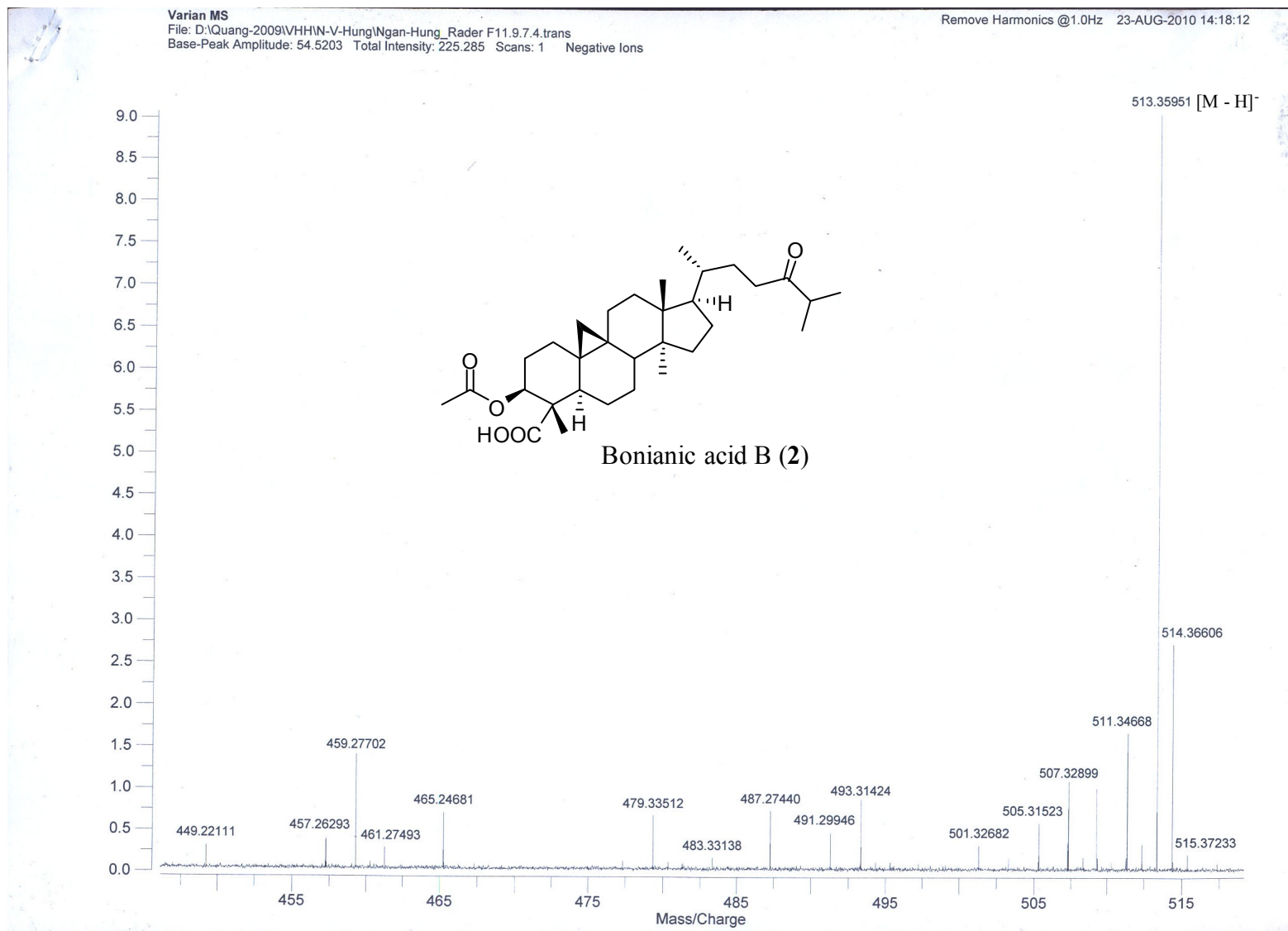
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 SW 9.000 ppm
 FnMODE States-TPPI

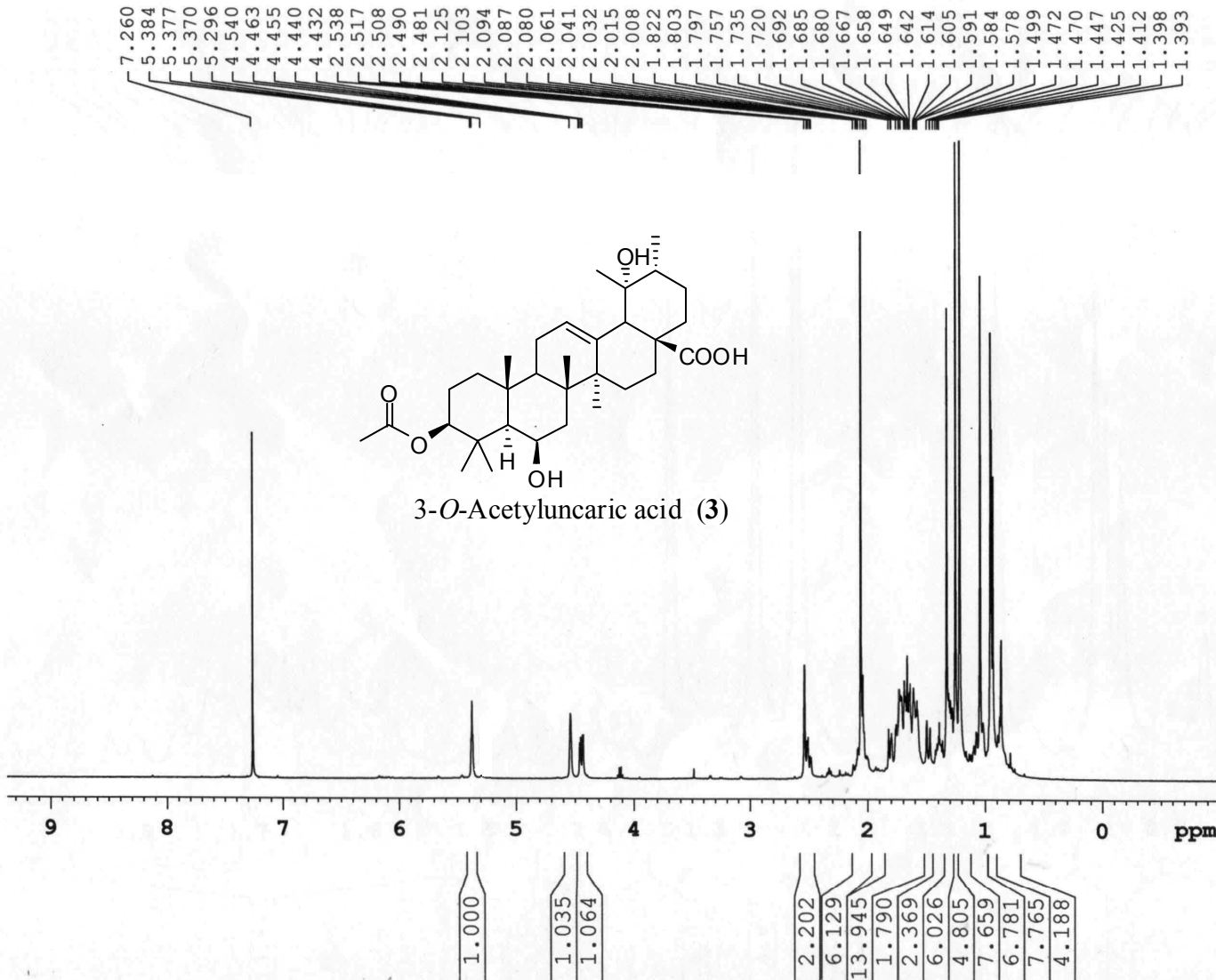
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 SSB 2
 LB 0.00 Hz
 GB 0
 PC 1.00

F1 - Processing parameters
 SI 1024
 MC2 States-TPPI
 SF 500.1303150 MHz
 WDW QSINE
 SSB 2
 LB 0.00 Hz
 GB 0

Negative HRESI mass spectrum of bonianic acid B (2)



¹H NMR spectrum (500.13 MHz, CDCl₃) of 3-O-acetylluncaric acid (3)



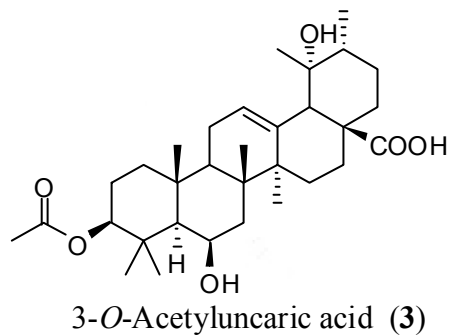
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EXPNO 1
PROCNO 1

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SOLVENT CDCl3
NS 16
DS 0
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2769001 sec
RG 181
DW 50.000 usec
DE 6.00 usec
TE 301.1 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

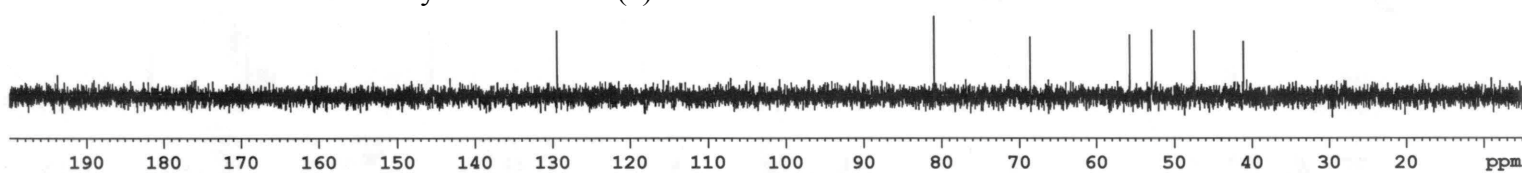
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SFO1 500.1323037 MHz

F2 - Processing parameters
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SF 500.1300134 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C NMR and DEPT spectra (125.76 MHz, CDCl_3) of 3-*O*-acetyluncaric acid (3)



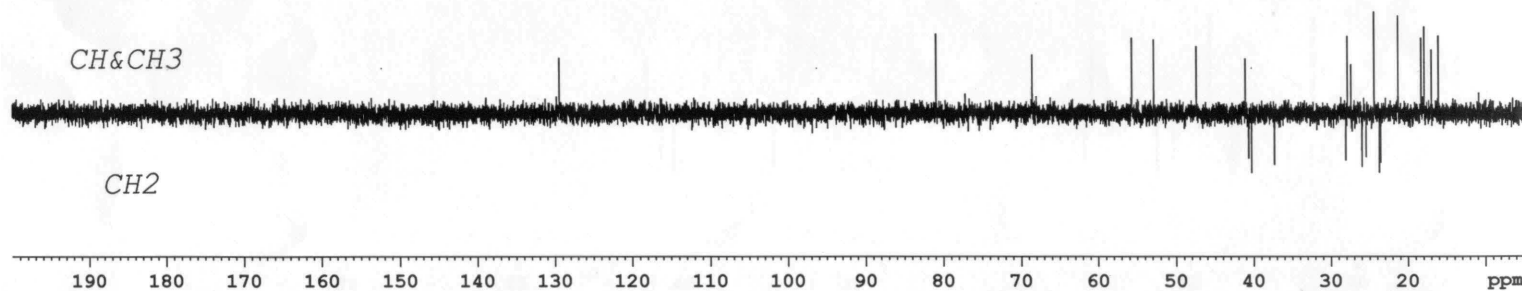
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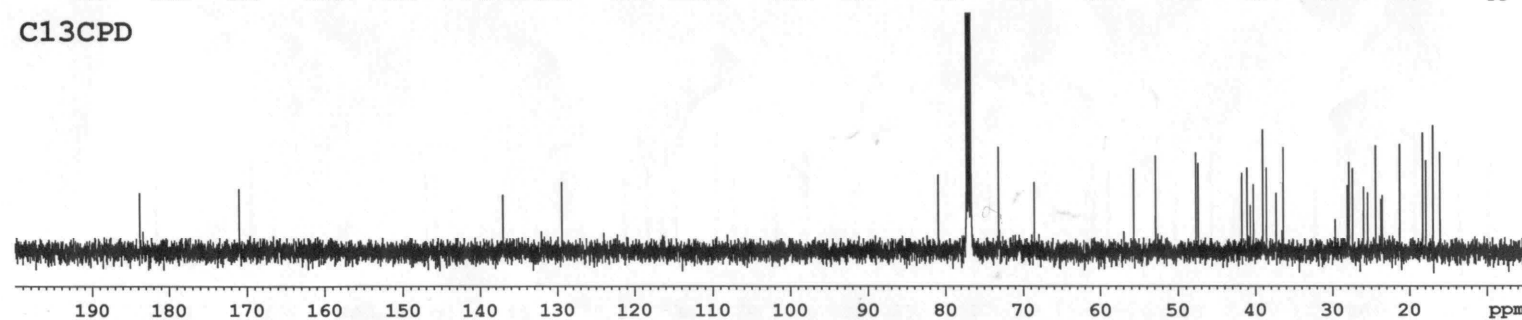
DEPT135

CH&CH₃

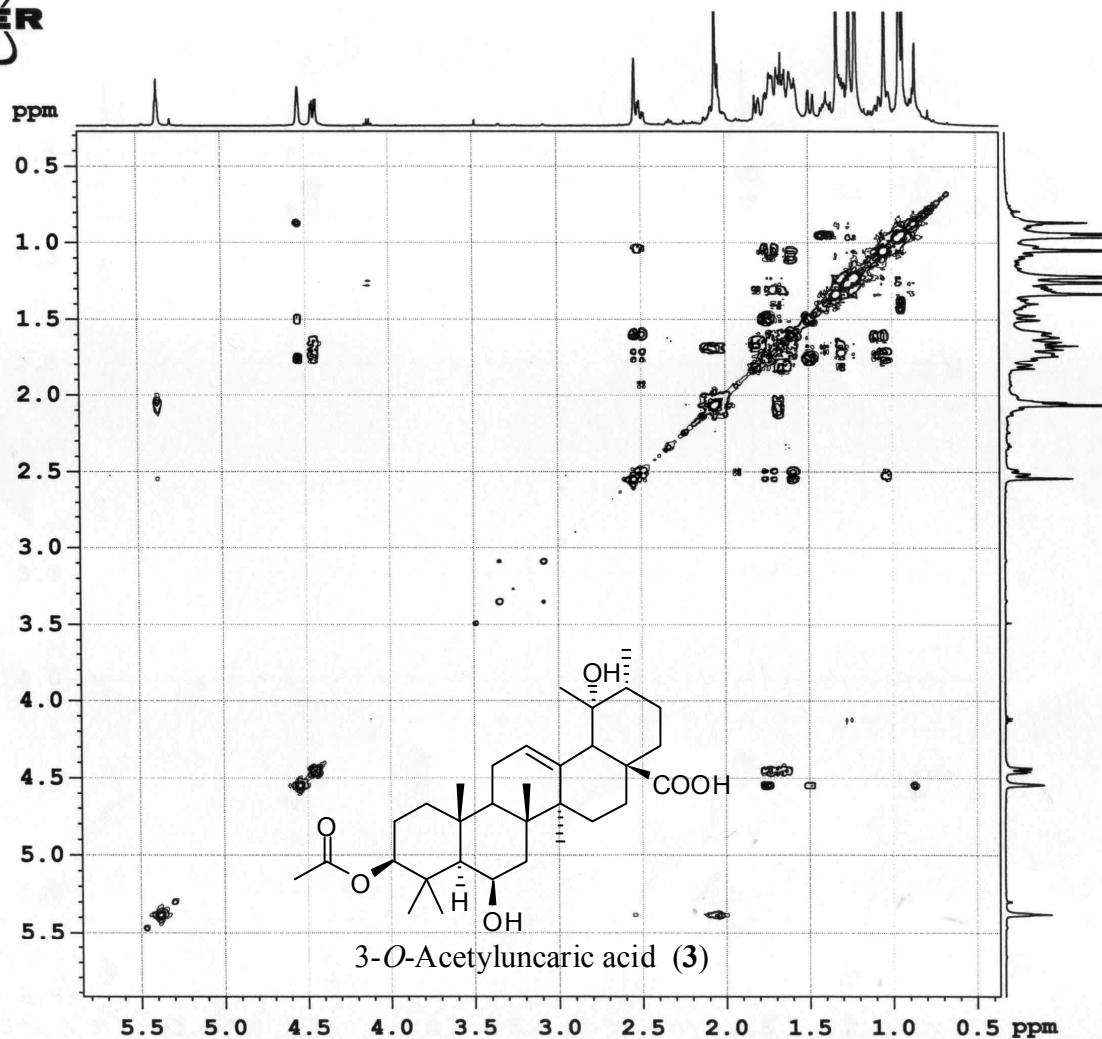
CH₂



C13CPD



^1H - ^1H COSY spectrum (500.13 MHz, CDCl_3) of 3-*O*-acetylluncaric acid (3)



Current Data Parameters
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 EXPNO 5
 PROCNO 1

F2 - Acquisition Parameters
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 PULPROG cosygpgf
 TD 2048
 SOLVENT CDCl_3
 NS 8
 DS 8
 SWH 5000.000 Hz
 FIDRES 2.441406 Hz
 AQ 0.2049500 sec
 RG 203.2
 DW 100.000 usec
 DE 6.00 usec
 TE 302.1 K
 d0 0.00000300 sec
 D1 1.48689198 sec
 d13 0.00000400 sec
 D16 0.00015000 sec
 INO 0.00020000 sec
 MCREST 0.00000000 sec
 MCWRK 1.48689198 sec

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 PL1 -3.00 dB
 SFO1 500.1322506 MHz

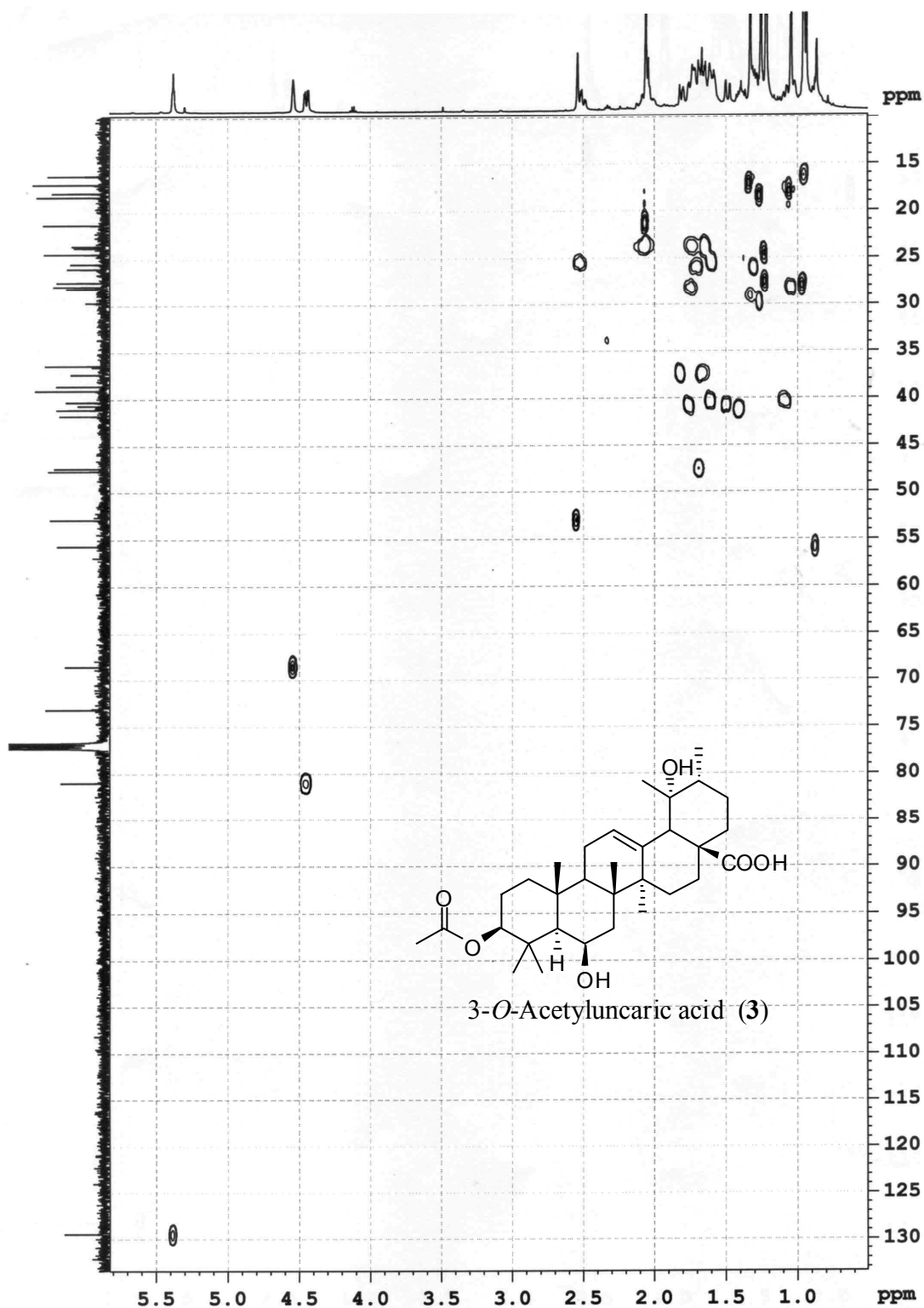
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 GPX2 0.00 %
 GPY1 0.00 %
 GPY2 0.00 %
 GPZ1 10.00 %
 GPZ2 10.00 %
 P16 1000.00 usec

F1 - Acquisition parameters
 ND0 1
 TD 256
 SFO1 500.1323 MHz
 FIDRES 19.531250 Hz
 SW 9.997 ppm
 FMODE QF

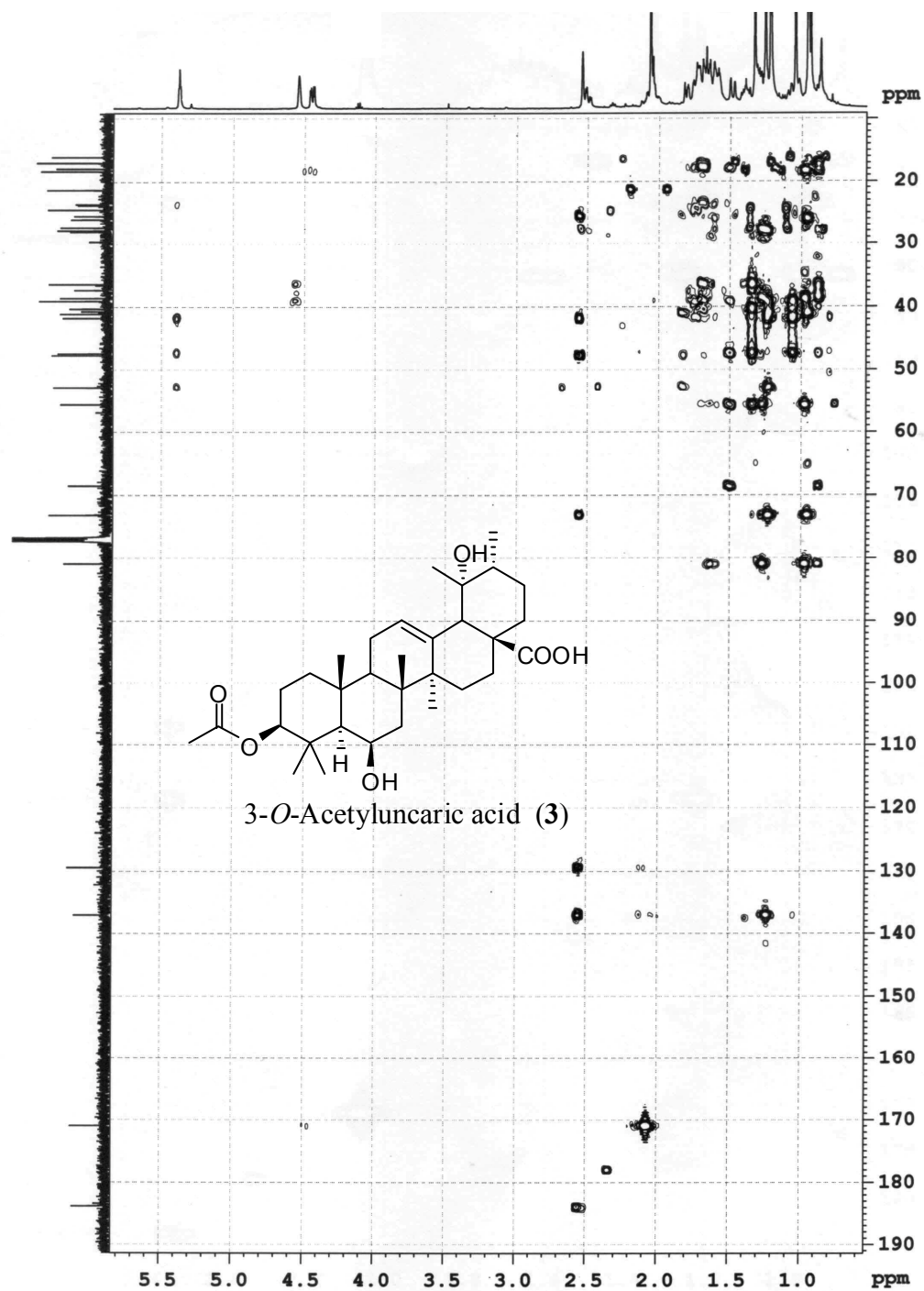
F2 - Processing parameters
 SI 1024
 SF 500.1300000 MHz
 WDW SINE
 SSB 0
 LB 0.00 Hz
 GB 0
 PC 1.40

F1 - Processing parameters
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 MC2 QF
 SF 500.1300000 MHz
 WDW SINE
 SSB 0
 LB 0.00 Hz
 GB 0

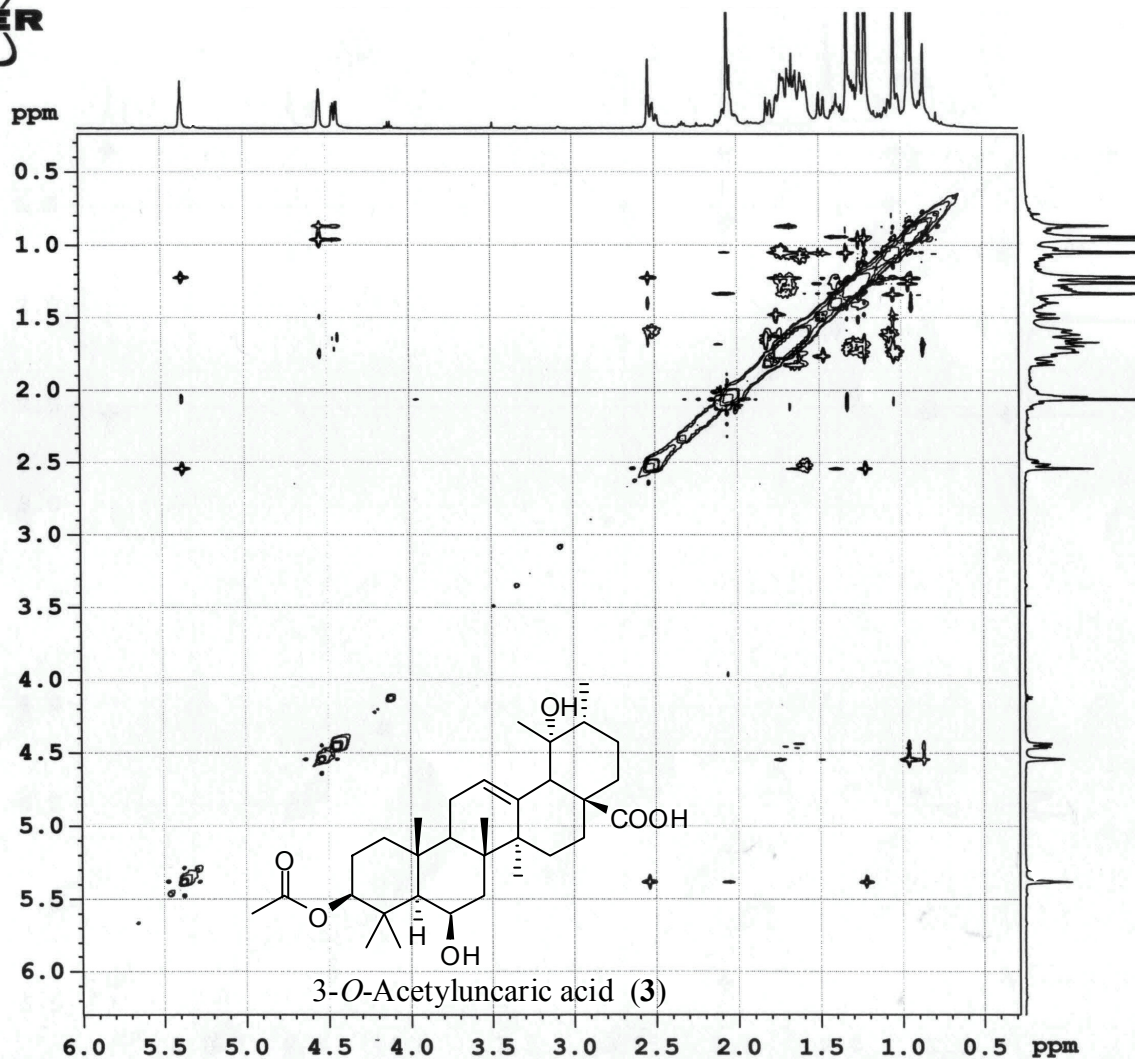
HSQC spectrum (^1H : 500.13 MHz, ^{13}C : 125.76 MHz, CDCl_3)
of 3-*O*-acetylluncaric acid (3)



HMBC spectrum (^1H : 500.13 MHz, ^{13}C : 125.76 MHz, CDCl_3)
of 3-*O*-acetyluncaric acid (3)



NOESY spectrum (500.13 MHz, CDCl₃) of 3-O-acetylluncaric acid (3)



Current Data Parameters
 NAME 2NGANHUNG.RADERF62
 EXPNO 8
 PROCNO 1

F2 - Acquisition Parameters
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 Time_ 0.44
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 PROBHD 5 mm Multinucl
 PULPROG noesyph
 TD 2048
 SOLVENT CDCl3
 NS 32
 DS 8
 SWH 5000.000 Hz
 FIDRES 2.441406 Hz
 AQ 0.2049500 sec
 RG 203.2
 DW 100.000 usec
 DE 6.00 usec
 TE 301.8 K
 d0 0.00008661 sec
 D1 1.50000000 sec
 D8 0.30000001 sec
 INO 0.00019995 sec
 MCREST 0.00000000 sec
 MCWRK 0.75000000 sec
 ST1CNT 128

----- CHANNEL f1 -----
 NUC1 1H
 P1 10.50 usec
 PL1 -3.00 dB
 SF01 500.1320005 MHz

F1 - Acquisition parameters
 NDO 1
 TD 256
 SF01 500.1323 MHz
 FIDRES 19.536135 Hz
 SW 10.000 ppm
 FnMODE States-TPPI

F2 - Processing parameters
 SI 1024
 SF 500.1300129 MHz
 WDW QSINE
 SSB 2
 LB 0.00 Hz
 GB 0
 PC 1.00

F1 - Processing parameters
 SI 1024
 MC2 States-TPPI
 SF 500.1303134 MHz
 WDW QSINE
 SSB 2
 LB 0.00 Hz
 GB 0

Positive HRESI mass spectrum of 3-O-acetylluncaric acid (3)

